

Chapter 2

Neuroprotective Agents

Classification of Neuroprotective Agents

A pharmacological classification of various neuroprotective agents is shown in Table 2.1. Approximately 80 categories are listed and include examples of over 400 neuroprotective agents. These approaches have been tested in animal experiments and clinical trials but some of the trials have not been successful. A few of the agents that are approved for non-neurological disorders have been used empirically as neuroprotectives in clinical practice. Some of these products will be described briefly in the following text.

Activity-Dependent Neuroprotective Protein

Activity-dependent neuroprotective protein (ADNP) is an essential protein for brain function and plays a role in normal cognitive performance. ADNP contains a homeobox profile and a peptide motif providing neuroprotection against a variety of cytotoxic insults (Gozes 2007). ADNP mRNA and protein expression responds to brain injury and fluctuates with phases of the estrus cycle. ADNP differentially interacts with chromatin to regulate essential genes. ADNP(+/-) mice exhibit cognitive deficits, significant increases in phosphorylated tau, tangle-like structures, and neurodegeneration compared with ADNP(+/+) mice. Increased tau hyperphosphorylation is known to cause memory impairments in neurodegenerative diseases associated with tauopathies, including AD. ADNP-deficient mice offer an ideal paradigm for evaluation of cognitive enhancers. Structure–activity studies have identified a short 8 amino acid peptide in ADNP, NAPVSIPQ (NAP), which interacts with microtubules and provides potent neuroprotection. NAP treatment partially ameliorated cognitive deficits and reduced tau hyperphosphorylation in the ADNP(+/-) mice (Vulih-Shultzman et al. 2007). An ADNP-based product, davunetide, is in phase II clinical trials to assess its effects on mild cognitive impairment and AD (see Chap. 9).

Table 2.1 A classification of neuroprotective agents

Category	Examples
Adenosine reuptake blockers	Dipyridamole Propentofylline
Anesthetics	Barbiturates Etomidate Gaseous anesthetics: isoflurane, xenon Local anesthetics: lidocaine Propofol
Angiotensin converting enzyme inhibitors/ nonantihypertensive Angiotensin-II inhibitors	Irbesartan (central AT ₁ receptor blocker) Perindopril Aliskiren
Antibiotics	β-Lactam antibiotics
Antidepressants	All categories
Antiepileptic agents	Phenytoin Topiramate Zonisamide
Anti-inflammatory agents	Acetylsalicylic acid (aspirin) Alpha-phenyl- <i>tert</i> -butylnitron COX-2 inhibitors: nimesulide Dipyrrone Doxycycline Gabexate mesilate Interleukin-1 antagonists Methylprednisolone Proteasome inhibitor MLN519 Synthetic fibronectin peptides Activated protein C Calpain inhibitors Caspase inhibitors: e.g., minocycline Cycloheximide DNA binding drugs: mithramycin A and chromomycin A3 Dopamine, noradrenaline, and ovarian steroid receptor agonists DP-b99 (metal ion chelator) GAPDH ligands JNK inhibitors L-Acetylcysteine Lithium Omega-3 fatty acids: docosahexaenoic acid (DHA) p53 inhibitors PARP (poly-(ADP-ribosyl) polymerase) inhibitors Transcription factor NF-κB TRO19622: prevents release of apoptotic factors from mitochondria

(continued)

Table 2.1 (continued)

Category	Examples
Antioxidants/free radical scavengers	α -Tocopherol (vitamin E) Ascorbic acid (vitamin C) Beta carotene (precursor of vitamin A) Cannabidiol (a cannabis compound) Cerovive Dexanabinol Dopamine D ₂ receptor agonists Dihydroergocryptine Ebselen Edaravone Flavonoids Glutathione peroxidase Idebenone (analog of coenzyme Q10) Neuroleptics: e.g., chlorpromazine Nicaraven Nitrones Pegorgotein Pyrrolopyrimidines Quercetin (a flavonoid in apples) Tirilazad mesylate Tauroursodeoxycholic acid
Atypical antipsychotics	Olanzapine
Cardiac glycosides	Neriifolin
CNS stimulants	Modafinil
Cytokines	Darbepoietin alfa Erythropoietin Granulocyte colony-stimulating factor Monocyte chemoattractant protein-1
Cell therapy	Cell transplants: neuronal stem cells, adult stem cells Cell transplants secreting neuroprotective substances Ceresine
Dichloroacetate/stimulates pyruvate dehydrogenase and lowers lactate	
Dopamine pathway inhibitor	Tetrabenazine
Endogenous vasoactive gases	Carbon monoxide
Flavones	Nitric oxide
GABA agonists	Epicatechin: found in cocoa and tea
Gene therapy	Clomethiazole Delivery of neurotrophic factors by genetically engineered cells CNTF delivered via tetracycline-regulated lentiviral vectors HSV vectors expressing virus-derived anti-apoptotic agents Oral genetic vaccine that targets a subunit of the NMDA receptor

(continued)

Table 2.1 (continued)

Category	Examples
Glutamate transport promoters	(<i>R</i>)-(-)-5-methyl-1-nicotinoyl-2-pyrazoline (MS-153) <i>N</i> -(4-Acetyl-1-piperazinyl)- <i>p</i> -fluorobenzamide monohydrate (FK960) Citicoline Ceftriaxone
Glutamate antagonists acting by multiple mechanisms	L-Phenylalanine <i>N</i> -Acylethanolamines
Glutamate blockade: presynaptic	619C89 [4-amino-2-(4-methyl-1-piperazinyl)-5-(2,3,5-trichlorophenyl)pyrimidine] Enadoline NAALADase (<i>N</i> -acetylated- α -linked-acidic-dipeptidase) inhibitors
Glutamate modulators: AMPA site	AMPA/kainate agonists AMPA/kainate antagonist AMPA receptor modulators
Glycine-proline-glutamate analogs (IGF-1 derivatives)	NNZ-2566 (Neuren Pharmaceuticals)
Heat shock proteins	HSP 40, HSP60, HSP70, HSP-90
Heme-degrading enzymes	Heme oxygenase-1
Herbal preparations	Rb extract of ginseng (<i>Panax quinquefolius</i>) Flavonoid wogonin (root of <i>Scutellaria baicalensis Georgi</i>)
Histamine H ₂ antagonists	Ranitidine
Hormones and related receptors	Corticosteroids Corticotrophin-releasing hormone Estrogens Insulin Progesterone SERMs (selective estrogen receptor modulators) Thyrotropin-releasing hormone analogs
Hydrogen sulfide-based	Sodium sulfide
Ion channel blockers:	Nimodipine
Ca ²⁺ channel blockers	Flunarizine
Ion channel blockers:	Carbamazepine
Na ⁺ channel blockers	Fosphenytoin Lamotrigine Phenytoin Riluzole Enecadin
Ion channel blockers:	
Ca ²⁺ and Na ⁺ channel blocker	
Ion channel blockers:	Amilorid
Acid-sensing ion channel blockers	
Ion channel blockers:	3-Bicyclo[2.2.1]hept-2-yl-benzene-1,2-diol
K ⁺ channel blockers	
Immunosuppressants	Mycophenolate Neuroimmunophilins (separate listing) Rapamycin

(continued)

Table 2.1 (continued)

Category	Examples
Iron chelator	Desferoxamine
Keap1/Nrf2 pathway activators	Neurite outgrowth-promoting prostaglandin compounds
Leukocyte adhesion inhibitors	Anti-ICAM antibody (Enlimomab) Neutrophil inhibitory factor Synthetic fibronectin peptides
MAO-A & B inhibitors	Lazabemide Selegiline
Mitochondrial protective agents	Methylene blue
Nanoparticulate neuroprotectives	Fullerene C60 (Buckyballs)
Neural regeneration protein	NNZ-4291
Neuroactive polyunsaturated lipids	LAX-101 (an ethyl-ester of eicosapentaenoic acid)
Neuroimmunophilins (see immunosuppressants also)	Cyclosporine GPI 1485 Tacrolimus (FK506) FKBP (FK binding proteins)
Neuropeptides	α -Melanocyte stimulating hormone Corticotropin-releasing hormone Thyrotropin-releasing hormone Vasoactive intestinal peptide
Neurotrophic factors and enhancing agents	Activity-dependent neurotrophic factor Angiogenesis growth factor-1 Brain derived neurotrophic factor Ciliary neurotrophic factor Fibroblast growth factors Insulin-like growth factor Leukemia inhibitory factor Nerve growth factor Neurotrophin 4/5 Pigment epithelium-derived factor
Neurotrophic factor-like neuroprotective agents	Clenbuterol (β 2-adrenoceptor agonist) Colivelin Gambogic amide Inosine Meteorin Neuregulin-1 Oxygen-regulated protein 150 kDa (ORP150) Prosaptide Siagoside (GM1 ganglioside)
Neurosteroids	Dehydroepiandrosterone Pregnenolone and allopregnanolone HF0220 (Hunter-Fleming)
Nicotine and nicotinic receptor agonists	Nicotine GTS-21 [3-(2,4-dimethoxybenzylidene)-anabaseine dihydrochloride]

(continued)

Table 2.1 (continued)

Category	Examples
Nitric oxide (NO) therapeutics	NO inhibitors: aminoguanidine, lubeluzole, selective nNOS inhibitors NO mimics: GT 015, GT 403, GT 094
NMDA antagonists: glycine site	ACEA 1021 Gavestinel
NMDA antagonists: polyamine site	Eliprodil Ifenprodil
NMDA receptor antagonists: competitive	1-(<i>cis</i> -2-carboxypiperidine-4-yl)-propyl-1-phosphonate 2-Amino-5-phosphonovalerate (APV) 2-Amino-7-phosphoheptanoate (APH) 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) Kynurenic acid derivatives
NMDA receptor antagonists: noncompetitive	3,3-bis(3-fluorophenyl) propylamine 5-Aminocarbonyl-10, 11-dihydro-5H-dibenzo [a,d]cyclohepten-5,10-imine Amantadine Aptiganel (previously known as Cerestat) Dextrophan Dextromethorphan Dizolcipine maleate (MK-801) Gacyclidine (GK-11) Ketamine Magnesium Memantine and neramexane Phencyclidine Remacemide Traxoprodil
Non-NMDA excitatory amino acid antagonists	5-HT agonists Opioid receptor antagonists: naloxone, nalmefene Cannabidiol
Nootropics	Dexanabinol Cerebrolysin Pyrrolidine derivatives: e.g., piracetam Ginkgo biloba
Nutraceuticals and food constituents	Creatine Curcumin Glyceryl triacetate Nicotinamide Reservatrol
Opioids	Delta opioid peptides Selective κ -opioid agonists
Osmotic diuretics	Frusemide Mannitol
Oxygen therapeutics	Hemoglobin-based oxygen carriers Hyperbaric oxygen Normobaric oxygen Perfluorocarbons

(continued)

Table 2.1 (continued)

Category	Examples
Phenothiazine-derivatives	Chlorpromazine, flufenazine, prochlorperazine, promazine, promethazine, propiomazine, thioridazine, trifluoperazine, trifluopromazine
Phosphodiesterase inhibitors	Ibutilast, denbufylline, sildenafil (Viagra)
Phosphatidylcholine precursor	Citicoline (CDP-choline)
Phytopharmaceuticals/natural derivatives	PYM50028 (Phytopharm plc), a phytosynthetic compound Sulforaphane (stimulates the expression of cytoprotective genes in brain)
Protease-activated receptor (PAR1) antagonist	BMS-200261
Proteins and peptides	Activated protein C Albumin (high dose) Cethrin™: a Rho antagonist recombinant protein C3: third complement component-derived peptide Fused protein transduction domain of the HIV Tat protein with FNK Glatiramer acetate Osteopontin PACAP38 (adenylate cyclase-activating polypeptide) Sir 2 group of proteins Uncoupling protein-2
Serine racemase antagonists	D-Amino acid oxidase
Signaling pathway activator	Fructose-1,6-bisphosphate
Statins: HMG-CoA (beta-hydroxy-beta-methylglutaryl coenzyme A reductase) inhibitors	Lovastatin Pravastatin
Thrombolytic agents for dissolving clots in cerebral arteries	Tissue plasminogen activator Desmoteplase
Toxins	Tetanus toxin
Vaccines	For autoimmune disorders such as multiple sclerosis For neurodegenerative disorders such as Alzheimer's disease
Vitamins	Vitamins A, B6, B12, C, D, and E
Nonpharmacological	Controlled hypoxia induced by sublethal doses of carbon monoxide Environmental enrichment Exercise Hypothermia Ketogenic diet Preconditioning-induced neuroprotection Transcranial magnetic stimulation

Adenosine Analogs

Adenosine (A), an inhibitory neuromodulator, is an endogenous neuroprotective agent. It is considered to be a link between brain energy and neuronal signaling. Adenosine receptors modulate neuronal and synaptic function in a range of ways that may make them relevant to the occurrence, development, and treatment of brain ischemic damage and degenerative disorders. A_1 adenosine receptors tend to suppress neural activity by a predominantly presynaptic action, while A_{2A} adenosine receptors are more likely to promote transmitter release and postsynaptic depolarization. Stimulation of A_1 receptors decreases excitatory amino acid transmission and stimulation of A_2 receptors inhibits platelet and neutrophil activation, thus promoting vasodilatation. In addition to affecting respiration and vascular tone, deviations from normal CO_2 alter pH, consciousness, and seizure propensity. In the hippocampal slice preparation, increasing CO_2 , and thus decreasing pH, increases the extracellular concentration of the endogenous neuromodulator adenosine and inhibits excitatory synaptic transmission (Dulla et al. 2005). These effects involve adenosine A_1 and ATP receptors and depend on decreased extracellular pH. In contrast, decreasing CO_2 levels reduced extracellular adenosine concentration and increased neuronal excitability via adenosine A_1 receptors, ATP receptors, and ATPase. Thus CO_2 -induced changes in neuronal function arise from a pH-dependent modulation of adenosine and ATP levels. ATP breakdown during ischemia produces adenosine, which effluxes out of the neurons.

The combined effects of adenosine on neuronal viability and inflammatory processes have also led to considerations of their roles in Lesch–Nyhan syndrome, Creutzfeldt–Jakob disease, Huntington disease, and multiple sclerosis (MS), as well as the brain damage associated with stroke. In addition to the potential pathological relevance of adenosine receptors, there are attempts in progress to generate ligands that will target adenosine receptors as therapeutic agents to treat some of these disorders (Stone et al. 2009). Propentofylline, an old drug, is a weak adenosine A_1 -receptor antagonist with neuroprotective effect.

Propentofylline

This is a neuroprotective glial cell modulator with multiple functional effects. The molecular mechanisms of action are by blocking adenosine transport and inhibition of cyclic adenosine monophosphate (cAMP) and cGMP-phosphodiesterase. Thus it reinforces the multiple cellular actions of endogenous adenosine and cellular second messengers, cAMP and cGMP. Propentofylline inhibits cytotoxic functions of activated microglia and also modulates astrocytic functions by stimulating nerve growth factor (NGF) synthesis and secretion. The drug was shown to exert neuroprotective effects in experimental models of global and focal brain ischemia. Postischemic administration of Propentofylline increases adenosine levels in the brain, reduces glutamate release, and improves glucose metabolism in all regions of

the brain. There is a therapeutic window of opportunity during which activation of an adenosine A₁ receptor is beneficial to ischemic neurons. This product reached phase III trials for vascular dementia and AD but further development was discontinued due to lack of efficacy.

Antidepressants

Antidepressants are used routinely in the management of neurodegenerative disorders. Several preclinical studies, using a variety of antidepressants, have shown neuroprotective effect but there is paucity of clinical evidence. In studies on rats, a single dose of fluoxetine provides long-lasting protection against MDMA-induced loss of serotonin transporter, and this neuroprotection is detectable in vivo by 4-¹⁸F-ADAM micro-PET (Li et al. 2010b).

Antidepressant-Induced Neurogenesis

New neurons are generated in the adult hippocampus of many species including rodents, monkeys, and humans. Conditions associated with major depression, such as social stress, suppress hippocampal neurogenesis in rodents and primates. In contrast, all classes of antidepressants stimulate neuronal generation, and the behavioral effects of these medications are abolished when neurogenesis is blocked. These findings generated the hypothesis that induction of neurogenesis is a necessary component in the mechanism of action of antidepressant treatments. In most of the studies, the effects of antidepressants on newborn neurons have been reported only in rodents and tree shrews. One study has shown that neurogenesis is increased in nonhuman primates after antidepressant treatment (Perera et al. 2007).

Neurogenesis Induced by Electroconvulsive Therapy

Adult monkeys have been subjected to repeated electroconvulsive shock (ECS), which is the animal analog of electroconvulsive therapy (ECT), the most effective short-term antidepressant. Compared with control conditions, ECS robustly increases precursor cell proliferation in the subgranular zone (SGZ) of the dentate gyrus in the monkey hippocampus. A majority of these precursors differentiate into neurons or endothelial cells, while a few mature into glial cells. The ECS-mediated induction of cell proliferation and neurogenesis is accompanied by increased immunoreactivity for the neuroprotective gene product B-cell chronic lymphocytic lymphoma 2 (BCL2) in the SGZ. The ECS interventions are not

accompanied by increased hippocampal cell death or injury. Thus ECS is capable of inducing neurogenesis in the nonhuman primate hippocampus and supports the possibility that antidepressant interventions produce similar alterations in the human brain.

Neuroprotective Effect of Selective Serotonin Reuptake Inhibitors

Selective serotonin reuptake inhibitors (SSRIs) increase the extracellular level of the neurotransmitter serotonin by inhibiting its reuptake and also have interactions with other receptors and neurotransmitters (Jain 2010k). Although the main action is on 5-HT receptors, a PET study has demonstrated that oral administration of fluvoxamine, but not paroxetine, could result in its binding to sigma-1 receptors in the healthy human brain, implicating a role of these receptors in the neuroprotective mechanism of action of fluvoxamine (Hashimoto 2009). Currently approved SSRIs include the following:

- Citalopram
- Dapoxetine (approved only in some European countries; in phase III trials elsewhere)
- Escitalopram
- Fluoxetine
- Fluvoxamine
- Paroxetine
- Sertraline
- Zimelidine

SSRIs are mainly used as antidepressants but are also indicated for several other neuropsychiatric disorders. SSRIs may protect against neurotoxicity caused by several toxic compounds. Fluoxetine suppresses kainic acid-induced neuronal loss in the rat hippocampus, and the neuroprotective effect is associated with its anti-inflammatory effects (Jin et al. 2009). SSRIs may promote the growth of new neural pathways or neurogenesis in experimental animals. The neuroprotective effect has not been demonstrated in humans. Repinotan, a serotonin agonist (5HT1A receptor subtype), was investigated as a neuroprotective agent in acute stroke, but further development was discontinued due to lack of efficacy.

Antiepileptic Drugs as Neuroprotectives

Some of the currently approved antiepileptic drugs (AEDs) also happen to have a neuroprotective effect. The neuroprotective effect of AEDs is classified according to their mechanism of action as shown in Table 2.2. Some of these drugs have an

Table 2.2 The neuroprotective effect of antiepileptic drugs

Mode of action	Drugs	Status
Na ⁺ channel blockers	Carbamazepine, fosphenytoin, lamotrigine, and phenytoin	Old, marketed
Interaction with voltage-sensitive calcium channels	Felbamate, lamotrigine, topiramate, and gabapentin	New, marketed
Ion channel modulation and selective binding to a synaptic vesicle protein	Levetiracetam	New, marketed
GABA agonists	Barbiturates, benzodiazepines, valproic acid	Old, marketed
	Gabapentin, felbamate, topiramate, tiagabine, vigabatrin, and zonisamide	New, marketed
Antiglutamate action	Felbamate and topiramate	New, marketed
	Remacemide hydrochloride	Phase III for refractory partial epilepsy. Also under investigation for the treatment of Parkinson's disease
	ADCI, a NMDA receptor channel blocker	Phase II clinical trials

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antiglutamate effect. Neuroprotective actions of tiagabine, topiramate, and zonisamide in cerebral ischemia are described in Chap. 3. The effect of remacemide as a neuroprotective in PD is discussed in Chap. 7.

Phenytoin

Phenytoin is one of the earliest AEDs. Cerebral protection with phenytoin has been reported in a variety of animal models of reduced oxygen delivery, including incomplete ischemia and global ischemia. Uncontrolled studies have investigated the use of phenytoin for its neuroprotective effect in patients with cardiac arrest, but the results are controversial. Phenytoin decreases cerebral blood flow but does not reduce cerebral metabolism. However, it stabilizes neuronal membranes, slows the release of K⁺ from ischemic neurons, and attenuates free fatty acid accumulation during complete global ischemia, thus preventing the cascade leading to the formation of free radicals. A study using DNA microarrays has shown that phenytoin exerts an effect on neuroprotection-related genes, namely the survival-promoting and antioxidant genes v-akt murine thymoma viral oncogene homolog 1, FK506-binding protein 12-rapamycin-associated protein 1 (Frap1), glutathione reductase, and glutamate cysteine ligase catalytic subunit (Mariotti et al. 2010).

Clinical role of phenytoin as a neuroprotective has not been established. Fosphenytoin was investigated as a neuroprotective in ischemic stroke in phase III

clinical trials but was found to be ineffective. The newer AEDs have a neuroprotective effect but no clinical trials are currently in progress to explore the neuroprotective effect in indications other than epilepsy.

Valproic Acid

Valproic acid (VPA), a drug widely used for the treatment of epilepsy, is also used for bipolar disorder and migraine prophylaxis. Phosphatidylinositol 3-kinase/Akt pathway and Sp1 are likely involved in heat shock protein (HSP)70 induction by histone deacetylase (HDAC) inhibitors, and induction of HSP70 by VPA in cortical neurons may contribute to its neuroprotective and therapeutic effects (Marinova et al. 2009). Neuroprotective actions of VPA in AD might be due to its anti-apoptotic action and slowing of neurofibrillary tangle formation through the inhibition of tau phosphorylation. This needs to be tested in mouse models of tauopathy as well as in a clinical trial of patients with AD. In a model of malonate toxicity in the rat striatum, augmentation of glutamate uptake contributes to VPA-mediated neuroprotection in striatum (Morland et al. 2004).

DP-VPA (D-Pharm) is a unique phosphatidyl-choline conjugate of VPA. It is a new chemical entity generated using D-Pharm's proprietary technology, Regulated Activation of Prodrugs (D-RAP), which enables precise control over drug action at the site of pathology. In particular, DP-VPA's antiseizure effects are regulated in direct response to "metabolic" activity at the epilepsy focus in the brain. Preclinical and phase I results demonstrate a unique safety and pharmacokinetic profile of the drug. It is in phase II clinical trials.

Levetiracetam

Levetiracetam is a pyrrolidone derivative and is not related to any of the AEDs currently in use. It is indicated as an adjunctive therapy in the treatment of partial seizures with or without secondary generalization in adults. It is approved as an adjunctive therapy in the treatment of myoclonic seizures in adults and adolescents with juvenile myoclonic epilepsy from 12 years of age.

Levetiracetam has been demonstrated to have neuroprotective effect in the rat middle cerebral artery model of stroke, which may be relevant to its antiepileptic effect (Jain 2010g). It has a neuroprotective effect, which is relevant to the prophylactic use of AEDs following subarachnoid hemorrhage (SAH) and traumatic brain injury (TBI). However, commonly used AEDs have multiple drug interactions, require frequent monitoring of serum levels, and are associated with adverse effects that may prompt discontinuation. A study has tested the neuroprotective effect of levetiracetam in clinically relevant models of SAH and TBI (Wang et al. 2006). A single dose of levetiracetam improved functional and histological outcomes after

TBI. This effect appeared specific for levetiracetam and was not associated with phenytoin treatment. Treatment with levetiracetam also improved functional outcomes and reduced vasospasm following SAH. Levetiracetam may be a therapeutic alternative to phenytoin following TBI in the clinical setting when seizure prophylaxis is indicated. It is now in a clinical trial to prevent seizures in TBI (see Chap. 4).

Anti-inflammatory Agents

A number of anti-inflammatory agents have been investigated for their neuroprotective effect. Nonsteroidal anti-inflammatory drugs (NSAIDs) have been reported to reduce the development of dementia in elderly subjects. One anti-inflammatory agent that has been used and investigated extensively as a neuroprotective is methylprednisolone, but its beneficial effect may also be due to free radical scavenging. Methylprednisolone is a synthetic glucocorticoid that has been used extensively in clinical trials as a high-dose neuroprotective in spinal cord injury (SCI) and brain injury patients. In spite of the controversy, this approach is still used in clinical practice.

Aspirin

Aspirin (acetylsalicylic acid) is a commonly prescribed drug with a wide pharmacological spectrum. At concentrations compatible with amounts in plasma during chronic anti-inflammatory therapy, acetylsalicylic acid and its metabolite sodium salicylate were found to be protective against neurotoxicity elicited by the excitatory amino acid glutamate in neuronal cultures and hippocampal slices. The site of action of the drugs appears to be downstream of glutamate receptors and to involve specific inhibition of glutamate-mediated induction of nuclear factor kappa B (NF- κ B). Additional studies that directly manipulate NF- κ B will be needed to prove that a decrease in NF- κ B activity is causally related to aspirin's neuroprotective effect. These findings are at conflict with other studies that indicate that activation of NF- κ B prevents neuronal cell death.

Interleukin-1 Antagonists

Interleukin-1 (IL-1) is induced immediately after insults to the brain, and elevated levels of IL-1 have been strongly implicated in the neurodegeneration that accompanies stroke, Alzheimer's disease (AD), and MS. When IL-1 is released into a tissue, it activates macrophages to move into the injury site and cause inflammation. Macrophages release substances that kill bacteria and viruses, and they ingest dead cells. They also release IL-1, which signals more macrophages to invade the

damaged tissue. The macrophage reaction is beneficial in regenerating tissues, but in a poorly regenerating tissue like the brain, it can be devastating. When macrophages release IL-1 and attract more of the scavenger cells to the brain, they become excited and overactive, causing harm to other nearby cells. This adds to the damage caused by the initial injury and destroys more healthy neurons.

Previous studies by several investigators have shown that IL-1 is elevated after TBI, MS, AD, and Down's syndrome and that mice with reduced IL-1 are significantly protected from ischemic injury. Antagonists of IL-1 protects neural cells in experimental models of stroke and MS by abrogating microglial/macrophage activation and the subsequent self-propagating cycle of inflammation. Cyclooxygenase (COX)-2 inhibitors have been shown to slow the onset of AD, and IL-1 is upstream of COX-2. If IL-1 is knocked out, not only are the effects of COX-2 lost, but other events that recruit cells to the brain are also abrogated. The ideal candidates to block IL-1 would be antibodies to IL-1 or small molecular inhibitors of this signaling cascade.

COX-2 Inhibitors

Cyclooxygenase (COX)-2 has been localized to neurons and in cells associated with the cerebral vasculature, where it is involved in the inflammatory component of the ischemic cascade, playing an important role in the delayed progression of the brain damage. Selective COX-2 inhibitors were developed for the treatment of inflammatory pain as an improvement on nonselective COX inhibitor NSAIDs, which have undesirable gastrointestinal complications including ulceration. Some COX-2 inhibitors such as nimesulide and NS-398 have neuroprotective properties. COX-2 inhibitors act as a neuroprotective in vivo by suppressing toxic actions of microglia/macrophages, and NS-398 may rescue neurons from hypoxia/reoxygenation damage by a mechanism independent of COX-2 inhibition. NS-398-induced phosphorylation through extracellular signal-regulated kinase pathway may contribute to the increased neuronal survival.

Certain products of COX-2 can both protect and damage the brain. Prostaglandins are involved in a wide variety of bodily activities, including relaxation and contraction of muscles and blood vessels, and control of blood pressure and inflammation. Defining which prostaglandin pathways are beneficial and which promote disease would help to design more specific therapeutics. Prostaglandin PGD₂, the most-produced prostaglandin in the brain, has a protective or harmful effect in the brain depending on where it docks on a brain cell's surface. After brain cells experience the laboratory equivalent of a stroke, PDG2 can protect them from being killed if it binds to one receptor on the cells' surface, but causes them to die in greater numbers if it binds to a second receptor instead (Liang et al. 2005). After strokes and other injuries to the brain, levels of glutamate rise, triggering a number of chemical reactions including an increase in COX-2 production and prostaglandin production. Increased COX-2 activity then leads to further neuronal death. Because PGD₂'s positive effects generally outweigh its negative ones, it may provide a potential

target for medicines to combat conditions involving brain damage, including stroke, PD, and AD. Further investigations will determine if these prostaglandins have a similar protective effect in mouse models of ALS, in which excessive glutamate is believed to damage neurons, and if the beneficial side of PGD2 activity can outweigh its toxic activity.

Nimesulide

Nimesulide, a selective COX-2 inhibitor, has been shown to delay neuronal death of hippocampal CA1 neurons following transient global cerebral ischemia in animal models. Nimesulide also rescues CA1 pyramidal neurons from ischemic death even when treatment is delayed until 24 h after ischemia. The neuroprotective effect of nimesulide is still evident a month after the ischemic episode, providing experimental evidence that COX-2 inhibitors confer a long-lasting neuroprotection. Oral administration of nimesulide is also able to reduce brain damage significantly, suggesting that protective effects are independent of the route of administration.

Nimesulide has neuroprotective effects against kainate (kainic acid, KA) excitotoxicity in vivo, but these are not mediated by direct free radical scavenging ability of this compound. It is much more likely that these effects are mediated by the inhibition of COX-2, a key source of free radicals during injury to the brain.

Gold Microparticles as Anti-neuroinflammatory Agents

Gold is a traditional remedy against inflammatory disorders such as rheumatoid arthritis. Auromedication by intracerebral application of metallic gold microparticles as a pharmaceutical source of gold ions represents a new medical concept that bypasses the BBB and enables direct drug delivery to inflamed brain tissues (Danscher and Larsen 2010). The systemic use of gold salts is limited by nephrotoxicity. However, implants of pure metallic gold release gold ions, which do not spread in the body, but are taken up by cells near the implant. This is a safer method of using gold to reduce local neuroinflammation. Release or dissolution of gold ions from metallic gold surfaces requires the presence of disolycytes, i.e., macrophages, and the process is limited by their number and activity. The method of delivery, however, is invasive and a gold implant could produce foreign body reaction, leading to an epileptic focus. This can be refined by the use of gold nanoparticles. The metallic gold treatment significantly increases the expression of the growth factors VEGF, FGF, LIF, and neurotrophin-4 (Pedersen et al. 2010). Furthermore, metallic gold has been found to reduce TNF- α expression, oxidative DNA damage, and proapoptotic signals after experimental brain injury, resulting in an overall neuroprotective effect.

Minocycline

Minocycline, a tetracycline derivative that is approved by the FDA for treatment of infections, has a high CNS penetration when taken orally. Minocycline is considered to be a neuroprotective agent that exerts its effects by several mechanisms. It is an anti-apoptotic agent (see the following section). It also suppresses inflammation in the nervous system. Lipopolysaccharide-induced inflammation, which gives rise to microglia activation in the area where the new neurons are born, strongly impairs basal hippocampal neurogenesis in rats. Activated microglia contribute to cognitive dysfunction in aging, dementia, epilepsy, and other conditions leading to brain inflammation. The increased neurogenesis triggered by a brain insult is reduced if it is associated with microglia activation caused by tissue damage or lipopolysaccharide infusion. The impaired neurogenesis in inflammation is restored by systemic administration of minocycline, which inhibits microglia activation. Minocycline affords substantial neuroprotection against hypoxic cell death, assessed by lactate dehydrogenase release and flow cytometry, while suppressing oxygen glucose deprivation-induced p38 MAP kinase activation (Guo and Bhat 2007).

Microglial activation contributes to NMDA excitotoxicity. Minocycline prevents the NMDA-induced proliferation of microglial cells and inhibits IL-1 β -converting enzyme and iNOS upregulation in animal models of ischemic stroke and HD. Intravenous minocycline at doses that are likely safe in humans reduces infarct size in a rat temporary middle cerebral artery occlusion (MCAO) model. The therapeutic time window of minocycline is 4–5 h, which would extend its potential therapeutic benefit to many stroke patients. The neuroprotective effect and safety profile of minocycline indicate that it may be an effective agent in acute ischemic stroke and support initiation of phase I trials of minocycline. In vivo, minocycline reduces infarct volume and neurological deficits, and markedly reduces BBB disruption and hemorrhage in mice after experimental stroke (Yenari et al. 2006). This effect is attributed to the inhibition of microglial activation by minocycline.

Nanomolar concentrations of minocycline protect neurons in mixed spinal cord cultures against NMDA excitotoxicity. There is evidence for the neuroprotective effect of minocycline in several animal models of neurological diseases. Oral minocycline alleviates neuronal damage induced by the AIDS virus in monkeys (Ratai et al. 2010). High penetration of the BBB, safety, and multiple mechanisms of make it a desirable candidate as therapy for acute neurological injury (Elewa et al. 2006). The neuroprotective affect of minocycline in animal models is shown in Table 2.3. The neuroprotective effect of minocycline in SCI is described in Chap. 5 and that in ALS in Chap. 10.

Anti-apoptosis Agents

Apoptosis is inherently programmed cell death and is distinct from cell necrosis. Apoptosis mediates cell deletion in tissue homeostasis, embryological development, and pathological conditions such as cerebral infarction and neurodegenerative

Table 2.3 Neuroprotective affect of minocycline in animal models

Disease model	Effect of minocycline on lesions and disease outcome
Amyotrophic lateral sclerosis	Increases lifespan of mice
Experimental autoimmune encephalopathy	Attenuates the extent of neuroinflammation, encephalomyelitis, and demyelination
HIV encephalitis	Reduces the severity of encephalitis, suppresses viral load in the brain, and decreases the expression of CNS inflammatory biomarkers
Huntington's disease	Delays progression of disease and extends lifespan
Intracerebral hemorrhage	Reduces volume of hemorrhagic lesion
Ischemic stroke	Decreases the size of infarcts
Parkinson's disease	Protects the nigrostriatal pathway
Spinal cord injury	Produces functional recovery, and decreases axonal, neuronal, and oligodendroglial loss
Traumatic brain injury	Decreases lesion size and improves performance on a rotarod test

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diseases. During conditions of degeneration, the cascade of events that is believed to result in cell death is initiated by an increase in synaptic glutamate levels, which results in an overstimulation of postsynaptic glutamate receptors. This results in a dramatic increase in intracellular calcium, which leads to the formation of free radicals, such as nitric oxide. The excitatory amino acids – *N*-methyl-D-aspartate and glutamic acid (NMDA) – believed to be a causal factor in many neurodegenerative diseases, have been found to induce apoptosis in neurons. Oxidative stress can also lead to apoptosis. There are several approaches to block apoptosis and some of the strategies mentioned under other categories of neuroprotectives have an anti-apoptotic effect.

Neuronal apoptosis probably underlies the damage caused by neurodegenerative disorders ranging from PD to MS. Therefore, strategies that inhibit components of the apoptotic pathway may prove useful in treating such conditions. Several anti-apoptosis agents are under investigation. Some of these are as follows:

- Activated protein C
- Immunosuppressants: FK506 and cyclosporine A
- Gene therapy: increasing the localized expression of bcl-2 protein in the brain.
- Neurotrophic factors
- Antisense therapy for modulation of p53 expression
- Apoptosis inhibitors: caspase inhibitors, calpain inhibitors, and poly (ADP-ribose) polymerase (PARPs) inhibitors
- Lithium

Activated Protein C

Activated protein C (APC), a serine protease with anticoagulant and anti-inflammatory activities, exerts direct cytoprotective effects on endothelium via endothelial protein

C receptor-dependent activation of PAR1. A modified version of APC is used to reduce inflammation or increase blood flow in patients with severe sepsis.

APC has been shown to protect mouse cortical neurons from two divergent inducers of apoptosis, NMDA and staurosporine (Guo et al. 2004). APC blocked several steps in NMDA-induced apoptosis downstream to nitric oxide, i.e., caspase-3 activation, nuclear translocation of apoptosis-inducing factor, and induction of p53, and prevented staurosporine-induced apoptosis by blocking caspase-8 activation. Intracerebral APC infusion dose dependently reduced NMDA excitotoxicity in mice. Direct neuroprotective effects of APC in vitro and in vivo require PAR1 and PAR3. Thus, PAR1 and PAR3 mediate anti-apoptotic signaling by APC in neurons, which may suggest novel treatments for neurodegenerative disorders.

Advantages of APC are that it is naturally present in the human body and is already being used to treat patients. A major side effect of APC is increased bleeding but neuroprotective effect of APC is separate from its ability to increase blood flow and reduce inflammation. This finding opens the possibility of creating a new compound that would protect brain cells without causing major side effects such as increased bleeding.

APC directly prevents apoptosis in hypoxic human brain endothelium through transcriptionally dependent inhibition of tumor suppressor protein p53, normalization of the proapoptotic Bax/Bcl-2 ratio, and reduction of caspase-3 signaling. These mechanisms are distinct from those involving upregulation of the genes encoding the anti-apoptotic Bcl-2 homolog A1 and inhibitor of apoptosis protein-1 (IAP-1) by APC in umbilical vein endothelial cells. Cytoprotection of brain endothelium by APC in vitro requires endothelial protein C receptor (EPCR) and protease-activated receptor-1 (PAR-1), similar to in vivo neuroprotective activity in a stroke model of mice with a severe deficiency of EPCR. This is consistent with a work showing the direct effects of APC on cultured cells via EPCR and PAR-1. Moreover, the in vivo neuroprotective effects of low-dose mouse APC seemed to be independent of its anticoagulant activity. Thus, APC protects the brain from ischemic injury by acting directly on brain cells.

Calpain Inhibitors

Calpain, a primary protease, was discovered more than 30 years ago as a Ca^{2+} -activated protease. Calpain activity is increased in TBI, cerebral ischemia, SCI, several neuromuscular disorders, and neurodegenerative diseases. Calpain proteolysis represents a later component of a pathway mediating apoptosis initiated by excitotoxicity and elevated Ca^{2+} levels. This would provide an advantage for calpain inhibitors over drugs with conventional targets such as ion channel blockers and glutamate antagonists.

Caspase Inhibitors

Apoptosis can be inhibited by agents that exhibit caspase activity. Caspases are an evolutionary conserved family of cysteine-dependent *aspartate*-directed proteases that cleave proteins after aspartic acid. Caspases comprise a family of 11 structurally related human enzymes, which are known to play specific roles in apoptosis (programmed cell death) and inflammation. Biochemical and genetic evidence indicates that initiator caspases proteolytically activate effector caspases, resulting in a proteolytic cascade that ultimately cleaves the cell to death. Caspase inhibitors are peptides resembling the cleavage site of known caspase substrates. Apart from naturally occurring caspase inhibitors, pharmacological caspase inhibitors can be reversible or irreversible. Several caspase inhibitors are being evaluated for neuroprotective effects in animal models of cerebral ischemia and various neurodegenerative diseases. Minocycline, an antibiotic, has a neuroprotective effect attributed to the inhibition of caspase-3.

DNA-Binding Drugs

Global inhibitors of RNA or protein synthesis such as actinomycin D or cycloheximide prevent neuronal apoptosis induced by numerous pathological stimuli *in vitro* and *in vivo* but clinical application to human neurological disease has been limited by the toxicities of these agents. Two sequence-selective DNA-binding drugs – mithramycin A and its structural analog chromomycin A3 – potentially inhibit apoptosis and DNA damage in cortical neurons caused by oxidative stress induced by DNA-damaging agents. This class of agents is believed to act, in part, by selectively inhibiting gene expression by displacing transcriptional activators that bind to G-C-rich regions of promoters. The complete prevention of cell death by this approach is probably by selective inhibition of the synthesis of one or more apoptotic pathway proteins.

Lithium

Lithium was introduced into psychiatry 50 years ago and remains a preferred treatment for acute mania and a preferred prophylactic therapy for manic-depressive illness. The therapeutic mechanisms of lithium for treating bipolar mood disorder remain poorly understood. Recent studies demonstrate that lithium has neuroprotective actions against a variety of insults. Neuroprotective effects of lithium against excitotoxicity have been demonstrated in cultured cerebral cortical neurons to coincide with the inhibition of NMDA receptor-mediated calcium influx. This action could also be relevant to its clinical efficacy for bipolar patients.

Lithium has also shown neuroprotective effect against striatal lesion formation in a rat model of HD and is associated with an increase in Bcl-2 protein levels. These results indicate that lithium may be considered as a neuroprotective agent in the treatment of neurodegenerative diseases such as HD and ALS (see Chap. 10). Lithium also has a neuroprotective effect in cerebral ischemia (see Chap. 3). Lithium chloride protects the retinal neural cells cultured with serum-free media to simulate the nutrient deprived state resulting from ischemic insult, which may be similar to the mechanism of cell death in glaucoma, and the improvement in DNA repair pathway involving ligase IV might have an important role in this neuroprotective effect (Zhuang et al. 2009).

Olesoxime

Olesoxime (Trophos SA's TRO19622) is a cholesterol-like small molecule with remarkable neuroprotective properties in vitro, as well as in vivo. It has demonstrated activity in four animal models, preventing neurodegeneration and accelerating neuroregeneration following neurotrauma. Investigation of its pharmacological action by Trophos and its academic partners has identified two potential mechanisms of action associated with binding sites for neurosteroids, GABA_A receptors, and mitochondria (Bordet et al. 2007). Olesoxime shows neuroprotective activity in the striatal model and may have the potential to become a more general neuroprotective drug. It is currently in phase III clinical trials for ALS and phase Ib trials for spinal muscular atrophy (SMA).

Omega-3 Fatty Acids

Omega-3 fatty acids, e.g., docosahexaenoic acid (DHA), regulate signal transduction and gene expression, and protect neurons from death. Omega-3 fatty acids demonstrate pronounced neuroprotective actions in rodent models and have been shown to mitigate cognitive dysfunction.

Docosahexaenoic Acid

Phosphatidylinositol 3-kinase (PI3K)/Akt signaling is a critical pathway in cell survival. Membrane alteration by the *n*-3 fatty acid status affects Akt signaling and impacts neuronal survival. DHA, a *n*-3 polyunsaturated fatty acid highly enriched in neuronal membranes, promotes neuronal survival by facilitating membrane translocation/activation of Akt through its capacity to increase phosphatidylserine (PS),

the major acidic phospholipid in cell membranes. The activation of PI3K and phosphatidylinositol triphosphate formation are not affected by DHA, indicating that membrane interaction of Akt is the event responsible for the DHA effect. In vivo reduction of DHA by dietary depletion of $n-3$ fatty acids decreases hippocampal PS and increases neuronal susceptibility to apoptosis in cultures. This mechanism may contribute to neurological deficits associated with $n-3$ fatty acid deficiency and support protective effects of DHA in pathological models such as brain ischemia or AD.

In cytokine-stressed human neural cells, DHA attenuates $A\beta$ secretion, an effect accompanied by the formation of neuroprotectin D1 (NPD1), a novel, DHA-derived 10,17S-docosatriene (Lukiw et al. 2005). DHA and NPD1 were reduced in AD hippocampal region but not in the thalamus or occipital lobes from the same brains. The expression of key enzymes in NPD1 biosynthesis, cytosolic phospholipase A2 and 15-lipoxygenase, was altered in AD hippocampus. NPD1 repressed $A\beta_{42}$ -triggered activation of proinflammatory genes, while upregulating the anti-apoptotic genes encoding Bcl-2, Bcl-x1, and Bfl-1(A1). Soluble APP- α stimulated NPD1 biosynthesis from DHA. These results indicate that NPD1 promotes brain cell survival via the induction of anti-apoptotic and neuroprotective gene-expression programs that suppress $A\beta_{42}$ -induced neurotoxicity.

Several studies demonstrate that in brain ischemia-reperfusion and in retinal pigment epithelial cells exposed to oxidative stress, stereospecific DHA-oxygenation pathways are activated and lead to the formation of docosanoid messengers. Two DHA-oxygenation pathways were identified: the first is responsible for the formation of the messenger NPD1 and the second pathway, which is active in the presence of aspirin, leads to the formation of the resolvin-type mediators (17R-DHA). NPD1 induces anti-apoptotic, anti-inflammatory signaling and is neuroprotective (Bazan 2007). This response aims to counteract proinflammatory, cell-damaging events triggered by multiple converging cytokines and in the case of AD, of amyloid peptide factors. Agonists of NPD1 biosynthesis, including dietary regimens enriched in omega-3 fatty acids or NPD1 analogs, may be useful for exploring new therapeutic strategies for stroke, head injury, AD, and related neurodegenerative diseases.

Poly(ADP-Ribose) Polymerase Inhibitors

PARP is an enzyme involved in the repair of damaged DNA and is very energy intensive. NO generation can cause excessive activation of PARP, which rapidly leads to a complete depletion of a cell's energy levels, resulting in cell death. PARP activation appears to be one of the final common steps in the neurotoxicity cascade, which results in neuronal cell death in stroke and neurodegenerative disorders. Thus, the inhibition of PARP offers a unique approach to the development of novel neuroprotective agents. Novel PARP inhibitors are being developed as neuroprotective agents for stroke and other disorders.

The activation of PARP by free radical-damaged DNA plays a pivotal role in mediating ischemia-reperfusion injury. In experimental studies, 3-aminobenzamide (a PARP inhibitor) has a neuroprotective effect if administered prior to reperfusion, but treatment after the reperfusion fails to produce a reduction in the volume of the damaged brain. These findings suggest that PARP activation sufficient to produce cellular damage occurs immediately after the reperfusion following cerebral ischemia.

In TBI, generation of NO and oxidative stress promotes PARP activation, contributing in post-traumatic motor, cognitive, and histological sequelae. Inactivation of PARP, either pharmacologically or using PARP null mice, induces neuroprotection in experimental models of TBI (Besson 2009). The mechanisms by which PARP inhibitors provide protection might not entirely be related to the preservation of cellular energy stores, but might also include other PARP-mediated mechanisms that need to be explored in a TBI context.

Excessive activation of PARP1 leads to NAD⁺ depletion and cell death during ischemia and other conditions that generate extensive DNA damage. When activated by DNA strand breaks, PARP1 uses NAD⁺ as a substrate to form ADP-ribose polymers on specific acceptor proteins. These polymers are in turn rapidly degraded by poly(ADP-ribose) glycohydrolase (PARG), a ubiquitously expressed exo- and endoglycohydrolase. PARG inhibitors do not inhibit PARP1 directly, but instead prevent PARP1-mediated cell death by slowing the turnover of poly(ADP-ribose) and thus slowing NAD⁺ consumption. PARG appears to be a necessary component of the PARP-mediated cell death pathway, and PARG inhibitors may have promise as neuroprotective agents.

Prevention of Apoptosis by Binding of proNGF to Sortilin

Sortilin, a protein whose function has been incompletely understood, plays a key role in conveying the message of apoptosis. Sortilin acts as a receptor for an unusual form of messenger proteins called proneurotrophins. These two proteins work together with a well-known receptor called p75 to initiate the death of both neurons and glia. This class of neurotrophin messenger proteins exhibits complex and even opposing actions. p75NTR (neurotrophin receptor) acts as a molecular signal switch that determines cell death or survival by three processes: (1) pro-nerve growth factor (proNGF) triggers cell apoptosis by its high-affinity binding to p75NTR, while NGF induces neuronal survival with low-affinity binding; (2) p75NTR mediates cell death by combining with co-receptor sortilin, whereas it promotes neuronal survival through combination with proNGF; and (3) release of the intracellular domain chopper or cleaved short p75NTR can independently initiate neuronal apoptosis.

Adding inhibitors that block binding of proNGF to sortilin can rescue cells from apoptosis, which occurs following TBI, SCI, MS, and neurodegenerative diseases

such as AD. It may be possible to develop a drug that can save healthy neurons from apoptosis by shutting down this cell death pathway over a period of hours to days but not years. This opens avenues for drug development that strongly contrast with past efforts to exploit neurotrophin actions. Earlier trials using chronic delivery of neurotrophins to promote neuronal survival in ALS patients were disappointing. The goal would be to inhibit the harmful signaling acutely, rather than to support survival actions chronically.

The cell self-destructive proNGF-p75NTR-sortilin signaling apparatus has been identified in ventral tier dopamine neurons of the substantia nigra, suggesting that p75NTR signaling might be involved in selective cell death mechanisms of substantia nigra neurons or disease progression of PD (Chen et al. 2008a). The proNGF-p75NTR-sortilin signaling complex may thus provide new target for neuroprotection of substantia nigra neurons and the therapeutic treatment of PD.

Antioxidants/Free Radical Scavengers

Free Radical Generation

Free radicals are formed during normal respiration and oxidation. Oxidation is a chemical reaction in which a molecule transfers one or more electrons to another. Biologically important oxygen-derived species include superoxide, hydrogen peroxide, hydroxyl radical, hypochlorous acid, heme-associated ferryl species, radicals derived from activated phagocytes, and peroxy radicals, both lipid soluble and water soluble. Stable molecules usually have matched pairs of protons and electrons, whereas free radicals have unpaired electrons and tend to be highly reactive, oxidizing agents. Free radicals vary in their reactivity, but some can cause severe damage to biological molecules, especially to DNA, lipids, and proteins.

Natural Defenses Against Oxidative Stress

Antioxidant defense systems (SOD, H_2O_2 -removing enzymes, and metal-binding proteins) scavenge and minimize the formation of oxygen-derived species, but they are not 100% effective. Mild oxidative stress often induces these antioxidant defense enzymes, but severe stress can cause oxidative damage to lipids, proteins, and DNA within cells, leading to such events as DNA strand breakage and disruption of Ca^{2+} metabolism. Prolonged oxidative stress including superoxide production may contribute to neuronal loss, as is probably the case in AD or the damaging effect of ischemia-reperfusion, although the latter may also involve the balance between nitric oxide and superoxide generation.

The enzyme SOD breaks down the free radical superoxide and plays a critical role in protecting the body against attack by reactive oxygen species. However, the

natural enzyme's high molecular weight, short half-life in circulation, inability to penetrate cells, and high cost of production limit its usefulness as a drug. Numerous attempts by many pharmaceutical companies in the past using various forms of the SOD enzyme failed to demonstrate the expected efficacy.

Effects of Oxidative Damage

Oxidative Damage and Aging

Oxidative stress and production of free radicals tend to increase with aging, whereas the body's natural antioxidant defenses decline. Cell damage caused by oxidative stress, therefore, tends to increase with age. This effect is most marked in the brain because of its high metabolic rate, the relatively low level of antioxidant protection in the brain, and the inability of neurons to regenerate. One of the consequences of this higher level of oxidative stress is that a significant percentage of the enzymes critical to normal neuron function may become oxidized, increasing susceptibility to debilitating and potentially fatal degenerative diseases such as PD and AD. In addition, age-associated changes may bring about increases in inflammation, leading to the production of cytokines and other cellular mediators in the brain that may induce certain genes to produce higher levels of neuronal toxins. These toxins may result in older individuals becoming more susceptible to sudden increases in oxidative stress caused by acute events such as stroke and trauma.

Exposure of brain neurons to oxidative-stress signals stimulates the activity of the protein MST1, which instructs neurons to die (Lehtinen et al. 2006). There is a tight link between MST1 and another family of molecules called FOXO proteins, which turn on genes in the nucleus. Once stimulated by oxidative stress, MST acts in its capacity as an enzyme to modify and thereby activate the FOXO proteins, instructing the FOXO proteins to move from the periphery of the cell into the nucleus of neurons. Once in the nucleus, the FOXO proteins turn on genes that commit neurons to apoptosis. The discovery of the MST–FOXO biochemical switch mechanism fills a gap in our understanding of how oxidative stress elicits biological responses in neurons, and may include besides cell death, neuronal dysfunction and neuronal recovery. Since oxidative stress in neurons and other cells in the body contributes to tissue damage in a variety of disorders, including stroke, ischemic heart disease, neurodegenerative diseases, and diabetes, identification of the MST–FOXO switch mechanism could provide potential new targets for the diagnosis and treatment of many common age-associated diseases.

Memory-related behavioral performance has been studied in transgenic mice over-expressing extracellular SOD (EC-SOD) and their wild-type littermates at different ages (Hu et al. 2006). EC-SOD transgenic mice exhibited better hippocampus-dependent spatial learning compared with their wild-type littermates. At the molecular level, aged EC-SOD transgenic mice had lower superoxide levels, a decrease in protein carbonyl levels, and a decrease in p38 and extracellular signal-regulated kinase 2 phosphorylation compared with that in aged wild-type mice. These findings

suggest that elevated levels of SOD contribute to aging-related impairments in memory, and that these impairments can be alleviated by overexpression of EC-SOD. Thus there is an age-dependent alteration in the role of SOD in modulating synaptic plasticity and learning and memory. This study supports the concept that high antioxidant levels, within reasonable limits, could promote longevity and reduce the risk of dementia associated with normal aging and neurodegenerative disorders such as AD.

Neuronal Damage by Free Radicals

Damage to cells caused by free radicals includes protein oxidation, DNA strand destruction, increase of intracellular calcium, activation of damaging proteases and nucleases, and peroxidation of cellular membrane lipids. Furthermore, such intracellular damage can lead to the formation of prostaglandins, interferons (IFNs), TNF- α , and other tissue-damaging mediators, each of which can lead to disease if overproduced in response to oxidative stress. Free radicals have been linked to numerous human diseases including neurodegenerative diseases and ischemia-reperfusion injury resulting from stroke.

Oxidative Damage and Neurodegenerative Disorders

The presence of nitrated α -synuclein in a variety of cells directly links oxidative damage to various neurodegenerative disorders. Aggregated α -synuclein proteins form brain lesions that are hallmarks of neurodegenerative synucleinopathies, and oxidative stress has been implicated in the pathogenesis of some of these disorders. Using antibodies to specific nitrated tyrosine residues in α -synuclein, extensive and widespread accumulations of nitrated α -synuclein have been demonstrated in the signature inclusions of PD, dementia with Lewy bodies, the Lewy body variant of AD, and multiple system atrophy brains. Nitrated α -synuclein is present in the major filamentous building blocks of these inclusions, as well as in the insoluble fractions of affected brain regions of synucleinopathies. The selective and specific nitration of α -synuclein in these disorders provides evidence to link oxidative and nitrative damage directly to the onset and progression of neurodegenerative synucleinopathies. These findings may pave the way for developing therapies to stop or slow the oxidative damage, and thus slow or reverse the progression of these diseases.

Measures to Control Oxidative Stress

Damaging effect of free radicals is controlled to some extent by the antioxidant defense systems and cellular repair mechanisms of the body, but at times it is overwhelmed. Enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, and vitamins such as α -tocopherol, ascorbate, and beta-carotene act to

quench radical chain reactions. Many of these agents have been investigated as potential therapeutic agents. Unfortunately, most studies testing naturally occurring antioxidants have resulted in disappointing results. Generally, natural antioxidants must be produced on site within the cell to be effective for disease prevention. Several of the synthesized antioxidant compounds have been tested in clinical trials. Some have failed to demonstrate efficacy as neuroprotectives in stroke and head injury, while others are still undergoing clinical trials or have completed the trials but none has been approved as yet (see Chaps. 3 and 4). However, some of the drugs used clinically are known to have neuroprotective effect, e.g., dopamine agonists used for the treatment of PD.

Categories of Therapeutic Antioxidants

Apart from the endogenous antioxidants, several other substances have antioxidant effect and have potential use as neuroprotectives. A classification of these is shown in Table 2.4.

Table 2.4 Classification of antioxidants or free radical scavengers with neuroprotective potential

Precursors or derivatives of endogenous antioxidant compounds

Acetylcysteine

Polyethylene glycol superoxide dismutase

Flavonoids

Metal chelators

Nanoparticles: e.g., fullerene C60

Desferoxamine

Substances derived from plants

Ginko biloba

Lycopene (in tomatoes)

Turmeric

Synthetic free radical compounds

21-Aminosteroids

Cerovive (NXY-059)

Pyrrolopyrimidines

Ebselen

Idebenone

Nitrones

Compounds with other effects but secondary antioxidant properties

Aspirin

Dihydroergocryptamine

Selegiline

Magnesium

Alpha-Phenyl-*tert*-Butylnitrone

Alpha-phenyl-*tert*-butylnitrone (PBN), a nitron-based free radical trap, has shown neuroprotective activity in several experimental neurodegenerative models. The basis of the neuroprotective activity of PBN is not its general free radical trapping or antioxidant activity per se, but its activity in mediating the suppression of genes induced by proinflammatory cytokines and other mediators associated with enhanced neuroinflammatory processes. Neuroinflammatory processes, induced in part by proinflammatory cytokines, yield enhanced reactive oxygen species and reactive nitric oxide species as well as other unknown components that have neurotoxic properties.

Coenzyme Q10

Coenzyme Q10 (CoQ10) serves as the electron acceptor for complexes I and II of the mitochondrial electron transport chain (ETC) and also acts as an antioxidant and neuroprotective (Jain 2010c). CoQ10 has the potential to be a beneficial agent in neurodegenerative diseases in which there is impaired mitochondrial function and/or excessive oxidative damage. The following are some of the effects that have been demonstrated in experimental studies:

- In animal models of PD, ALS, and HD, CoQ10 can protect against striatal lesions produced by the mitochondrial toxins malonate and 3-nitropropionic acid. These toxins have been utilized to model the striatal pathology that occurs in HD.
- It also protects against 1-methyl-1,2,3,6-tetrahydropyridine toxicity in mice. CoQ10 significantly extended survival in a transgenic mouse model of ALS.
- Administration of CoQ10 combined with mild hypothermia increases survival and could improve neurologic outcome following cardiac arrest and cardiopulmonary resuscitation.

CoQ10 is currently under investigation for the treatment of neurodegenerative disorders. Although not yet approved by the FDA, the product is available from health food stores.

Idebenone [2,3-dimethoxy-5-methyl-6-(10-hydroxydecyl)-1,4-benzoquinone] is a synthetic analog of CoQ10, the vital cell membrane antioxidant and essential constituent of the ATP-producing mitochondrial ETC. Idebenone is a potent antioxidant, with the ability to operate under low oxygen tension situations. Because of its ability to inhibit lipid peroxidation, idebenone protects cell membranes and mitochondria from oxidative damage. Its antioxidant properties protect against cerebral ischemia and nerve damage in the CNS. Idebenone also interacts with the ETC, preserving ATP formation in ischemic states. It has been tested in phase III clinical trials in the treatment of AD and other neurodegenerative diseases but has not been approved for use in any of these.

Dihydroergocryptine

Dihydroergocryptine (DHEC, hydergine) is a hydrogenated ergot derivative with pharmacological actions mainly related to its dopaminomimetic activity. In experimental studies, DHEC antagonizes both the neuronal death produced by acute exposure to glutamate and the normal age-dependent degeneration in culture. The effect of DHEC might be mediated by a free radical scavenger action, as suggested by the finding that the compound reduces the formation of intracellular peroxides formed following exposure to glutamate. This action is apparently not mediated entirely by interactions with the dopamine D2 receptors. DHEC is now classified as an antioxidant. The neuroprotective action suggests that DHEC might be a potentially useful drug in the therapy and/or prophylaxis of acute and chronic neurodegenerative diseases related to excitotoxic damage.

DHEC is used almost exclusively for treating patients with dementia or “age-related” cognitive symptoms. Since the early 1980s, there have been over a dozen more clinical trials, yet hydergine’s efficacy remains uncertain. However, hydergine shows significant treatment effects when assessed by either global ratings or comprehensive rating scales. The activity in slowing disease progression was shown in 1998 in a double-blind, randomized, placebo-controlled study in patients with AD, but no recent clinical trial results have been reported. Studies of the use of α -DHEC in combination with levodopa in patients with PD show that this is a well-tolerated and efficacious treatment option. DHEC is a promising compound for dementia of PD for which there is no approved therapy.

Flavonoids

Flavonoids are naturally occurring polyphenolic compounds that are present in a variety of fruits, vegetables, cereals, tea, and wine, and are the most abundant antioxidants in the human diet. Several studies report the neuroprotective actions of dietary flavonoids. While there has been a major focus on the antioxidant properties, there is an emerging view that flavonoids and their *in vivo* metabolites do not act as conventional hydrogen-donating antioxidants, but may modulate cell functions through actions at protein kinase and lipid kinase signaling pathways. Flavonoids, and more recently their metabolites, have been reported to act at PI 3-kinase, Akt/protein kinase B (Akt/PKB), tyrosine kinases, protein kinase C (PKC), and mitogen-activated protein kinase (MAPK) signaling cascades. Inhibitory or stimulatory actions at these pathways are likely to affect cellular function profoundly by altering the phosphorylation state of target molecules and by modulating gene expression. An understanding of the mechanisms of action of flavonoids, either as antioxidants or as modulators of cell signaling, is a key to the evaluation of these potent biomolecules as inhibitors of neurodegeneration.

Mitochondria-Targeted Antioxidants

These compounds are accumulated by mitochondria within living tissue due to the presence of a triphenylphosphonium cation attached, through an alkyl chain, to the antioxidant component. The delocalized nature of these lipophilic phosphonium cations enables them to permeate lipid bilayers easily and they accumulate several hundredfold within mitochondria, driven by the large membrane potential across the mitochondrial membrane. Mitochondrial membrane potential is central to mitochondrial biology. It defines the transport of ions and provides the driving force for oxidative phosphorylation. This selective uptake of bioactive molecules should greatly enhance their efficacy and specificity of molecules while also decreasing harmful side effects.

Mitoquinone is a potent, orally active antioxidant that targets coenzyme Q in the mitochondria, where it is capable of blocking oxidative reactions that may lead to neurodegeneration with an increase in potency over 1,000-fold. In contrast to current treatments that have no impact on disease progression, mitoquinone shows the potential to become the first disease-modifying agent to treat both PD and Friedreich ataxia. Phase II trials are in progress.

Nanoparticles as Neuroprotective Antioxidants

Three of the most-studied nanoparticle redox reagents at the cellular level are rare earth oxide nanoparticles (particularly cerium), fullerenes, and carbon nanotubes. Ceria nanoparticles from anthanide series have several unique properties that make them highly efficient redox reagents. Several studies have reported the ability of ceria nanoparticles to mitigate oxidative stress at the biological level. Ceria nanoparticles also protect neurons from free radical-mediated damage initiated by ultraviolet (UV) light, H_2O_2 , and excitotoxicity, leading to the hypothesis that the mechanism of action is one of free radical scavenging (Rzigalinski et al. 2006). When compared with single doses of other free radical scavengers, such as vitamin E, melatonin, and *n*-acetylcysteine, ceria nanoparticles demonstrated significantly greater neuroprotection after a 5- and 15-min UV insult. A single dose of nanoparticles delivered up to 3 h post-injury also afforded neuroprotection. Ceria nanoparticles were also effective in reducing cell death associated with γ -irradiation. In another study, nanoparticles were shown to decrease free radical production directly (Schubert et al. 2006). No toxicity was observed with ceria nanoparticle sizes of 6 and 12 nm, and yttrium oxide nanoparticles were even more effective than ceria. Ceria nanoparticles larger than 30 nm or nitrates and sulfates of cerium did not have any significant effects. Several studies also suggest that ceria nanoparticles are potent anti-inflammatory agents. Microglia, the immune cells of the brain, are “activated” in response to neuronal damage and show an inflammatory response with the release of NO as well as IL-1 β . Treatment of injured organotypic cultures with ceria nanoparticles reduced their ability to activate microglia. Furthermore, treatment

of activated microglia with ceria nanoparticles reduces the production of soluble factors that promote death in uninjured neurons, including NO and IL-1 β . Delivery of nanoparticles to the uninjured neurons also directly affords neuroprotection from the damaging effects of activated microglia. Thus, it appears that nanoparticles may blunt the inflammatory response in immune cells, as well as reduce inflammatory injury to nonimmune cells.

Water-soluble derivatives of buckminsterfullerene C60 derivatives are a unique class of nanoparticle compounds with potent antioxidant properties. Studies on one class of these compounds, the malonic acid C60 derivatives (carboxyfullerenes), indicated that they are capable of eliminating both superoxide anion and H₂O₂, and were effective inhibitors of lipid peroxidation, as well. Carboxyfullerenes demonstrated robust neuroprotection against excitotoxic, apoptotic, and metabolic insults in cortical cell cultures. They were also capable of rescuing midbrain dopaminergic neurons from both MPP(+) and 6-hydroxydopamine-induced degeneration. Although there is limited *in vivo* data on these compounds, systemic administration of the C3 carboxyfullerene isomer has been shown to delay motor deterioration and death in a mouse model familial ALS. Ongoing studies in other animal models of CNS disease states suggest that these novel antioxidants are potential neuroprotective agents for other neurodegenerative disorders including PD.

Neuroleptics as Antioxidants

Neuroleptic drugs such as chlorpromazine, prochlorperazine, metoclopramide, methotrimeprazine, and haloperidol may be able to exert antioxidant and/or pro-oxidant actions *in vivo* and *in vitro*. All except haloperidol are very powerful scavengers of hydroxyl radicals, reacting at an almost diffusion-controlled rate with little reaction with the superoxide radical. Chlorpromazine shows some ability to inhibit iron ion-dependent hydroxyl radical formation. Chlorpromazine, methotrimeprazine, promethazine, and prochlorperazine are also powerful inhibitors of iron ion-dependent liposomal lipid peroxidation, scavengers of organic peroxy radicals, and inhibitors of hem protein/hydrogen peroxide-dependent peroxidation of arachidonic acid.

Nitrones

Specific nitrones have been used for more than 30 years in analytical chemistry and biochemistry to trap and stabilize free radicals for the purpose of their identification and characterization. PBN, one of the more widely used nitrones for this purpose, has been shown to have potent pharmacologic activities in a number of aging-related disease models, most notably the neurodegenerative diseases of stroke and AD. Studies in cell and animal models strongly suggest that PBN has potent antiaging activity. It has also shown efficacy in the prevention of memory dysfunction associated with normal aging in a mouse model. Mechanistic studies have shown that the neuroprotective activity of nitrones is not due to mass-action free radical-trapping

activity, but due to cessation of enhanced signal transduction processes associated with neuroinflammatory processes known to be enhanced in several neurodegenerative conditions. Enhanced neuroinflammatory processes produce higher levels of neurotoxins, which cause death or dysfunction of neurons. Therefore, quelling of these processes is considered to have a beneficial effect, allowing proper neuronal functioning. The possible antiaging activity of nitrones may reside in their ability to quell enhanced production of reactive oxygen species associated with age-related conditions. On the basis of novel ideas about the action of secretory products formed by senescent cells on bystander cells, it is postulated that nitrones will mitigate these processes and that this may be the mechanism of their antiaging activity.

Translation of Antioxidant Neuroprotection from Preclinical to Clinical

Numerous studies of antioxidant agents in animal models of neurological conditions with significant oxidative stress components generally show neuroprotective effects. In contrast, antioxidant efficacy in human clinical trials has been mostly disappointing. Some of the explanations for the poor translation of results are as follows (Kamat et al. 2008):

- Many substances can be antioxidants in vitro under conditions that are not relevant in vivo.
- Dosages used in animal models may not be achieved safely and practically in humans.
- There is problem in identification and extrapolation of the key biomarkers of disease from animal research to humans.
- Design of experiments with early intervention in animals does not reproduce the course of chronic neurodegenerative diseases and changes that may be irreversible.

Carbon Monoxide and Heme Oxygenase

Carbon monoxide (CO), a chemically stable gas, occurs in nature as a product of the oxidation or combustion of organic materials. CO also arises in cells and tissues as a byproduct of heme oxygenase (HO), a rate-limiting enzyme that degrades heme into CO, iron, and antioxidants biliverdin/bilirubin. CO acts as a vasorelaxant and may regulate other vascular functions such as platelet aggregation and smooth muscle proliferation. CO has also been implicated as a neurotransmitter in the CNS. Many of the effects of CO depend on the activation of guanylate cyclase, which generates guanosine 3',5'-monophosphate (cGMP), and the modulation of MAPK signaling pathways. Poisoning by CO inhalation is associated with disorders of the nervous system (see Chap. 11). Actions of CO in the nervous system thus range from the physiological to the pathological.

HO has two active isoforms: HO-1 and HO-2. HO-2 is highly expressed in cerebral microvessels as a constitutive isoform, whereas the inducible form, HO-1, is not detectable. HO-1, however, can be induced by various insults. Several investigators have postulated that it has cytoprotective activities, although its role in the nervous system is not fully understood, especially considering that normally HO-2 accounts for the vast majority of HO activity in the brain. Both HO-1 and HO-2 have anti-apoptotic effects against oxidative stress-related glutamate toxicity in cerebral vascular endothelium.

CO, when applied at low concentration, exerts potent cytoprotective effects mimicking those of HO-1 induction, including downregulation of inflammation and suppression of apoptosis. HO-1 has been shown to provide neuroprotection against acute excitotoxicity, suggesting that potential intervention that can increase HO-1 activity within the brain should be considered as a therapeutic target in acute and potentially chronic neurological disorders (Ahmad et al. 2006). Researchers at Johns Hopkins have shown that brain damage was reduced by as much as 62.2% in mice that inhaled low amounts of CO after an induced stroke (Zeynalov and Doré 2009).

Cell Transplants

Cells Secreting Neuroprotective Substances

Transplantation of cells with specific functions is a recognized procedure for the treatment of human diseases, particularly for those in which a specific hormone or other substances secreted by the cells are missing. When applied to the CNS, the procedure is referred to as neurotransplantation and is described in more detail elsewhere (Jain 2010a). The beneficial effect is explained by two mechanisms: (1) a diffusible factor within the transplanted tissue and (2) establishment of neural connections between the transplanted tissue and the host. Use of genetically engineered encapsulated cells is considered to be a form of gene therapy.

Several neurodegenerative disorders can be potentially treated by the administration of neuroactive agents in a controlled manner through the use of living cells that secrete these molecules. Cells can function as biological pumps for the release of neuroactive compounds such as neurotransmitters and neurotrophic factors. Neurotransplantation can be used as a neuroprotective measure for stroke, neurodegenerative conditions, and CNS injury. Following are some of the tissue types used for neurological disorders.

Fetal tissues. Human neural fetal tissue has been used for transplantation into the striatum of patients with neurodegenerative disorders such as PD. The limiting factors are the ethical issues involved in obtaining human fetal tissues and the need for immunosuppression for the transplanted tissue to survive.

Neurons. Clinical grade human neuronal cells are available for potential therapeutic use in a variety of CNS diseases including stroke and neurodegenerative disorders.

Stem Cells

An alternative to fetal tissue transplants is the use of neuron-derived stem cells that have been made to proliferate in culture under the influence of bFGF prior to transplantation into the CNS. These can differentiate into either glial or neuronal cells to replace the neurons that degenerate in AD and PD.

An in vitro model of neuronal hypoxia that affords the possibility to investigate both apoptotic neuronal cell death and neuroprotective therapies has been used to study neuroprotective properties of human umbilical cord blood (UCB) cells (Hau et al. 2008). Therapeutic influence of human UCB mononuclear cells (MNCs) was investigated on the progression of apoptotic cell death. The neuroprotective effect of MNC was due to anti-apoptotic mechanisms related to direct cell–cell contacts with injured neuronal cells and distinct changes in neuroprotective, inflammatory cytokines as well as to the upregulation of chemokines within the cocultures.

Stem Cell Activation for Neuroprotection/Regeneration by Glucocorticoids

Regenerative medicine holds the promise of replacing damaged tissues largely by stem cell activation. Hedgehog signaling through the plasma membrane receptor Smoothened (Smo) is an important process for regulating stem cell proliferation. The development of hedgehog-related therapies has been impeded by a lack of FDA-approved Smo agonists. Using a high-content screen with cells expressing Smo receptors and a β -arrestin2-GFP reporter, four FDA-approved drugs were identified as Smo agonists that activate hedgehog signaling: halcinonide, fluticasone, clobetasol, and fluocinonide (Wang et al. 2010b). These drugs demonstrated an ability to bind Smo, promote Smo internalization, activate Gli, and stimulate the proliferation of primary neuronal precursor cells alone and synergistically in the presence of sonic hedgehog protein. Halcinonide, fluticasone, clobetasol, and fluocinonide provide an opportunity to promote and protect neural stem cell populations involved in tissue repair in conditions such as SCI and PD.

Cytokines

Erythropoietin

Erythropoietin (EPO) and its receptor function as primary mediators of the normal physiological response to hypoxia. EPO is approved by the FDA for the treatment of anemia that may result from a variety of conditions, including the anemia associated with chronic renal failure. EPO modulates a broad array of cellular processes

Table 2.5 Role of erythropoietin in the nervous system

Clinical condition	Role of erythropoietin
Cerebral ischemia	Increase of neuronal survival and development of ischemic tolerance
Retinal ischemia	Decrease of apoptosis in retinal ganglion cell
Spinal cord injury	Decrease of motor neuronal apoptosis and inflammation Improvement of neuronal function
Subarachnoid hemorrhage	Improvement of neuronal function and blood flow
Peripheral nerve injury	Decrease of spinal neuronal apoptosis and improvement of myelin repair
Oxidative stress injury	DNA fragmentation, free radical production, and caspase activity decreased
Glutamate toxicity	Glutamate release decreased and neuronal survival increased
Cerebral inflammation	Microglial inflammation decreased and cytokine release diminished

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including progenitor stem cell development, cellular integrity, and angiogenesis. As a result, cellular protection by EPO is robust and EPO inhibits the apoptotic mechanisms of injury, including the preservation of cellular membrane asymmetry to prevent inflammation. Role of EPO in the nervous system is shown in Table 2.5.

As further investigations are planned for clinical applications for EPO that maximize efficacy and minimize toxicity progresses, a deeper appreciation for the novel roles that EPO plays in the brain and heart and throughout the entire body should be acquired. Studies in which recombinant human EPO (rhEPO, epoetin alfa) is injected directly into ischemic rodent brain show that EPO also mediates neuroprotection. Abundant expression of the EPO receptor (EpoR) has been observed at brain capillaries, which could provide a route for circulating EPO to enter the brain. Systemic administration of epoetin alfa before or up to 6 h after experimental focal brain ischemia reduces injury by 50–75%. Epoetin alfa also limits the extent of concussive brain injury, the immune damage in experimental autoimmune encephalomyelitis, and excitotoxicity induced by kainate. Thus, systemically administered epoetin alfa in animal models has neuroprotective effects, demonstrating its potential use after TBI and in MS. It is evident that EPO has biological activities in addition to increasing red cell mass.

EpoR binding mediates neuroprotection by endogenous Epo or by exogenous rhEpo. The mechanisms underlying the protective effects of EPO on ischemic/hypoxic neurons are not fully understood. Activation of EpoR suppresses ischemic cell death by inhibiting the reduced Ca^{2+} -induced glutamate release from cultured cerebellar granule neurons. Pretreatment with EPO protects neurons in models of ischemic and degenerative damage due to excitotoxins and consequent generation of free radicals, including nitric oxide. Activation of neuronal EpoR prevents apoptosis induced by NMDA or NO by triggering cross talk between the signaling pathways involved in transcription of neuroprotective genes. In vitro experiments which showed that EPO attenuated neuronal damage caused by chemical hypoxia

at lower extracellular concentrations suggest that EPO prevents delayed neuronal death possibly through upregulation of Bcl-x(L), which is known to facilitate neuronal survival. Features of EPO as a neuroprotective agent can be summarized as follows.

- It is expressed in the human CNS.
- The transcription factor hypoxia-inducible factor-1 (HIF-1) upregulates EPO following hypoxic stimuli.
- It has demonstrated remarkable neuroprotective potential in cell culture and animal models of disease.
- It has multiple protective effects (anti-apoptotic, neurotrophic, antioxidant, anti-inflammatory, and angiogenic).
- EPO and IGF-I exert cooperative actions that afford acute neuroprotection via activation of the PI3K-Akt pathway.
- Neuroprotective effect has been demonstrated in the CNS as well as in diabetic peripheral neuropathy.

The level of EpoR gene expression may determine tissue responsiveness to Epo. Thus, harnessing the neuroprotective power of Epo requires an understanding of the Epo–EpoR system and its regulation. A study using environmental manipulations in normal rodents demonstrates the strict requirement for induction of EpoR expression in brain neurons to achieve optimal neuroprotection (Sanchez et al. 2009). The results indicate that regulation of EpoR gene expression may facilitate the neuroprotective potential of rhEPO.

Thrombopoietin (TPO), a stimulator of platelet formation, acts in the brain as a counterpart of EPO. During hypoxia, EPO and its receptor are rapidly re-expressed, whereas neuronal TPO and its receptor are downregulated. TPO is strongly proapoptotic in the brain, causing death of newly generated neurons through the Ras-extracellular signal-regulated kinase 1/2 pathway. This effect is inhibited by EPO.

Given the excellent safety profile of epoetin alfa, a clinical trial was conducted to evaluate systemically administered epoetin alfa as a neuroprotective treatment in stroke, but it failed to protect from damages induced by cerebral ischemia (see Chap. 3).

Nonerythropoietic EPO Variants and Mimics

Enthusiasm for rhEPO as a potential neuroprotective therapeutic is tempered, however, by the knowledge that it also enlarges circulating red cell mass and increases platelet aggregability. Asialoerythropoietin (asialoEPO), generated by total enzymatic desialylation of rhEPO, possesses a very short plasma half-life and is fully neuroprotective. In contrast with rhEPO, this molecule does not increase the hematocrit of mice or rats at doses and frequencies at which rhEPO exhibited erythropoiesis. Radioiodine-labeled asialoEPO appears promptly within the CSF after intravenous administration and binds to neurons within the hippocampus and cortex in a pattern corresponding to the distribution of the EpoR. It exhibits a

broad spectrum of neuroprotective activities, as demonstrated in models of cerebral ischemia, spinal cord compression, and sciatic nerve crush. These data suggest that nonerythropoietic variants of rhEPO can cross the BBB and provide neuroprotection.

Darbepoietin alfa is a novel erythropoiesis-stimulating protein developed for treating anemia. In animal models, exogenous rhEPO has been reported to be beneficial in treating experimental cerebral ischemia. Darbepoietin was shown to reduce infarct volume and total infarct areas significantly at multiple levels in a rat model of transient focal cerebral ischemia (Belayev et al. 2005).

Carbamylated EPO (CEPO) is a modified EPO molecule not affecting hematocrit. Extensive preclinical studies show that CEPO is a neuroprotective agent (Lapchak 2008). A proprietary preparation, Lu AA24493 (Lundbeck), is in clinical trials for Friedreich ataxia.

Tissue-protective domains within EPO have been narrowed down to short peptide sequences. Helix B (amino acid residues 58–82) of EPO, which faces the aqueous medium when EPO is bound to the receptor homodimer, is both neuroprotective in vitro and tissue protective in vivo in a variety of models, including ischemic stroke, diabetes-induced retinal edema, and peripheral nerve trauma (Brines et al. 2008). This study also found that an 11-aa peptide composed of adjacent amino acids forming the aqueous face of helix B is also tissue protective, as confirmed by its therapeutic benefit in models of ischemic stroke. Furthermore, this peptide simulating the aqueous surface of helix B also exhibits EPO's trophic effects by accelerating wound healing and augmenting cognitive function in rodents. Neither helix B nor the 11-aa peptide is erythropoietic in vitro or in vivo. Thus, the tissue-protective activities of EPO are mimicked by small, nonerythropoietic peptides that simulate a portion of EPO's 3D structure.

EPO, CEPO, and helix B peptide EPO analogs exhibited significant neuroprotective activity in preclinical stroke models. However, in view of the adverse effect of EPO in a clinical trial, preclinical safety studies of EPO molecules in embolic stroke models that parallel acute ischemic stroke in humans are warranted (Lapchak 2010).

Granulocyte Colony-Stimulating Factor

Granulocyte colony-stimulating factor (G-CSF) is a hematopoietic factor that is used to treat neutropenia associated with chemotherapy. G-CSF is a cytokine that has effects on the nervous system as well. G-CSF displays strong anti-apoptotic activity in mature neurons and activates multiple cell survival pathways. Both G-CSF and its receptor are widely expressed by neurons in the CNS, and their expression is induced by ischemia, which suggests an autocrine protective signaling mechanism. G-CSF receptor is also expressed by adult neural stem cells, and G-CSF induces neuronal differentiation in vitro. In cell culture experiment, G-CSF exhibits a significant neuroprotective effect after glutamate-induced excitotoxicity.

G-CSF also passes the intact BBB and reduces infarct volume in two different rat models of acute stroke (Schneider et al. 2005). G-CSF markedly improved long-term behavioral outcome after cortical ischemia, while stimulating neural progenitor response in vivo, providing a link to functional recovery. Thus, G-CSF is an endogenous ligand in the CNS that has a dual activity beneficial both in counteracting acute neuronal degeneration and in contributing to long-term plasticity after cerebral ischemia. G-CSF is a potential new drug for stroke and neurodegenerative diseases.

Delta-Opioid Receptor Agonists

Mammalian cortical neurons are highly sensitive to hypoxic stress. Depending on severity and duration, hypoxia can lead to serious neurological disease. A poor understanding of the mechanisms underlying hypoxic neuronal injury has hampered the development of adequate therapies.

Delta-opioid receptor (DOR) activation protects cortical neurons against glutamate-induced injury. Because glutamate is a mediator of hypoxic injury in neurons, it is hypothesized that DOR is involved in neuroprotection during oxygen deprivation and that its activation/inhibition may alter neuronal susceptibility to hypoxic stress. DOR antagonists, on the contrary, exacerbate damage caused by hypoxic stress. This mechanism of neuronal protection may be useful in the development of novel solutions for diseases related to hypoxic injury. DOR agonists may prevent and treat injury related to hypoxia. The following strategies could be implemented.

- Develop small-molecule agonists to target specifically DOR in the brain
- Increase DOR expression in the brain through gene therapy

FK960

FK960 with the chemical structure [*N*-(4-acetyl-1-piperaziny)]-*p*-fluorobenzamide monohydrate] is a putative anti-dementia drug with a novel mechanism of action. FK960 facilitates hippocampal neurotransmission in normal mice and also in mice lacking the glial glutamate transporter, GLT-1, but to a lesser extent. FK960 enhanced glutamate release from cultured hippocampal astrocytes from normal rats and mice, while the drug had no effect on the release from cultured rat hippocampal neurons. FK960 causes an increase in intracellular Ca^{2+} concentrations by stored Ca^{2+} release in cultured rat hippocampal astrocytes. FK960 stimulates glutamate release from astrocytes, likely as a result of raising intracellular Ca^{2+} concentrations via a PKA pathway. The FK960 action would increase synaptic glutamate concentrations, in part responsible for the facilitation of hippocampal neurotransmission. These findings provide a new idea that agents targeting astrocytes could serve as

anti-dementia drugs. FK680 showed signs of clinical efficacy in phase II clinical trials for AD and there were no safety concerns. However, the manufacturer (Fujisawa) decided to put these studies on a hold and develop this drug for the prevention of cognitive decline in schizophrenia.

Gene Therapy

Gene therapy is defined as the transfer of defined genetic material to specific target cells of a patient for the ultimate purpose of preventing or altering a particular disease state. It has three components: (1) identification of the gene that is mutated in the disease and to obtain a healthy copy of that gene; (2) carrier or delivery vehicle called vectors to deliver the healthy gene to a patient's cells; and (3) additional DNA elements that turn on the healthy gene in the right cells and at the right levels. Gene therapy usually involves in situ production of therapeutic proteins, but some approaches require suppression of gene expression to achieve therapeutic effects. Applications of gene therapy would be narrow if confined only to transfer of defined genetic material to specific target cells using vectors, which are usually viral but several nonviral vectors are used as well. Genes and DNA can be introduced without the use of vectors, and various techniques are being used to modify the function of genes in vivo without gene transfer, e.g., gene repair. Gene medicines may modify the effects of genes. If one includes cell therapy, particularly with the use of genetically modified cells, the scope of gene therapy becomes much broader. Gene therapy can now be combined with antisense and RNA interference (RNAi), further increasing the therapeutic scope. Techniques of gene therapy are described in a special report on this topic (Jain 2010b).

Herpes simplex virus (HSV) has been extensively genetically modified for gene transfer to nerve and other tissues, to create vectors that are devoid of viral gene expression and toxicity. Recombinant vectors have been engineered to express genes that protect neurons against toxic insults resulting in cell death, including NGF and anti-apoptotic genes (e.g., bcl-2). HSV vectors expressing these gene products and their potential protective role in ameliorating neurodegenerative processes have been demonstrated in animal model systems.

Increasing knowledge of neuronal death mediators has led to gene therapy techniques for neuroprotection. Overexpression of numerous genes enhances survival after necrotic or neurodegenerative damage. Nonetheless, although encouraging, little is accomplished if a neuron is spared from death, but not from dysfunction. Variations in gene delivery systems, including virus type and latency between damage onset and vector delivery, probably impact the therapeutic outcome. Additionally, functional sparing might depend on factors related to insult severity, neuron type involved, or the step in the death cascade that is targeted. There are many instances in which gene therapy could spare neurons from insult-induced dysfunction. Overexpression of anti-necrotic and anti-apoptotic proteins, growth factors, and enzymes involved in neurotransmitter system can protect biochemical pathways, promote neurite regrowth, preserve synaptic plasticity, and reinstate normal behavior.

Gene transfer technologies have been applied to define the exact nature of cell death following a neurological insult and devise neuroprotective strategies. The role of particular apoptotic signals in cultured rat hippocampal neurons following acute excitotoxicity, metabolic poisoning, and heat stress has been investigated by using a modified HSV vector to deliver viral anti-apoptotic genes specifically. A battery of viral genes that have evolved to suppress suicidal host responses to infection were examined as inhibitors of various apoptotic insults. Each viral agent tested had a unique profile in its ability to protect hippocampal neurons following acute neurological insults. No genes tested have been shown to protect against metabolic poisoning by cyanide.

Gene therapy is under investigation as a neuroprotective in cerebrovascular disease (Jain 2010d). Gene therapy can also be applied to repair, regenerate, and protect the CNS following trauma.

Glucagon-Like Peptide

Glucagon-like peptide-1 (GLP-1) is a gut peptide that, together with its receptor, GLP-1R, is expressed in the brain. It is a hormone derived from tissue-specific posttranslational processing of proglucagon gene in intestinal L cells and exerts effects on glucose-dependent insulin secretion. GLP-1R-deficient mice have a phenotype characterized by a learning deficit that is restored after hippocampal Glp1r gene transfer. In addition, rats overexpressing GLP-1R in the hippocampus show improved learning and memory. It has been suggested that molecules that facilitate learning and memory may also help to protect the brain against various insults.

GLP-1R-deficient mice also have enhanced seizure severity and neuronal injury after kainate administration. Systemic administration of [Ser(2)]exendin(1–9) in wild-type animals prevents kainate-induced apoptosis of hippocampal neurons. Brain GLP-1R thus represents a promising new target for both cognition-enhancing and neuroprotective agents.

Glatiramer Acetate

Glatiramer acetate, approved for the treatment of MS, consists of synthetic polypeptides containing four naturally occurring amino acids: L-glutamic acid, L-lysine, L-alanine, and L-tyrosine (Jain 2010e). The rationale for its use is that it stimulates myelin basic protein, a natural component of the myelin sheath. It is being investigated as a potential neuroprotective agent in various disorders, e.g., ALS.

Unlike other antigens, glatiramer acetate induces T-cell-mediated neuroprotection without the risk of causing autoimmune disease. Multiple animal models of neurological diseases including models of SCI, optic nerve injury, TBI, and ALS have demonstrated that glatiramer acetate formulated as a vaccine can induce a beneficial T-cell-mediated effect. The regimen for glatiramer administration in

individuals with autoimmune disease (daily injection) differs from that required for treatment after CNS injury. Future studies will aim to establish the optimal regimen for glatiramer administration in individuals with diseases that are both autoimmune and neurodegenerative, to achieve both neuroprotection against degeneration and prevention of disease by arrest of the demyelination process. Emerging data from immunological and imaging studies that quantify axonal injury in the brain point toward neuroprotective abilities of glatiramer acetate (Perumal et al. 2006).

Glutamate Antagonists

Glutamate is the principal excitatory neurotransmitter in the CNS. Transmitter glutamate is taken up and stored in synaptic vesicles by an ATP-dependent process and, upon depolarization, it is released into the synaptic cleft in a calcium-dependent fashion. Glutamate is rapidly cleared from the extracellular space by sodium-dependent transporters located on glial and neuronal cell membranes. However, during cerebral ischemia, there is increased synaptic release and impaired cellular reuptake of glutamate, resulting in large increases in extracellular glutamate.

Neuronal death following excessive glutamate-mediated excitation, often referred to as excitotoxicity, is a critical feature of acute cerebral infarction. Increase in synaptic glutamate levels mediates apoptosis in neurodegenerative disorders. Overactivity of the excitatory amino acid neurotransmission is also associated with traumatic injuries of the CNS.

Glutamate receptors comprise two large families: (1) ligand-gated ion channels called ionotropic glutamate (iGlu) receptors (see Table 2.6) and (2) G-protein-coupled receptors (GPCRs) called metabotropic glutamate receptors (mGlu), which are divided into eight subtypes (mGluR1–GluR8) that have been cloned and classified into three groups according to sequence homology, signal transduction mechanism, and agonist selectivity (Table 2.7).

iGluRs mediate neurotransmission within milliseconds of activation by direct action on ion channels, whereas mGluRs exert a less direct and much slower effect. mGluRs are GPCRs that activate a second-messenger mechanism (i.e., the G-protein on the inner surface of the postsynaptic membrane). mGluRs modulate

Table 2.6 Ionotropic glutamate receptors

Receptor	Agonists	Effector system
NMDA (<i>N</i> -methyl-D-aspartate)	Glutamate, aspartate, NMDA, quinolinate, and ibotenate	Ion channels: Na ⁺ , K ⁺ , Ca ²⁺
Kainate	Kainate, domoate, glutamate, acromelate	Ion channels: Na ⁺ , K ⁺
AMPA	AMPA, quisqualate, and glutamate	Ion channels: Na ⁺ , K ⁺

Table 2.7 Classification of metabotropic glutamate receptors (mGluRs)

Group	mGluRs	Signal transduction/coupling
I	mGluR1, mGluR5	Phosphoinositide hydrolysis/ Ca^{2+} mobilization – stimulatory
II	mGluR2, mGluR3	Adenyl cyclase – inhibitory
III	mGluR4, mGluR6, mGluR7, mGluR8	Adenyl cyclase – inhibitory

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processes in the neuronal cell body that are far downstream from the synapse; and their effects on metabolic processes, gene expression, and ionic diffusion may require from seconds to (possibly) hours to occur. The difference in mechanisms of action – iGluRs mediate glutamate neurotransmission; mGluRs modulate glutamate neurotransmission – may prove to be the chief factor that enables the successful launch of a new class of neuropharmacologic agents based on mGluRs.

The largest segment of the pharmaceutical neuroprotective drug development activity is focused on the anti-excitotoxicity approach. Stroke was the first therapeutic area to be investigated. Some of the antiglutamate agents still in clinical development are briefly described here, whereas others are discussed in chapters dealing with neuroprotection in various conditions such as cerebral ischemia and neurodegenerative disorders.

Neuroprotection by Scavenging Blood Glutamate

Excess glutamate in brain fluids characterizes acute brain insults such as TBI and stroke. Its removal could prevent the glutamate excitotoxicity that causes long-lasting neurological deficits. Blood glutamate scavenging has been demonstrated to increase the efflux of excess glutamate from brain into blood. The hypothesis that oxaloacetate-mediated blood scavenging causes neuroprotection has been tested in a pathological situation such as TBI, in which there is a well-established deleterious increase of glutamate in brain fluids. Highly significant improvements were observed of the neurological status of rats submitted to TBI following an intravenous treatment with oxaloacetate, a glutamate scavenger, which decreased blood glutamate levels by 40% (Zlotnik et al. 2007). Oxaloacetate provided neuroprotection when administered before TBI or at 60 min post-TBI, but not at 120 min post-TBI. Since neurological recovery from TBI was highly correlated with the decrease of blood glutamate levels, it was concluded that blood glutamate scavenging affords brain neuroprotection. Blood glutamate scavenging may now open new therapeutic options for neuroprotection. Furthermore, administration of this blood glutamate scavenger may display in man a therapeutic effect which has so far not been observed for glutamate receptor antagonists. One possible benefit is that the enhanced brain-to-blood glutamate efflux produced by blood glutamate scavenging

is self-limiting since it slows down in parallel with the decrease in brain glutamate. Thus, it will eliminate excess glutamate without preventing glutamate from exerting a role in neurorepair, a factor that has been suggested to account for the failure of glutamate receptor antagonists in human clinical studies.

N-Acylethanolamines for Protection Against Glutamatergic Excitotoxicity

The *N*-acylethanolamines (NAEs) and 2-arachidonoylglycerol (2-AG) are bioactive lipids that can modulate inflammatory responses and protect neurons against glutamatergic excitotoxicity. A model of focal cerebral ischemia in young adult mice was used to investigate the relationship between focal cerebral ischemia and endogenous NAEs (Degn et al. 2007). Over the first 24 h after induction of permanent MCAO, a time-dependent increase was observed in all the investigated NAEs, except for anandamide. Accumulation of NAEs did not depend on the regulation of *N*-acylphosphatidylethanolamine-hydrolyzing phospholipase D or fatty acid amide hydrolase. Treatment with the fatty acid amide hydrolase inhibitor URB597 (cyclohexyl carbamic acid 3'-carbamoyl-biphenyl-3-yl ester) 1.5 h before arterial occlusion decreased the infarct volume in this model system. The results suggest that NAEs and 2-AG may be involved in the regulation of neuroprotection during focal cerebral ischemia in mice.

Glutamate Transporters

Five human glutamate transporters that have been isolated and named are as follows:

- EAAT1 and EAAT2, found in cortical astroglia, are associated with ALS, AD, HD, and PD.
- EAAT3, found in cortical neurons and astroglia, is associated with epilepsy.
- EAAT4, found in cerebellar Purkinje cells, is associated with spinocerebellar ataxia.
- EAAT5, found in retinal neurons, is associated with glaucomatous neurodegeneration.

The gene promoter responsible for EAAT2 production can be significantly activated by small-molecule compounds, resulting in the desired measurable transcription, translation, trafficking of protein to the cell surface, and functional activity in vitro and in vivo. The data suggest that if the density of functional EAAT2 glutamate transporters can be increased significantly, the destruction of neurons and the progression of the neurodegenerative process may be slowed. This needs to be reproduced in humans.

Glutamate Transporter-Mediated Neuroprotective Effect of Drugs

Reducing plasma glutamate is difficult and an alternative approach is to stimulate the EAAT of the BBB that removes glutamate from the extracellular fluid. Glutamate transporters are important in preventing glutamate neurotoxicity by removing glutamate from nerves. Several well-known drugs, mentioned in other parts of this report, act via glutamate transporters. These include citicoline, ceftriaxone, FK960, isoflurane, and VPA. Many β -lactam antibiotics are potent stimulators of GLT1 expression. One of these, ceftriaxone, is neuroprotective in vitro when used in models of ischemic injury and motor neuron degeneration. Two novel neuroprotective agents that stimulate the transport of glutamate are MS-153 and pyroglutamate.

(R)-(-)-5-methyl-1-nicotinoyl-2-pyrazoline (MS-153) facilitates glutamate uptake through GLT-1, a Na^+/K^+ -dependent glial glutamate transporter. The cerebroprotective effect of MS-153 in an ischemic model in vivo is due to the specific reduction of the glutamate concentration in the extracellular space, which can probably be attributed to the acceleration of glutamate uptake by the indirect modulation of the glutamate transporter activity. MS-153 inhibits high voltage-gated calcium channels through interactions with PKC, thereby preventing massive release of glutamate from nerve terminals in ischemic conditions. There was no further development of MS-153 as a neuroprotective.

Pyroglutamate (oxoprolinone), an intracellular product of gamma-glutamyl cycle, stimulates Na^+ -dependent transport of glutamate by 46%, whereas facilitative glutamate uptake in luminal membranes is inhibited (Hawkins et al. 2006). This may be part of a regulatory mechanism that accelerates glutamate removal from brain. Pyroglutamate, by stimulating the activity of the EAAT, speeds the recovery of normal glutamate concentrations in extracellular fluid.

Neuroprotection by Targeting KAI Subunit of Kainate Receptor

To study the mechanism of excitotoxic cell death in the hippocampus following ischemic brain injury, laminin gamma1 (lamgamma1) expression was disrupted in the hippocampus (Chen et al. 2008b). Lamgamma1 knockout (KO) and control mice had similar basal expression of KA receptors, but the lamgamma1 KO mice were resistant to KA-induced neuronal death. After KA injection, KA1 subunit levels increased in control mice but were unchanged in lamgamma1 KO mice. KA1 levels in tissue plasminogen activator (tPA)-KO mice were also unchanged after KA administration, indicating that both tPA and laminin were necessary for KA1 upregulation after KA injection. Infusion of plasmin-digested laminin-1 into the hippocampus of lamgamma1 or tPA-KO mice restored KA1 upregulation and KA-induced neuronal degeneration. Interfering with KA1 function with a specific anti-KA1 antibody protected against KA-induced neuronal death both in vitro and in vivo. The treated mice did not suffer the severe side effects that come with blocking

the entire glutamate receptor. This finding suggests that drugs targeting a specific subunit of the complex glutamate receptor might be able to slow brain damage without disrupting other crucial brain functions such as consciousness, which is involved in coma induced for neuroprotection by complete blockade of glutamate receptors. Whether this discovery would translate into neuroprotection in stroke patients remains uncertain at present.

Glycine-Proline-Glutamate Analogs

Glycine-proline-glutamate is an endogenous neuroprotective tripeptide. A series of glycine-proline-glutamate analogs, including modifications at the Pro and/or Glu residues, was prepared and evaluated for their NMDA binding and neuroprotective effects. Results suggest that the pyrrolidine ring puckering of the Pro residue plays a key role in the biological responses, while the preference for *cis* or *trans* rotamers around the Gly-Pro peptide bond is not important. Glycine-proline-glutamate is the N-terminal tripeptide of IGF-1, which is naturally cleaved in the plasma and brain tissues. Glycine-proline-glutamate reduces neuronal loss from hypoxic-ischemic brain injury following systemic administration as it crosses the blood-CSF and the functional CSF-brain barriers (Baker et al. 2005). The longer half-life in the CNS may be due to its unique enzymatic stability.

NNZ-2566 (Neuren Pharmaceuticals) is a proprietary small-molecule analog of glycine-proline-glutamate with good oral bioavailability and high neuroprotective potency. It is in phase II clinical trials for acute and recovery phase treatment of TBI.

Herbal Preparations

Herbs in the Traditional Eastern Medicine that have been documented to have a neuroprotective effect on in vitro and in vivo ischemic model systems, and the neuroprotective compounds produced by them have been isolated. The neuroprotective effect of medicinal plants that are regarded as having therapeutic effects for stroke in Korean traditional medicine were studied using both in vitro and in vivo cerebral ischemia models (Chun et al. 2008). Following two-vessel occlusion in gerbils, extracts of *Pedicularis densiflora* and *Vitis vinifera* significantly increased the number of surviving cells/mm² of the CA1 region by more than twofold.

Flavonoid Wogonin

Wogonin (5,7-dihydroxy-8-methoxyflavone), a flavonoid originated from the root of a medicinal herb *Scutellaria baicalensis* Georgi, has been previously shown to

have anti-inflammatory activities in various cell types including macrophages. Wogonin was shown to inhibit inflammatory activation of cultured brain microglia by diminishing lipopolysaccharide-induced TNF- α and IL-1 β , and suppressing NO production by inhibiting inducible NO synthase (iNOS) induction and NF- κ B activation in microglia (Lee et al. 2003). Inhibition of the inflammatory activation of microglia by wogonin led to the reduction in microglial cytotoxicity toward cocultured PC12 cells, supporting a neuroprotective role for wogonin in vitro. The neuroprotective effect of wogonin was further demonstrated in vivo using two experimental brain injury models: transient global ischemia by four-vessel occlusion and excitotoxic injury by systemic KA injection. In both animal models, wogonin conferred neuroprotection by attenuating the death of hippocampal neurons, and the neuroprotective effect was associated with the inhibition of the inflammatory activation of microglia. These results indicate that wogonin exerts its neuroprotective effect by inhibiting microglial activation, which is a critical component of pathogenic inflammatory responses in neurodegenerative diseases.

Ginseng

American ginseng (*Panax quinquefolius*) and Asian species of ginseng contain ginsenosides which are considered to have several therapeutic properties including antioxidant action. Pretreatment with a preparation of ground leaves and stems of American ginseng, which contains greater levels of Rb extract than the ground root, improved the behavioral score and reduced the volume of the striatal lesion in an animal model of neurodegeneration induced by 3-nitropropionic acid, an inhibitor of succinate dehydrogenase (Lian et al. 2005). There was no motor impairment and deaths in the treated animals. The results demonstrate that some but not all of the ginsenosides have neuroprotective activity and that purification of whole ginseng should be done to extract the neuroprotective components. The postulated ability of the ginsenosides to scavenge free radicals might explain the neuroprotective effects of ginseng and the results of this study, but further research is needed to elucidate the precise mechanism for the neuroprotection.

Hydrogen Sulfide

Hydrogen sulfide (H₂S) is an endogenously produced gas in the CNS and is considered to be the body's third gaseous signaling molecule after NO and CO. H₂S is involved in the regulation of (1) intracellular signaling molecules such as protein kinase A, receptor tyrosine kinases, and mitogen kinases, and oxidative stress signaling; (2) ion channels such as calcium (L-type, T-type, and intracellular stores), potassium (K_{ATP} and small conductance channels), and cystic fibrosis transmembrane conductance

regulator chloride channels; and (3) the release and function of neurotransmitters such as GABA, NMDA, glutamate, and catecholamines (Tan et al. 2010). The role of H_2S as an important mediator of numerous neural functions has potential for neuroprotection.

Deliberate exposure to sublethal doses of H_2S in ischemia-hypoxia in rats prevents oxygen radical-induced tissue damage by replacing oxygen, providing an opportunity for later resuscitation. Further studies are in progress with injectable sodium sulfide.

NMDA Receptor Ion Channel Complex

The NMDA receptor is coupled to a membrane permeable to either Na^+ or K^+ that can be blocked by magnesium (Mg^{2+}) attached within the ion channel (see Fig. 2.1). Glutamate-binding sites can be competitive or noncompetitive such as the glycine-binding site and ion channel-binding sites. Increase in glutamate and aspartate levels after ischemia allows Ca^{2+} and water influx into the neurons via the NMDA receptors, resulting in cellular edema and neuronal death. Thus, the NMDA receptor could be involved in many neurological disorders including stroke, AD, and epilepsy, making it an important drug target.

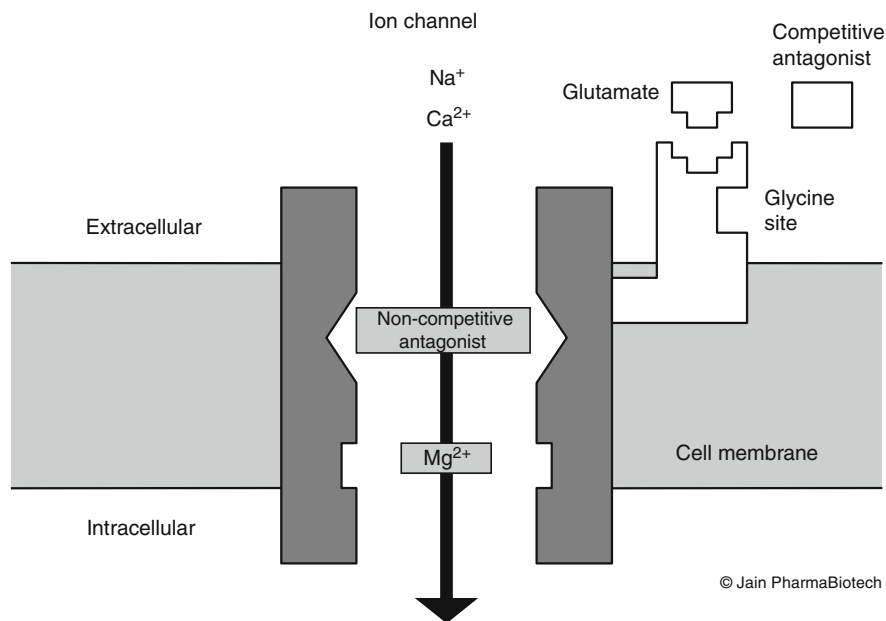


Fig. 2.1 NMDA receptor ion channel complex. Glutamine binding is facilitated by glycine and antagonized by competitive antagonists

There are three sites of action for the attenuation of damage mediated by excitatory amino acids such as glutamate.

- Upstream from membrane receptors or presynaptic, e.g., to reduce the release or enhance the uptake of glutamate.
- At receptor level to antagonize the effect of glutamate.
- Downstream or postsynaptic, i.e., drugs could be used to offset the neurotoxic events set in motion following glutamate receptor stimulation.

One site that is particularly attractive for intervention is located in the channel itself. Some drugs act only if the channel is open because the antagonist can gain access to the channel only in the open state. The agent also has to remain in the channel for some time to block the effect of glutamate overstimulation.

Modulation of the NMDA receptor carries some risk. Total receptor blockade with drugs such as MK801 (dizocilpine, Merck & Co.) leads to unacceptable neurobehavioral adverse effects. Glycine site antagonists were developed to avoid this problem. Although they were promising in experimental studies, they did not prove to be effective in controlled clinical trials. An example of this is gavestinel. Development of agonists of NMDA receptor is also problematic. These compounds could enhance memory in early stages of AD, but they are not neuroprotective and carry the risk of producing neuronal damage associated with excessive calcium flow through the NMDA receptor channel. Partial NMDA agonists at glycine site might be safer and more effective. Some modulators of NMDA receptor will be described in the following pages.

NMDA Receptor Antagonists

NMDA antagonists block the harmful effects of glutamate and are divided into competitive and noncompetitive antagonists. Competitive NMDA antagonists bind competitively and directly to the postsynaptic glutamate receptor. In contrast, noncompetitive NMDA antagonists bind to the NMDA-linked receptor channel. Most competitive NMDA antagonists are hydrophilic and require direct cerebral administration to obtain high levels in the brain. Noncompetitive antagonists are extremely lipophilic and reach high levels in the brain after systemic administration.

NMDA NR2B Subunit Receptor Antagonists

NMDA NR2B subunits contribute to the striatal damage caused by in vivo and in vitro ischemia and play a critical role in the induction of postischemic long-term potentiation (LTP) and in the suppression of activity-dependent LTP in the ischemic penumbra (Picconi et al. 2006). A number of NMDA NR2B subunit receptor antagonists have been investigated as neuroprotectives, including ifenprodil, traxoprodil (CP101,606), Ro 25-6981, and Ro 63-1908, for various conditions including TBI and cerebral ischemia.

Ifenprodil

Ifenprodil is a novel NMDA receptor antagonist that selectively inhibits receptors containing the NR2B subunit. As such, it has been widely used as a tool to study subtypes of NMDA receptors both *in vitro* and *in vivo*, and as a tool for molecular studies of the properties and regulation of NMDA receptors. Ifenprodil has an unusual form of activity dependence and its mechanism of action may involve an increase in proton inhibition of NMDA receptors. These properties are shared by analogs or derivatives of ifenprodil, some of which may be lead compounds for therapeutically useful NMDA antagonists. Such antagonists have potential as neuroprotectants, anticonvulsants, and analgesics, and for the treatment of PD and other disorders of the nervous system. The location of the ifenprodil-binding site on NMDA receptors and the structural and mechanistic basis of its effects are still unknown, but that at least part of the ifenprodil-binding site is located in the R1/R2 domain of the NR1 subunit. This region, like the S1/S2 agonist-binding domain, shares homology with bacterial periplasmic binding proteins.

Memantine as a Neuroprotective Agent

Memantine is approved for the treatment of AD and has a good safety record. The molecular basis for memantine efficacy in neurological diseases is at least in part due to the correction of overactivation of NMDA receptors (Rs) that causes excessive Ca^{2+} influx through the receptor's associated ion channel and consequent free radical formation. Memantine is a noncompetitive and low-affinity NMDA antagonist, with a high voltage dependency and fast receptor kinetics, but none of the undesirable effects associated with NMDA antagonists. In experimental studies in rats, it was shown that memantine infusion at low doses leads to steady-state serum levels within a therapeutic range and provides neuroprotection as well as cognitive enhancement. A nitro group is attached to memantine to target NMDA receptors of overly active neurons. These drugs, called nitromemantines, represent second-generation memantine derivatives that are designed to have enhanced neuroprotective efficacy without sacrificing safety (Lipton 2006). Use of memantine in AD is described in Chap. 8. Other potential clinical uses are in vascular dementia and AIDS–dementia complex.

Magnesium

The magnesium ion blocks the NMDA channel in a voltage-dependent fashion but electrophysiologically, extracellular magnesium behaves as a noncompetitive NMDA antagonist. The neuroprotective effect of intra-arterial magnesium sulfate has been shown in animal models of reversible focal cerebral ischemia. This compound may be useful for limiting infarction if given intra-arterially before the induction of reversible ischemia during cerebrovascular surgery. The advantages of

using magnesium are that it is widely available, is cheap, and has an established safety profile. A randomized, placebo-controlled, double-blind, pilot trial of intravenous magnesium sulfate in acute stroke patients showed an improved early outcome in magnesium sulfate recipients. A phase III trial is currently in progress.

The Field Administration of Stroke Therapy-Magnesium (FAST-MAG) pilot trial showed the feasibility and safety of initiating administration of neuroprotective agents by paramedics. The drug can be started within 2 h of symptom onset. It is possible to envision large-scale neuroprotective trials in which all patients are treated in the field within 2 h of stroke onset, allowing the human treatment situation to closely parallel that in animal models, in which treatment is effective.

NAALADase Inhibitors

These compounds act as antiglutamate agents by the inhibition of the enzyme *N*-acetylated- α -linked-acidic-dipeptidase (NAALADase) at presynaptic level and have been shown to protect against neurodegeneration in a number of in vitro and in vivo animal models. A newly described NAALADase inhibitor – 2-(phosphonomethyl)pentanedioic acid (2-PMPA) – protects against ischemic injury in a neuronal culture model of stroke and in rats after transient MCAO. Neuroprotection has been shown in a rodent model of MPTP PD as well. Because NAALADase inhibitors do not interact with postsynaptic glutamate receptors, they are devoid of the behavioral toxicity associated with postsynaptic glutamate antagonists.

Gacyclidine

Gacyclidine (G-11), a phencyclidine derivative, is a noncompetitive NMDA antagonist. As a neuroprotective agent, it has been investigated for the treatment of SCI but its development for this indication was discontinued due to the lack of efficacy in phase III clinical trials. It has also been considered for the treatment of organophosphate poisoning based on the concept that excitatory amino acid glutamate also plays a prominent role in the maintenance of organophosphate-induced seizures and in the subsequent neuropathology, especially through an overactivation of the NMDA receptor subtype.

***N*-Alkylglycines**

A family of *N*-alkylglycines has been identified that selectively blocks the NMDA receptor. Notably, compound 3,3-diphenylpropyl-*N*-glycinamide (referred to as N20C) inhibited NMDA receptor channel activity with micromolar affinity, fast on–off blockade kinetics, and strong voltage dependence. Molecule N20C did not act as a competitive glutamate or glycine antagonist. In contrast, saturation of the blocker-binding site with N20C prevents dizolcipine (MK-801) blockade of the NMDA

receptor, implying that both drugs bind to the same receptor site. The *N*-alkylglycine efficiently prevents in vitro excitotoxic neurodegeneration of cerebellar and hippocampal neurons in culture. Attenuation of neuronal glutamate/NMDA-induced Ca^{2+} overload and subsequent modulation of the glutamate–nitric oxide–cGMP pathway seems to underlie N20C neuroprotection. Noteworthy, this molecule exhibited significant in vivo neuroprotectant activity against an acute, severe, excitotoxic insult. Taken together, these findings indicate that *N*-alkylglycine N20C is a novel, low molecular weight, moderate-affinity NMDA receptor open channel blocker with in vitro and in vivo neuroprotective activity, which may become a tolerated drug for the treatment of neurodegenerative diseases.

AMPA Receptor Modulators

AMPAKINES (Cortex Pharmaceuticals) are proprietary AMPA receptor modulators. They enhance the functioning of the AMPA-type glutamate receptor by increasing the amount of current flow that takes place when glutamate binds to the receptor. The compounds improve the encoding of memory by facilitating LTP. One compound of this category, CX516 (Cortex Pharmaceuticals), has been shown to facilitate short-term memory in rats. It had a positive effect on memory in clinical trials on healthy volunteers and in phase II studies in patients with AD. Treatments with the ampakine CX614 markedly and reversibly increases BDNF mRNA and protein levels in cultured rat hippocampal slices, and comparable results are reported with a second, structurally distinct ampakine CX546. Ampakine-induced upregulation was broadly suppressed by AMPA, but not NMDA receptor antagonists. These findings raise the possibility of using positive AMPA modulators to regulate neurotrophin levels in the aged brain. AMPA modulators have potential for use as neuroprotectants in acute insults to the brain such as stroke, trauma and prolonged status epilepticus, but some of the limitations of their use are as follows:

- Prophylactic use of AMPAKINES as neuroprotectants in chronic degenerative diseases is problematic.
- AMPAKINES have the potential to modify other brain functions such as memory and cognition, but further studies are needed to determine the receptor subtypes and the circuits.
- Potential toxic effects of AMPA/kainate receptor manipulation, particularly neuronal death due to activation.

Metabotropic Glutamate Receptor Modulators

mGlu receptors have been considered as potential targets for neuroprotective drugs, but the lack of specific drugs has limited the development of neuroprotective strategies

in experimental models of acute or chronic CNS disorders in the past. The advent of potent and centrally available subtype-selective ligands has overcome this limitation, leading to an investigation of the role of mGlu receptor subtypes in neurodegeneration. Pharmacological blockade of mGlu1 or mGlu5 receptors or pharmacological activation of mGlu2/3 or mGlu4/7/8 receptors produces neuroprotection in a variety of in vitro or in vivo models. mGlu1 receptor antagonists are promising drugs for the treatment of cerebral ischemia. mGlu5 receptor antagonists may limit neuronal damage induced by a hyperactivity of NMDA receptors, because mGlu5 and NMDA receptors are physically and functionally connected in neuronal membranes.

There are potential applications of mGlu5 receptor antagonists in chronic neurodegenerative disorders such as ALS and AD. mGlu2/3 receptor agonists not only inhibit glutamate release, but also promote the synthesis and release of neurotrophic factors in astrocytes. These drugs may, therefore, have a broad application as neuroprotective agents in a variety of CNS disorders. The advantage of all these drugs with respect to NMDA or AMPA receptor agonists derives from the evidence that mGlu receptors do not “mediate,” but rather “modulate,” excitatory synaptic transmission. Therefore, it can be expected that mGlu receptor ligands are devoid of the undesirable effects resulting from the inhibition of excitatory synaptic transmission, such as sedation or an impairment of learning and memory.

Efforts are being made toward finding and optimizing novel mGluR4-positive allosteric modulators (PAMs). Preclinically in rodent models, mGluR4 activation has offered much promise as a novel treatment of PD (Lindsley et al. 2009). It is anticipated that continued progress in this area will further our understanding of the potential of mGluR4 modulation as a novel symptomatic and potentially disease-modifying treatment for PD. Although several mGlu5 receptor antagonists are in development for psychiatric disorders, no drug of this category has been translated into a clinical neuroprotective agent.

Cannabinoids

Endocannabinoids are involved in neuroprotection through numerous biochemical pathways. Endocannabinoid 2-arachidonoyl glycerol (2-AG) is released in mouse brain after closed head injury, and treatment with exogenous 2-AG exerts neuroprotection via the central cannabinoid receptor CB₁. This process involves inhibition of inflammatory signals that are mediated by activation of the transcription factor NF- κ B. A recent study shows that 2-AG decreases BBB permeability and inhibits the acute expression of the main proinflammatory cytokines: TNF- α , IL-1 β , and IL-6 (Panikashvili et al. 2005). It also augmented the levels of endogenous antioxidants.

Cannabinoid type 1 (CB1) receptors play a central role in the protection against excitotoxicity induced by the treatment of mice with kainic acid. Inactivation of CB1 receptor function in mice blocks KA-induced increase of brain-derived

neurotrophic factor (BDNF) mRNA levels in hippocampus. Both genetic ablation of CB1 receptors and pharmacological blockade with the specific CB1 receptor antagonist SR14 increase the susceptibility of the in vitro cultures to KA-induced excitotoxicity, leading to extensive neuronal death (Khaspekov et al. 2004). Furthermore, the application of SR141716A to hippocampal cultures from wild-type mice abolishes the KA-induced increase in BDNF protein levels. BDNF prevents KA-induced neuronal death after blockade of CB1 receptor signaling. These results strongly suggest that BDNF is a key mediator in CB1 receptor-dependent protection against excitotoxicity, and further underline the physiological importance of the endogenous cannabinoid system in neuroprotection. An indirect evidence for the role of CB1 receptors in neuroprotection is a study showing that in aging mice, the decline in cognitive functions is accelerated in the absence of CB1 receptors and is accompanied by a loss of neurons in the CA1 and CA3 regions of the hippocampus (Bilkei-Gorzo et al. 2005).

Cannabidiol, a nonpsychoactive constituent of marijuana, has been shown to reduce glutamate toxicity in cortical neuron cultures exposed to toxic levels of the excitatory neurotransmitter glutamate. Cannabidiol is a stronger protective against glutamate neurotoxicity than either ascorbate or α -tocopherol, indicating that it is a potent antioxidant. Cannabidiol is a potentially useful therapeutic agent for neuroprotection.

Dexanabinol

Dexanabinol (Pharmos) is a proprietary synthetic cannabinoid derivative. Pharmos has completed extensive preclinical work on dexanabinol, which demonstrated that the compound holds a unique position among putative neuroprotective agents. It offers a combination of three mechanisms of action in one molecule:

1. Blockade of glutamate neurotoxicity through noncompetitive inhibition of the NMDA receptor channel
2. Anti-inflammatory action through the inhibition of TNF- α
3. Antioxidant activity in several animal models, including those of closed head injury, focal and global forebrain ischemia, and optic nerve crush. In all of these models, a single injection of dexanabinol given after the insult confers significant long-term functional improvement and increased neuronal survival

Because dexanabinol inhibits neuroinflammation and is also able to block cell-death cascades in the brain, it a powerful agent for the treatment of CNS diseases in which neuroinflammatory cascades are involved, such as TBI and stroke. It is devoid of the undesirable psychotropic effects of natural cannabis derivatives and has proven to be safe in healthy volunteers as well as in a phase III trial of severe TBI (see Chap. 4). It is in phase IIa trial as a neuroprotective for the prevention of cognitive impairment in heart bypass surgery. The use of

dexanabinol circumvents certain legal and social challenges to the therapeutic use of marijuana by virtue of a configuration that avoids the psychotropic effects of natural cannabis.

Glutathione

The cytoprotective effects of glutathione are well known and it plays an important role in neuroprotection from neurotoxicity. It protects against selective toxicity of 2-chloropropionic acid to the cerebellum by modulating brain thiol status. This subject is a complex one as certain chemicals may be converted to neurotoxins following conjugation with glutathione such as in case of (+/-)-3,4-methylenedioxymphetamine-mediated serotonergic neurotoxicity.

Heat Shock Proteins

HSPs comprise the majority of a group known as stress proteins that, themselves, are the largest members of a group of proteins known as molecular chaperones – a key part of the cellular protection, maintenance, and repair mechanism. Synthesis of proteins is a normal part of every cell's activity and molecular chaperones perform manufacturing support and quality control roles, ensuring that newly synthesized proteins are complete, taken to the correct position within the cell's structure, and correctly folded. Where there is a problem anywhere during this process that cannot be overcome, HSPs will also direct a nonfunctional protein for degradation. HSPs are classified according to molecular weight (e.g., HSP40, HSP60, HSP70, HSP90, etc.). For instance, members of the HSP 70 family are found in the cytoplasm, nucleus, mitochondria, and endoplasmic reticulum where they bind to proteins in their intermediate folded states. They then “chaperone” or translocate them across the cell membranes to their correct site of operation where they assist the protein to fold into its proper native and functional shape. The heat shock response and more specifically the increase in intracellular HSP levels are highly cytoprotective. This protective capability is based upon the following mechanisms:

- Stabilization of cellular membrane
- Heat stress and ischemia damage the cytoskeleton. Increased HSP levels help avoid loss of the intermediate filament network, degradation of the cytoplasmic network, and disruption of the microtubules and mitotic spindle.
- Increase in biologically available NO by reduction in free radical generation and other oxidative intermediates are highly destructive to cells and a major factor in many disease states.
- Under severe stress conditions, intracellular calcium levels can rapidly rise to toxic levels. Increased levels of HSPs bind to different ion channels and restrict this increase.

Hormones

Estrogen and Neuroprotection

Estrogen and progesterone, long considered for their roles as primary hormones in reproductive and maternal behavior, are now being studied as neuroprotective and neuroregenerative agents in stroke and TBI. These hormones reduce the sequelae of the injury cascade by enhancing antioxidant mechanisms, reducing excitotoxicity (altering glutamate receptor activity, reducing immune inflammation, providing neurotrophic support, and stimulating axonal remyelination), and enhancing synaptogenesis and dendritic arborization. Estrogen receptor-dependent activation of MAPK is involved in estrogen-mediated anti-inflammatory pathways in microglial cells and offers an explanation of how estrogen may attenuate the progression of neurodegenerative disease.

Evidence from basic research strongly indicates that the use of estrogenic drugs that can mimic the anti-inflammatory activity of 17 β -estradiol (E2) might trigger beneficial effects against neurodegeneration in addition to carrying out their specific therapeutic function (Pozzi et al. 2006). Although some of estrogen's neuroprotective effects may depend upon the estrogen receptor, this effect may occur without hormonal side effects. Estrogen replacement therapy has also been used clinically in neuroprotection in AD in women, but recent evidence indicates that it may increase the risk of dementia in women over the age of 65 years (see section on Alzheimer's disease).

Neuroprotective Effect of Estrogen Receptor Ligands

There is some controversy about the role of estrogen receptors (ERs), but both ER α and ER β can couple to rapid signaling events that mediate estrogen-elicited neuroprotection. Another explanation for the neuroprotective effect of estrogen is that ERs and insulin-like growth factor-1 receptor (IGF-IR) are interdependent in the promotion of neuronal differentiation. In adults, ERs and IGF-IR interact in the induction of synaptic plasticity. Furthermore, both in vitro and in vivo studies have shown that there is an interaction between ERs and IGF-IR in the promotion of neuronal survival and in the response of neural tissue to injury, suggesting that a parallel activation or co-activation of ERs and IGF-IR mediates neuroprotection.

Effects of treatment with an ER α have been compared with that of an ER β ligand in experimental autoimmune encephalomyelitis model of MS (Tiwari-Woodruff et al. 2007). ER α ligand treatment abrogated disease at the onset and throughout the disease course. In contrast, ER β ligand treatment had no effect at disease onset but promoted recovery during the chronic phase of the disease. ER α ligand treatment was anti-inflammatory in the systemic immune system, whereas ER β ligand treatment was not. Also, ER α ligand treatment reduced CNS inflammation, whereas ER β ligand treatment did not. However, treatment with either the

ER α or the ER β ligand was neuroprotective, as evidenced by reduced demyelination and preservation of axon numbers in white matter and decreased neuronal abnormalities in gray matter. Thus, by using the ER β selective ligand, the anti-inflammatory effect was dissociated from the neuroprotective effect of estrogen treatment, indicating that neuroprotective effects of estrogen treatment do not necessarily depend on anti-inflammatory properties. Together, these findings suggest that ER β ligand treatment should be explored as a potential neuroprotective strategy in MS and other neurodegenerative diseases, particularly because estrogen-related toxicities such as breast and uterine cancer are mediated through ER α .

Selective Estrogen Receptor Modulators

The nigrostriatal dopaminergic system of male mice is more sensitive to the neurotoxic effects of methamphetamine, and the basis for this difference can be related to estrogen, which has the capacity to function as a neuroprotectant against neurotoxins that target the nigrostriatal dopaminergic system. In addition to 17 β -estradiol (E2), some selective estrogen receptor modulators, including tamoxifen, afford neuroprotection in various experimental models of neurodegeneration. Tamoxifen, used in the management of breast cancer, is a selective estrogen receptor modulator (SERM) that can function as a nigrostriatal dopaminergic neuroprotectant against methamphetamine-induced neurotoxicity in intact female and male mice. Tamoxifen, like estrogen, can protect ovariectomized rats from stroke-like injuries. Both E2 and tamoxifen provide effective neuroprotection against manganese-induced toxicity in rat cortical primary cultures of neurons and astrocytes (Lee et al. 2009). Long-term administration of estrogen or tamoxifen to ovariectomized rats affords neuroprotection to hippocampal neurons by modulating the expression of apoptotic proteins Bcl-2 and Bax (Sharma and Mehra 2008). Furthermore, the estrogen-like effects of tamoxifen indicate its potential as a neuroprotectant in neurodegenerative disorders, particularly in postmenopausal women.

GeneChip assay is being used to identify genes that are up- or downregulated by the tamoxifen treatment. Tamoxifen regulates several genes in the rat cerebral cortex including cell adhesion genes such as contactin 1, regeneration genes including stem cell factor K-1 and 5-HT 7 receptor, and transcription and signaling factors including Fos-related antigen and hairless protein. Tamoxifen also downregulates the expression of PKC- Δ , a gene implicated in neuronal cell death, by nearly fourfold. Further research will help elucidate the mechanisms by which tamoxifen exerts its neuroprotective effect, and the information will help design drugs that mimic estrogen, but without estrogen's undesirable effects.

Mitochondrial Mechanisms of Estrogen Neuroprotection

Determination of the molecular targets of estrogen action on mitochondrial function, whether localized to the organelle or elsewhere, is critical for understanding

estrogen neuroprotection and for optimization of drugs for the treatment of neurodegenerative diseases. Studies of structure–activity relationships and mitochondrial function have led to a mechanistic model in which these steroidal phenols intercalate into cell membranes, where they block lipid peroxidation reactions and are in turn recycled. Estrogens have multiple effects on mitochondrial function that are expressed under stress. They preserve ATP production, prevent the production of ROS, moderate excessive cellular and mitochondrial Ca^{2+} loading, and preserve mitochondrial membrane potential during insults. All of these effects are important in maintaining the viability of mitochondria during insults. The neuroprotective and mitoprotective potencies for a series of estrogen analogs are significantly correlated, suggesting that these compounds prevent cell death in large measure by maintaining functionally intact mitochondria (Simpkins and Dykens 2008). This therapeutic strategy is germane not only to sudden mitochondrial failure in acute circumstances, such as during a stroke or myocardial infarction, but also to gradual mitochondrial dysfunction associated with chronic degenerative disorders such as AD.

Insulin

Preischemic hyperglycemia is recognized to aggravate the effects of cerebral ischemia, although postischemic administration of glucose may be neuroprotective. Various mechanisms of the neuroprotective effect of insulin are as follows:

- Inhibition of neuronal metabolism.
- Inhibition of neuronal uptake of GABA with accumulation in the extracellular space.
- Insulin stimulates Na^+/K^+ -ATPase activity, which decreases neuronal excitability.
- Insulin increases noradrenergic tone by enhancing norepinephrine release and inhibits its uptake. This has a neuroprotective effect.
- Insulin can act in a neurotrophic manner in the same way as the insulin-like growth factors.

The mechanisms are essentially of two types: one in which insulin interacts directly with brain tissue and one in which insulin acts indirectly by reducing peripheral blood glucose levels. Protective effect of insulin has been explored in focal and global ischemic models. The direct mechanism appears to predominate in global ischemia and is mediated by insulin-like growth factor-1 receptors. In focal ischemia, unlike that in global ischemia, the effect of insulin is predominantly via peripheral hypoglycemia, because neuroprotection is largely annulled by the coadministration of glucose. In clinical situations where transient focal ischemia to the hemisphere can be anticipated, insulin-induced hypoglycemia of a mild degree may be beneficial. The two clinical counterparts of global and focal ischemic models are, respectively, cardiac arrest encephalopathy and focal ischemic stroke. Insulin use in both these clinical situations could be evaluated in clinical trials that attempt to reduce

ischemic brain damage, because insulin has a long and safe history of human use in diabetes treatment. Severe hypoglycemia, however, can produce brain damage and this is discussed further in Chap. 11.

Ion Channel Modulators

Ion channels are protein pores in the cell membrane that allow the passage of ions down their respective electrochemical gradients. Ion channels are classified according to the ion passing through them (e.g., sodium, potassium, calcium, or chloride) and the mechanisms by which they are opened or closed. Classical roles of ion channels in the nervous system are signal propagation along a cell surface and signal translation (by release of transmitters) into the cytoplasm of the cell (Jain 2010f). Some examples of the role of ion channels in neuroprotection are as follows.

Calcium Channel Blockers

Ca²⁺ overload may play a critical role in ischemia as well as various neurodegenerative disorders. Thus, it appears that the development of improved strategies to prevent Ca²⁺ overload will be important for neuroprotection. Cognitive and functional decline with age is correlated with deregulation of intracellular calcium, which can lead to neuronal death in the brain. Antagonists of the low-voltage T-type Ca²⁺ channel, which form a family of FDA-approved AEDs, comprise three members (Cav3.1, Cav3.2, and Cav3.3) based on their respective main pore-forming alpha subunits: $\alpha 1G$, $\alpha 1H$, and $\alpha 1I$. Among these three subunits, $\alpha 1H$ is highly expressed in hippocampus and certain cortical regions. However, T-type Ca²⁺ channel blockers can protect neurons derived from $\alpha 1H$ -/- mice, suggesting that neuroprotection demonstrated by these drugs is not through the $\alpha 1H$ subunit (Wildburger et al. 2009). In addition, T-type Ca²⁺ channel blockers were not able to confer any protection to neurons in long-term cultures, while L-type Ca²⁺ channel blockers could protect neurons. These data indicate a new function of T-type calcium channel blockers, and also suggest different mechanisms to regulate neuronal survival by calcium signaling pathways. These findings have important implications in the development of new treatment for age-related neurodegenerative disorders.

Ca²⁺ channel blockers, particularly those that block excitatory amino acid release, have been shown to be neuroprotective following experimental ischemic insult to the brain. The success of Ca²⁺ antagonists in cardiovascular indications has encouraged research into their therapeutic potential in cerebrovascular conditions. Data from an epidemiological study suggest a potential neuroprotective role for centrally acting L-type calcium channel blockers of the dihydropyridine class in PD (Ritz et al. 2010).

Ziconotide

Ziconotide (Prialt), the synthetic form of cone snail peptide ω -conotoxin MVIIA, is a neuron-specific N-type calcium channel blocker with an analgesic and neuroprotective effect. Intrathecal ziconotide has been approved by the FDA for the management of chronic pain (Jain 2000). Ziconotide has a protective effect against focal ischemia in animal models of stroke. The neuroprotective effect is difficult to explain because ziconotide's inhibition of glutamate release is weak and it exerts protection even if administered after Ca and glutamate levels become normal. This indicates that the mechanism underlying neuronal damage is more complicated than that proposed in the glutamate–calcium overload hypothesis.

Ziconotide penetrates the brain after intravenous injection, although the mechanism has not yet been elucidated. The amount found in the brain after intravenous injection is consistent with the biological potency of the peptide and enables it to exert neuroprotective effect against cerebral ischemia. Intrathecal infusion of ziconotide was shown to be protective against injurious intervals of spinal ischemia in experimental animals. Based on these findings, ziconotide was expected to provide both neuroprotection and preemptive analgesia in aortic aneurysm surgery. Ziconotide was investigated as a neuroprotective in phase III clinical studies for the treatment of acute ischemic stroke and in phase II trials for TBI, but further development was discontinued due to lack of efficacy.

Na⁺ Channel Blockers

In cerebral ischemia, energy demand exceeds the supply. Neuroprotection may be achieved not only by restoring cerebral perfusion but also by decreasing the metabolic demands of the neural tissues. Downregulation of the Na⁺ channels is another effective way of reducing energy demand, because a large part of the energy consumed by the brain is used for the maintenance of ion gradients across the cellular membranes. A key component linking energy metabolism to transmembrane ion transport is the Na⁺/K⁺-ATPase, an enzyme that uses ATP to transport Na⁺ back out of the cell and K⁺ back into the cell, thus restoring the ionic gradient across the cellular membrane. Downregulation of the Na⁺ channels in ischemia reduces the Na⁺ influx into the brain cell with resulting energy preservation. This also prevents the intrinsic neurotoxicity of the acute Na⁺ influx and the linked Ca²⁺ influx.

Various pharmacological agents that exert their neuroprotective effect by downregulating Na⁺ channels are lamotrigine, phenytoin, and riluzole. Fosphenytoin was tested in clinical trials in ischemic stroke patients but failed to show a neuroprotective effect. Carbamazepine, one of the older anticonvulsants, has a neuroprotective effect that has been demonstrated in animal models of stroke but has not been clinically evaluated. Lamotrigine inhibits the presynaptic release of glutamate by blockade of voltage-dependent Na⁺ channels, and its effectiveness was demonstrated in cardiac arrest-induced global cerebral ischemia with reperfusion in rats.

Neuroprotective Potassium Channel Inhibitors

Potassium (K^+) channels, the most widely distributed class of ion channels, are transmembrane proteins that play a pivotal role in electrical activity of all excitable tissues. Some types of K^+ channels present in the plasma membrane of various cells have been found in the inner mitochondrial membrane as well. Abnormalities in K^+ currents result in cardiovascular, neurological, renal, and endocrine pathology. The discovery of apoptosis mediation by K^+ currents points to the role of K^+ homeostasis in cellular proliferation and degeneration. Pharmacological modulation of specific K^+ currents is recognized as a major strategy in the treatment of a broad range of disorders. Various K^+ channels are recognized as potential therapeutic targets in the treatment of PD, AD, and stroke. In addition to their importance as therapeutic targets, certain K^+ channels are known for their beneficial role in neuroprotection. It has been shown that increased K^+ flux into brain mitochondria induced by either the ATP-regulated $\text{mitoK}_{\text{ATP}}$ channel or the Ca^{2+} -regulated $\text{mitoBK}_{\text{Ca}}$ channel affects the beneficial effects on neuronal cell survival under pathological conditions (Bednarczyk 2009).

Kynurenine Inhibitors

Kynurenine (KYN) is a constituent of the human and rodent brain. Neuroactive kynurenine metabolites play a role in the normal physiology of the human brain. Two of these metabolic products, quinolinic acid and kynurenic acid (KYNA), act as an agonist and an antagonist, respectively, at receptors of excitatory amino acids. 3-Hydroxykynurenine also acts an intracellular neurotoxin by producing free radicals and is involved in the pathology of neurodegenerative disorders.

Altered KYNA metabolism in the red blood cells (RBC) is linked to the pathogenesis of PD. The increased activity of kynurenine aminotransferase (KAT) II in correlation with the elevated KYNA level in the RBC may mediate a consecutive protective response against excitatory neurotoxic effects in PD. There is decreased expression of KAT-I- immunoreactivity in the substantia nigra of mice after administration of MPTP (complex I inhibitor) in animal models of PD. Immunohistochemical depletion of KAT-I- immunoreactivity after administration of 3-nitropropionic acid to adult animals may contribute to the pathological processes in HD. Furthermore, the effect of L-kynurenine in increasing CBF might be mediated by the activation of cholinergic and NO pathways. The alterations of two main KYN metabolites, KYNA and quinolinic acid, seem to be associated with the impairment of the cognitive function in AD patients, which offers therapeutic opportunities for the development of new compounds for neuroprotection (Gulaj et al. 2010).

The discovery of the pathophysiological importance of KYNs has led to the development of KYN inhibitors as neuroprotectives. KYN 3-mono-oxygenase inhibitors attenuate postischemic brain damage in animal models of stroke by reducing the formation of 3OH-KYN and quinolinic acid.

Leukocyte Adhesion Inhibitors

Leukocytes are involved in CNS ischemic injury by two mechanisms: (1) direct microvascular occlusion after endothelial and basement membrane adhesion and (2) transendothelial migration of leukocytes with secondary CNS tissue infiltration and neuronal cytotoxic injury. Adhesion of leukocytes to microvascular endothelium is essential for the initiation of either of these mechanisms. These mechanisms potentiate reperfusion injury as well. Both the microcirculation obstruction and the brain infiltration can be decreased by the use of special monoclonal antibodies directed against leukocyte adhesion receptors. The following have been used:

Antibodies directed against the CD18 complex. The leukocyte membrane glycoprotein complex predominantly responsible for endothelial adherence is termed CD18 or β_2 -integrin. The use of special mouse monoclonal antibodies directed at various components of the CD18 complex is associated with a reduction in ischemic injury and decreased leukocyte infiltration in the brain following MCAO in the mouse. One compound of this category, Hu23F2G (LeukArrest), reached phase III clinical trials as a neuroprotective in stroke before it was discontinued.

Anti-ICAM-1 antibodies (Enlimomab). The corresponding counter receptor for the CD/integrin complex is the intercellular adhesion molecule ICAM-1. Several studies using anti-ICAM-1 monoclonal antibodies in animal models of stroke have shown reduction in the effects of cerebral ischemia only when significant reperfusion injury was involved. This treatment also reduces leukocyte infiltration when administered within 6 h of onset of stroke symptoms. A phase III study concluded that the product was clearly of no benefit and might have had an adverse effect on the outcome. Further development has been discontinued.

Neutrophil inhibitory factor (NIF). A peptide derived from NIF has been shown to block adherence to polymorphonuclear leukocytes that was dependent on both Mac-1 and LFA-1 integrins. By preventing the activation of neutrophils and their subsequent migration from blood into damaged tissue, NIF may prevent the acute inflammatory response during reperfusion injury that can exacerbate damage to the areas of the brain affected by the stroke. Phase IIb clinical trials of recombinant NIF (UK-279,276) for the treatment of stroke reperfusion injury showed safety but not efficacy. Further development for this indication was discontinued.

Synthetic fibronectin peptides. Fibronectin is an extracellular matrix molecule found in plasma, cells' matrix, basal lamina, and cell surface. Fibronectin has been

shown to support leukocyte adhesion to endothelial cells. Synthetic fibronectin peptides corresponding to the cell- and heparin-binding sequences of fibronectin that disturb leukocyte adhesion molecules have been shown to be effective in neuronal protection after transient focal cerebral ischemia in rats.

Modafinil

Modafinil is an approved drug to improve wakefulness in patients with excessive daytime sleepiness associated with narcolepsy as it acts selectively in areas of the brain believed to regulate normal wakefulness (Jain 2010h). The mechanism of action of modafinil is considered to involve hypocretin, histamine, GABA, and glutamate. The selective CNS activity of modafinil is distinct from the action of amphetamine and methylphenidate. Functional MRI studies have shown that low cortical activation levels in both normal and narcoleptic subjects are increased following the administration of modafinil. Modafinil increases daytime wakefulness but does not interfere with the integrity or architecture of nighttime sleep. It significantly improves patients' ability to remain awake while engaged in daily activities.

Modafinil has a neuroprotective effect, not only in dopaminergic neurons, but also in the nondopaminergic nerve cell population of the substantia nigra. Modafinil was able to prevent the MPTP-induced neuronal damage in marmosets partially (van Vliet et al. 2008). Modafinil may have a therapeutic potential in neurodegenerative diseases such as PD.

Neural Regeneration Protein

A novel rodent gene encoding for a neural regeneration protein (NRP) has been identified with an activity spectrum similar to that of the chemokine stroma-derived factor (SDF)-1, but with much greater potency (Gorba et al. 2006). The *Nrp* gene is first expressed in neural stem cells and expression continues in glial lineages. Recombinant NRP and NRP-derived peptides possess biological activities including induction of neural migration and proliferation, promotion of neuronal survival, enhancement of neurite outgrowth, and promotion of neuronal differentiation from neural stem cells. NRP exerts its effect on neuronal survival by phosphorylation of the ERK1/2 and Akt kinases, whereas NRP stimulation of neural migration depends solely on p44/42 MAPK activity. Taken together, the expression profile of *Nrp*, the existence in its predicted protein structure of domains with similarities to known neuroprotective and migration-inducing factors, and the high potency of NRP-derived synthetic peptides acting in femtomolar concentrations suggest it to be a novel gene of relevance in cellular and developmental neurobiology. The scope of their therapeutic potential appears to be broad at this stage.

Beneficial effects have been observed in vitro and in animal models at extremely low concentrations. It has potential for development as a neuroprotective agent in peripheral neuropathy.

Neurite Outgrowth-Promoting Prostaglandin Compounds

Electrophilic neurite outgrowth-promoting prostaglandin (NEPP) compounds protect neurons from oxidative insults. Part of the neuroprotective action of NEPPs lies in induction of HO-1, which, along with other phase II enzymes, serve as a defense system against oxidative stress. A study using fluorescent tags and immunoprecipitation assays found that NEPPs are taken up preferentially into neurons and bind in a thiol-dependent manner to Keap1, a negative regulator of the transcription factor Nrf2 (Sato et al. 2006). By binding to Keap1, NEPPs prevent Keap1-mediated inactivation of Nrf2 and, thus, enhance Nrf2 translocation into the nucleus of cultured neuronal cells. In turn, Nrf2 binds to antioxidant/electrophile-responsive elements of the HO-1 promoter to induce HO-1 expression. Consistent with this concept, NEPP induction of an HO-1 reporter construct is prevented if the antioxidant-responsive elements are mutated. NEPPs are shown to be neuroprotective both in vitro from glutamate-related excitotoxicity and in vivo in a model of cerebral ischemia/reperfusion injury (stroke). These results suggest that NEPPs prevent excitotoxicity by activating the Keap1/Nrf2/HO-1 pathway. Because NEPPs accumulate preferentially in neurons, they may provide a category of neuroprotective compounds, distinct from other electrophilic compounds such as *tert*-butylhydroquinone, which activates the antioxidant-responsive element in astrocytes. NEPPs thus represent a therapeutic approach for stroke and neurodegenerative disorders.

Neuroimmunophilins

Neuroimmunophilin ligands are small molecules that have been shown in preclinical experiments to repair and regenerate damaged nerves without affecting normal, healthy nerves. Neuroimmunophilin ligands may have application in the treatment of a broad range of diseases, including PD, SCI, TBI, and peripheral nerve injuries such as that leading to post-prostatectomy erectile dysfunction. The immunosuppressants tacrolimus (FK506) and cyclosporin are in clinical use for the treatment of allograft rejection following organ transplantation. Immunophilins can regulate neuronal survival and nerve regeneration, although the molecular mechanisms are poorly understood. Anti-ischemic activity of FK506 and cyclosporine is explained by the anti-inflammatory action. Other explanations are suppression of calcium-dependent signal transduction pathway that promotes IL-2 gene transcription in helper T cells, direct neuronal action, inhibition of nitric oxide synthase (NOS) activity, and suppression of apoptosis. The immunosuppressant mycophenolate mofetil inhibits

proliferation and activation of microglia and astrocytes, and protects neurons after excitotoxic injury. FKBP52 is a component of the copper efflux machinery, and in so, may also promote neuroprotection from copper toxicity (see Chap. 11).

Cyclosporin-A

Maas BiolAB have discovered neuroprotective uses for cyclosporin-A (CsA), which inhibits Ca^{2+} -induced mitochondrial permeability transition (mPT), but might be unrelated to a reduction in post-traumatic Ca^{2+} accumulation (Mirzayan et al. 2008). mPT can only form if it has cyclophilin-D available. CsA binds up all cyclophilin-D within each mitochondrion. CsA-treated mitochondria continue to function normally, even while under attack from conditions damaging the brain. In animal experiments, intravenous CsA achieves therapeutic levels in brain parenchyma, and 10 mg/kg is the most effective dose in attenuating axonal damage after TBI (Okonkwo et al. 2003). The NIH has funded multicenter human trials for treating TBI with CsA. Potential applications are as follows:

- Acute: TBI, SCI, stroke, and cerebral vasospasm following SAH.
- Chronic: neurodegenerative diseases such as ALS, HD, PD, and AD.
- Selective neuronal brain protection from radiotherapy for brain cancer.

CsA is the active ingredient in Maas Biolab's Mitogard® intrathecal formula developed to treat ALS, which is in preclinical stage. Maas' partner NeuroVive Pharmaceutical AB is producing NeuroSTAT®, an intravenous CsA formula for the treatment of TBI. In a prospective, blinded, placebo-controlled, randomized, dose-escalation trial of intravenous CsA initiated within 8 h of TBI, the rate of mortality or other adverse events was not significantly different from that in the placebo group (Hatton et al. 2008).

FK506

The immunosuppressant FK506 (tacrolimus) is known to be neuroprotective following cerebral ischemia. Studies with FK506 in a model of transient focal cerebral ischemia in rats suggest that it exerts a neuroprotective effect by inhibiting apoptosis and suppressing inflammatory reactions (Furuichi et al. 2004). Another study in an ischemic model found that administration of FK506 was associated with a significant downregulation of IL-1 β expression in astrocytes and microglia suggesting that these are targets for FK506, and that modulation of glial response and inflammation may be a mechanism of FK506-mediated neuroprotection in ischemia (Zawadzka and Kaminska 2005). FK506 was also shown to protect against demyelination and axonal loss markedly in a model of MS through immunosuppression and neuroprotection (Gold et al. 2004). Its use has also been explored in TBI and SCI.

Rapamycin

Rapamycin, an immunosuppressant used to prevent transplant rejection, binds to mammalian target of rapamycin (mTOR). A high-density cell array with quantitative and automated readout of cell fitness has been used to map the relation between genes and cell fitness in response to rapamycin (Xie et al. 2005). An unexpected “rapamycin-enhanced” phenotype was identified in several genes whose deletion allowed cells to grow better in the presence of rapamycin than in its absence. Most of these genes encode mitochondrial proteins. mTOR is also associated with multiple mitochondrial enzymes. Because mitochondrial dysfunction is known to underlie the pathogenesis of a wide range of neurodegenerative disorders due to impaired energy production, increased oxidative damage, and apoptosis, these results suggest that rapamycin may be useful in preventing the progression of neurodegenerative diseases.

Modification of rapamycin at mTOR-binding region yields immunophilin ligands, WYE-592 and ILS-920, which have potent neurotrophic activities in cortical neuronal cultures, efficacy in a rodent model for ischemic stroke, and significantly reduced immunosuppressive activity. Both compounds show higher binding selectivity for FKBP52 versus FKBP12, in contrast to that of previously reported immunophilin ligands. Affinity purification has revealed two key binding proteins, the immunophilin FKBP52 and the β 1-subunit of L-type voltage-dependent Ca^{2+} channels (Ruan et al. 2008). Electrophysiological analysis indicates that both compounds can inhibit L-type Ca^{2+} channels in rat hippocampal neurons and F-11 dorsal root ganglia/neuroblastoma cells. These immunophilin ligands can protect neurons from Ca^{2+} -induced cell death by modulating Ca^{2+} channels and promote neurite outgrowth via FKBP52 binding.

Neurotrophic Factors

Neurotrophic factors (NTFs) regulate the proliferation, survival, migration, and differentiation of cells in the nervous system. NTFs are synthesized by and released from target cells of the neurons, bind to specific receptors, internalized, and retrogradely transported by the axon to the cell soma where multiple survival-promoting effects are initiated. Several different NTFs have been shown to prevent death of cortical and hippocampal neurons induced by excitotoxic and oxidative insults in cell culture and in vivo (Jain 2010i). Various studies of NTFs have shown that they have a neuroprotective action and that they enhance cellular systems involved in the maintenance of Ca^{2+} homeostasis and free radical metabolism. Several other compounds that mimic the action of NTFs by activating signal transduction cascades involving tyrosine phosphorylation have proven to be beneficial in animal studies of ischemic brain injury and provide opportunities for the development of therapeutic approaches for ischemic stroke. Further studies are required

to determine the relative potency of various NTFs, therapeutic windows, modes of administration, and doses. This section will describe well-known NTFs and NTF-like substances.

Activity-Dependent Neurotrophic Factor

Activity-dependent neurotrophic factor (ADNF) is a novel, femtomolar-acting, glial-derived polypeptide known to protect neurons from a variety of toxic insults. The active site for ADNF function is localized to a 9-amino acid stretch (SALLRSIPA; ADNF-9). A novel ADNF-9-like active peptide (NAP) has been identified and shown to be expressed in the CNS and exhibit an activity profile similar to that of ADNF-9. Such studies suggest that ADNF-9 and NAP might function like other known neurotrophins and play a role in neural development and maintenance. ADNF-9 and NAP increased synaptophysin expression in both rat hippocampal and cortical cultures (Smith-Swintosk et al. 2005). These results suggest that ADNF-9 and NAP might contribute to neuronal plasticity associated with the development and repair after injury.

ADNF enhances basal glucose and glutamate transport, and attenuates oxidative impairment of glucose and glutamate transport induced by A β and Fe²⁺ in neocortical synaptosomes. ADNF can act locally in synaptic compartments to suppress oxidative stress and preserve the function of glucose and glutamate transporters. Such synaptoprotective actions indicate possible therapeutic applications of agents that stimulate local synaptic (transcription-independent) NTF signaling pathways.

Bone Morphogenetic Proteins

Bone morphogenetic proteins (BMPs), with 20 members, are a rapidly expanding subclass of TGF- β superfamily. BMP ligands and receptors exert a broad range of effects during multiple stages of neural development. In addition, BMPs act on more lineage-restricted embryonic CNS progenitor cells to promote regional neuronal survival and cellular differentiation.

Growth differentiation factor (GDF)-5 is a novel member of this subclass. It is expressed in the developing CNS, including the midbrain, and acts as a neurotrophic, survival-promoting molecule for rat dopaminergic midbrain neurons. Recombinant human GDF-5 supports dopaminergic neurons in culture to the same extent as both TGF- β 3 and GDNF. However, in contrast to TGF- β 3 and GDNF, GDF-5 augments members of astroglial cells in cultures, suggesting that it may act indirectly. Also, it may act through pathways different from those triggered by TGF- β 3 and GDNF. GDF-5 also protects dopaminergic neurons against *N*-methylpyridinium ion-induced toxicity that selectively damages dopaminergic neurons.

Brain-Derived Neurotrophic Factor

BDNF and its receptor are widely expressed in the developing and the adult nervous system. BDNF signaling in the brain can increase peripheral insulin sensitivity, suggesting a mechanism whereby the brain can control lifespan. The expression of BDNF is modulated by the activity of GABAergic and cholinergic systems, and BDNF, in return, can influence neuronal synaptic activity. One form of BDNF protein enters the nucleus and may directly influence transcription, whereas another fraction travels by retrograde axonal transport out of the synthesizing cell and can be detected in the basal forebrain cholinergic neurons. BDNF has a neuroprotective effect because of its ability to reverse the NMDA-induced inactivation of PKC in cortical neurons.

BDNF secreted from the endothelium participates in the homeostatic interactions between endothelium and cerebral parenchyma and protects neurons against oxygen-glucose deprivation, oxidative damage, endoplasmic reticulum stress, hypoxia, and amyloid neurotoxicity (Guo et al. 2008). Endothelial production of BDNF is sustained by β -1 integrin and integrin-linked kinase (ILK) signaling. Noncytotoxic levels of oxidative stress disrupt ILK signaling and reduce endothelial levels of neuroprotective BDNF.

Intraventricular injection of BDNF has been shown to reduce infarct size in a rat model of focal cerebral ischemia. Potential mechanisms of the neuroprotective role of BDNF in focal cerebral ischemia include protective effects against glutamate toxicity and attenuation of apoptosis.

Ciliary Neurotrophic Factor

Ciliary neurotrophic factor (CNTF) is released from Schwann cells and astrocytes in response to injury and is considered to play a key role in nerve cell maintenance and repair. The human CNTF gene has been mapped to chromosome 11 and contains a single 1-kb intron within the coding domain. Axokine-1 is an rCNTF with substitution of alanine for cysteine at position 63 and deletion of 13 C-terminal amino acids. The synthesis of CNTF occurs mainly in the Schwann cells and astrocytes and appears to be regulated by either direct or indirect signals from neurons. The exact mechanism of release of CNTF is not known. The following biological actions have been attributed to CNTF:

- Support of survival of all peripheral nervous system neurons plus many CNS neurons
- Induction of neurite outgrowth
- Promotion of cholinergic phenotype in sympathetic neurons
- Arrest of division of neuronal precursor cells

- Inhibition of mitosis and induction of vasoactive intestinal peptide
- Induction of differentiation of type II astrocytes from glial progenitor cells of the optic nerve
- Activation of signal transducers and transcription factors may be one of the intracellular signaling pathways employed by CNTF to mediate survival and differentiation effects on CNS neurons

CNTF activates astrocytes, redistributes their glutamate transporters, and improves glutamate handling in vivo (Escartin et al. 2006). CNTF has been shown to protect hippocampal neurons from excitotoxic damage. It also has potential clinical applications in the treatment of ALS.

Fibroblast Growth Factors

The fibroblast growth factor (FGF) family encompasses at least 23 members and of these, 10 are expressed in the developing CNS, along with 4 FGF receptors. Acid fibroblast growth factor (aFGF) is present in a select set of neurons, including some that are at risk for developing neurodegenerative disorders, whereas basic fibroblast growth factor (bFGF) is widely distributed in tissues. Its gene is located on chromosome 4 of the human genome. bFGF is present in most astrocytes and a specific hippocampal neuronal population. Immunoreactivity of bFGF is expressed weakly in the neurons and strongly in the choroid plexus, whereas glial cells express little of these proteins.

bFGF is a potent dilator of systemic blood vessels and cerebral pial arterioles. Intravenous bFGF is able to cross the damaged BBB and exert a direct trophic effect on the ischemic brain tissue. Intracisternal bFGF has also been shown to enhance behavioral recovery following focal cerebral infarction in the rat. The mechanism of infarct reduction may include direct cytoprotective and vasoactive effects. No angiogenesis has been demonstrated in any of the studies although bFGF is known to have angiogenic properties. Although the exact mechanisms of the beneficial effect of bFGF in ischemic stroke have not been proven, there is evidence that delayed administration enhances functional recovery. The mechanism of action of bFGF as a neuroprotective agent involves the activation of survival signals, which, in turn, enhance anti-apoptotic proteins, antioxidant enzymes, and calcium-binding proteins.

In neurodegenerative disorders such as AD and human immunodeficiency virus (HIV) encephalitis, the neuroprotective activity of bFGF against several neurotoxic agents might involve regulation of glycogen synthetase kinase- β , a pathway important in determining cell fate. bFGF has a synergistic effect with citicoline (cytidine 5'-diphosphate choline), as a neuroprotective in animal models of focal cerebral ischemia.

Glial Cell Line-Derived Neurotrophic Factor

Glial cell line-derived neurotrophic factor (GDNF) is a neurotrophic polypeptide and is distantly related to TGF- β . Purified recombinant GDNF promotes neurite outgrowth and is considered to be a NTF for developing peripheral neurons. It has also been implicated in the survival, and morphological and functional differentiation of midbrain dopaminergic neurons in vitro. GDNF travels by retrograde transport via midbrain dopamine neurons of the nigrostriatal pathway. The pattern of retrograde transport following intrastriatal injections indicates that there may be subpopulations of neurons that are GDNF responsive.

In addition to its role in the dopaminergic system, GDNF may act on several types of nondopaminergic neurons such as noradrenaline neurons, cerebral Purkinje cells, and neurons in peripheral ganglia. GDNF may function as a target-derived trophic factor for neuronal populations innervating skeletal muscle, including sensory neurons and spinal cord motor neurons. Based on a comprehensive review of the pharmacology of GDNF, it appears that this molecule may be useful in the treatment of neurodegenerative diseases such as Parkinson disease, ALS, and cholinergic deficit-related dementia. Recent studies indicate that GDNF can protect the cerebral hemispheres from damage induced by middle cerebral arterial ligation, and that such neuroprotective effects are mediated through specific GDNF receptor $\alpha 1$.

Delivery of NTFs into tissues and across the BBB is severely limited by their size and biochemical properties. The 11-amino acid HIV-TAT protein transduction domain is able to cross cell membranes and the BBB, even when coupled with larger peptides. TAT-GDNF fusion protein has been shown to have a protective role in focal cerebral ischemia when delivered both before and after an ischemic insult. This approach may be of clinical interest because such fusion proteins can be intravenously applied and reach the ischemic brain regions.

Insulin-Like Growth Factor

Insulin-like growth factor (IGF) is of considerable interest because it is not only a potent neuroprotective trophic factor but also a survival factor for cells of the oligodendrocyte lineage, and possesses a potent myelinogenic capacity. It has been investigated for neuroprotective effect in several neurological disorders: MS, amyotrophic lateral sclerosis, and animal models of cerebral ischemia. The IGF system is complex and includes not only IGF-1 and IGF-2 and their receptors but also modulating IGF-binding proteins (IGFBPs), of which six have been identified.

The potential involvement of IGF-I in brain diseases associated with neuronal death is strongly supported by its neuroprotective role. Furthermore, the unexplained high incidence of glucose metabolism dysregulation in brain diseases makes insulin also a strong candidate in neuropathological research. Because mounting evidence suggests a complementary role of insulin and IGF-I in the brain,

unveiling the cellular and molecular pathways involved in brain insulin/IGF-I actions helps to establish potentially new therapeutic targets, and its exploitation may lead to new treatments for a wide array of brain diseases (Davila et al. 2007).

Nerve Growth Factor

NGF is the prototype of a target-driven neurotrophin. It is essential for the development and differentiation of peripheral sympathetic and neural crest-derived sensory nerve cells. NGF also plays a role in the CNS as a trophic agent for basal forebrain cholinergic neurons. NGF binds to TrkA receptor and triggers the activation of numerous signaling cascades, which play critical roles in neuronal plasticity, survival, and neurite outgrowth. NGF is internalized by binding to the TrkA receptor and travels by retrograde axonal transport of sensory and sympathetic neurons to reach the cell body. There, it activates the second messenger system to influence transcription of several genes. Altered amounts of specific proteins are transported by anterograde transport in the axons. Various agents that regulate NGF are as follows:

- Cytokines mediate injury-induced rise of NGF mRNA.
- Glucocorticoid hormones decrease NGF mRNA levels in glial cells, but increase it in neurons.
- Vitamin D3 increases NGF mRNA.
- Serum is a powerful stimulator of NGF synthesis in both cell and organ cultures.
- Depolarization due to high extracellular concentrations of potassium increases NGF mRNA.
- Propentofylline (xanthine derivative) upregulates NGF in cultured neurons.

NGF exerts a modulatory role on sensory nociceptive nerve physiology in the adult brain and appears to correlate with hyperalgesic phenomena occurring in tissue inflammation. In addition to its classical actions in the nervous system, NGF maintains a balanced interplay between the nervous, immune, and endocrine systems. Examples of NGF interaction with the neuroendocrine system are as follows:

- Stress that activates hypothalamic–pituitary–adrenocortical axis raises circulating NGF levels.
- Conversely, administration of NGF can activate the hypothalamic–pituitary–adrenocortical axis and increase circulating corticosteroid levels.
- Using NGF antibody treatment to neutralize circulating NGF blocks the stress-induced rise of circulating glucocorticoid level.
- NGF deprivation during development leads to deficits in thyroid and adrenal functions.

The potential of NGF as a treatment for degenerative neurologic disorders and peripheral neuropathies is being explored. Intracerebroventricular NGF administration has been shown to induce complete recovery of cortical muscarinic receptors

in rat models of cerebral infarction. It is not clear if this effect arises from protection of the cholinergic pathway or from an independent action of NGF on cortical muscarinic receptors. There is no evidence of functional recovery, and the use of NGF for stroke remains questionable. However, NGF has been shown to be useful in AD.

Neurotrophins

The family of the neurotrophins, which also includes NGF and BDNF, is well known to enhance the survival and to stabilize the phenotype of different populations of neurons in the nervous system. Neurotrophin-3 (NT-3) is expressed in noradrenergic neurons of the locus coeruleus and acts as a trophic factor for these neurons. NT-3 prevents degenerative changes in striatal projection neurons after excitotoxicity *in vivo* and has the potential to treat HD. Adeno-associated viral vector-mediated NT-3 gene transfer in the injured adult rat spinal cord has been shown to improve hind-limb function, indicating potential applications in the treatment of SCI.

NT-4/5 includes NT-4 and NT-5, which were originally described separately but are considered to be similar in several respects. The biological actions of NT-4/5 are believed to be mediated by trkB receptor tyrosine kinase. The mRNA for trkB is expressed in hippocampal cells *in vivo*, and may play a neuroprotective role against excitotoxic damage in the hippocampus and the cerebral cortex by enhancement of the neuronal calcium homeostatic system. NT-6 has a spectrum of actions similar to those of NGF on experimental animal models, although it is less potent. Interaction of NT-6 with heparin-binding molecules may modulate its action in the nervous system.

The neurotrophin signaling network is critical for the development and survival of many neuronal populations. Cholinergic neurons in the basal forebrain are progressively lost in AD as they are particularly sensitive to imbalances in the neurotrophin system. Therapeutic use of neurotrophins to prevent this loss is hampered, however, by a number of pharmacological challenges. These include a lack of transport across the BBB, rapid degradation in the circulation, and difficulty in production.

Osteogenic Protein-1

Osteogenic protein-1 (OP-1) is a member of TGF- β superfamily and plays an important role in the formation of new bone. OP-1 is a powerful regulator of genes for neural cell adhesion molecules and promotes the development of adrenergic phenotypes in neural cell cultures. It induces dendritic growth in rat sympathetic neurons in the presence of NGF as a cofactor. In the presence of optimal concentration

of NGF, OP-1-induced dendritic growth from cultured perineural neurons is comparable to that observed in situ. OP-1 enhances recovery of motor function following stroke in an animal model.

Pigment Epithelium-Derived Factor

Pigment epithelium-derived factor (PEDF) is a NTF that aids the development, differentiation, and survival of neural retina and motor neurons of the human spinal cord. PEDF has been tested in a postnatal culture model of motor neuron degeneration and shown to be highly neuroprotective. The morphological appearance and number of motor neurons were preserved. The level of motor neuron choline acetyltransferase (ChAT) was maintained but not increased, indicating that the observed effect was neuroprotective and not merely an upregulation of motor neuron ChAT. PEDF has the potential for further development as a therapeutic agent for motor neuron diseases such as ALT.

Transforming Growth Factor- β 1

Transforming growth factor- β 1 (TGF- β 1) has been shown to be protective in animal models of cerebral ischemia. The possible mechanism of this effect is by counteraction of glutamate neurotoxicity and oxidative stress by increase of bcl-2 protein expression. Further studies are warranted to determine if activation of TGF- β 1 after ischemic stroke would be of therapeutic benefit. OP-1 (related to TGF- β 1) also shows promise as a novel therapeutic agent for stroke.

Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF), identified originally as an angiogenic and vascular permeability factor, is also a neurotrophic and neuroprotective factor. VEGF can also stimulate neurogenesis. Taken together with prior evidence for survival promoting, protective effects of VEGF on neurons suggest that VEGF regulates a broad range of functions in neurons because neurons express some of the same VEGF receptors and VEGF receptor-coupled signal transduction pathways that are found in the endothelium. Neurogenesis and angiogenesis appear to be mechanistically linked, and VEGF might provide such a linkage. The ability of VEGF to stimulate the proliferation of neuronal precursors may be of interest in the context of lesion-induced neurogenesis, which occurs after excitotoxicity, seizures, oxidative stress, and cerebral ischemia.

Neurotrophic Factor-Related Neuroprotective Agents

Some agents modulate the expression of the neurotrophins themselves, while others act by enhancing neurotrophin action. Retinoids, AMPA receptor agonists, SSRIs, serotonin/norepinephrine reuptake inhibitors, and tricyclic antidepressants all increase neurotrophin expression. Among the agents that act by enhancing neurotrophin action, xaliproden is a combined NGF potentiator and serotonin 5-HT_{1A} receptor agonist that also reduces chemotherapy-induced peripheral sensory neuropathy but has not yet shown efficacy in cognitive decline in AD. Its ability to enhance NGF action in vitro is mimicked by tyrosine kinase inhibitors. NO donors also contribute to neuronal survival through transactivation of TrkA. The protective effect requires cyclic GMP and protein kinase G, but the downstream target is not known. Activation of G-protein coupled receptors by adenosine, or pituitary adenylate cyclase activating peptide (PACAP), also leads to transactivation of TrkA, stimulation of downstream signaling, and neuroprotection.

Amitriptyline as a TrkA and TrkB Receptor Agonist

Amitriptyline, an antidepressant drug, directly binds TrkA and TrkB and triggers their dimerization and activation (Jang et al. 2009). Amitriptyline, but not any other tricyclic or SSRI antidepressants, promotes TrkA autophosphorylation in primary neurons and induces neurite outgrowth in PC12 cells. Amitriptyline binds the extracellular domain of both TrkA and TrkB and promotes TrkA–TrkB receptor heterodimerization. Truncation of amitriptyline-binding motif on TrkA eliminates the receptor dimerization by amitriptyline. Administration of amitriptyline to mice activates both receptors and significantly reduces kainic acid-triggered neuronal cell death. Inhibition of TrkA, but not TrkB, abolishes amitriptyline's neuroprotective effect without impairing its antidepressant activity. Thus, amitriptyline acts as a TrkA and TrkB agonist and possesses marked neurotrophic activity.

Colivelin

A neuroprotective peptide named colivelin has been constructed by attaching activity-dependent neurotrophic factor (ADNF) to the N-terminus of a potent Humanin derivative, AGA-(C8R)HNG17 (Chiba et al. 2007). HN was originally identified from an AD brain as an endogenous neuroprotective peptide that suppresses AD-relevant toxicity. Colivelin protects neurons from death relevant to neurodegenerative diseases by activating two independent prosurvival signals: an ADNF-mediated Ca²⁺/calmodulin-dependent protein kinase IV pathway and an HN-mediated STAT3 pathway. Intracerebroventricular colivelin dose-dependently improved motor performance and prolonged survival of G93A-SOD1 transgenic ALS mice, indicating that it is a promising treatment for ALS.

Gambogic Amide

Gambogic amide is the major active ingredient of gamboge, a traditional Chinese medicine. To mimic NGF functions pharmacologically, a high-throughput screening assay was developed to identify small-molecule agonists for TrkA receptor (Jang et al. 2007). The most potent compound, gambogic amide, selectively binds to TrkA, but not TrkB or TrkC, and robustly induces its tyrosine phosphorylation and downstream signaling activation, including Akt and MAPKs. Furthermore, it strongly prevents glutamate-induced neuronal cell death and provokes prominent neurite outgrowth in PC12 cells. Gambogic amide specifically interacts with the cytoplasmic juxtamembrane domain of TrkA receptor and triggers its dimerization. Administration of this molecule in mice substantially diminishes kainic acid-triggered neuronal cell death and decreases infarct volume in the transient MCAO model of stroke. Thus, gambogic amide might not only establish a powerful platform for dissection of the physiological roles of NGF and TrkA receptor but also provide effective treatments for neurodegenerative diseases and stroke.

Inosine

The purine nucleoside inosine has been shown to induce axon outgrowth from primary neurons in culture through a direct intracellular mechanism. Inosine applied with a minipump to the rat sensorimotor cortex has been shown to stimulate extensive sprouting of the axons of the intact pyramidal cells into the denervated spinal cord white matter and adjacent neutrophil. Axon growth was visualized by anterograde tracing with biotinylated dextran amine and by immunohistochemistry with antibodies to GAP-43. In adult rats with unilateral cortical infarcts, inosine stimulated neurons on the undamaged side of the brain to extend new projections to denervated areas of the midbrain and spinal cord. This growth is paralleled by improved performance on several behavioral measures. Thus, inosine, a naturally occurring metabolite without known side effects, might help to restore essential circuitry after injury to the CNS. Inosine is in preclinical development for SCI, TBI, and stroke by Alseres Pharmaceuticals Inc., and there are plans for clinical trials.

Meteorin

Meteorin (NsGene) is a novel neurotrophic protein with protective and regenerative effects on the nervous systems. Several molecular tools such as recombinant protein, polyclonal antibodies, and lentiviral vectors have been generated to study this novel therapeutic factor in detail. In mouse primary ventral mesencephalon (VM) cultures, meteorin has been shown to increase the number of neurons compared with normal growth medium. The presence of meteorin specifically increases the number of dopaminergic cells and the level of dopamine. The effect on the dopaminergic system has been supported by experiments with immortalized human

VM cell lines, where the presence of meteorin increased the number of dopaminergic neurons by two- to threefold. In parallel with the studies on the dopaminergic system, the therapeutic effects of meteorin have been evaluated in several predictive cell culture systems and animal models for neurological disorders. Meteorin plays important roles in both glial cell differentiation and axonal network formation during neurogenesis. Genzyme Corporation has been granted the exclusive right to evaluate meteorin for the treatment of ALS.

Oxygen-Regulated Protein 150 kDa

Oxygen-regulated protein 150 kDa (ORP150) is a novel endoplasmic reticulum-associated chaperone induced by hypoxia/ischemia. Although ORP150 is sparingly upregulated in neurons from human brain undergoing ischemic stress, there is robust induction in astrocytes. Cultured neurons overexpressing ORP150 are resistant to hypoxemic stress, whereas astrocytes with inhibited ORP150 expression are more vulnerable. Mice with targeted neuronal overexpression of ORP150 have smaller strokes compared with controls. Neurons with increased ORP150 demonstrate suppressed caspase-3-like activity and enhanced BDNF under hypoxia signaling. These data indicate that ORP150 is a neuroprotective agent and that it exerts this effect by increasing the availability of BDNF in the extracellular space.

Prosaptide

Prosaptide TX14, a prosaposin-derived peptide, is both a precursor of sphingolipid activator proteins and a secreted neurotrophic and myelinotrophic factor. It protects peripheral nerves in diabetic neuropathy and also enhances nerve regeneration (Jolivald et al. 2008). Prosaptide is BBB permeable and has also been shown to reduce brain infarct area in a rat model of reversible MCAO.

Siagoside

Gangliosides are naturally occurring complex glycopospholipids that are important components of mammalian cell membranes, particularly those of the nerve cells. Siagoside (Fidia Pharmaceutical's Sygen), a monosialoganglioside (GM1), functions as a possible NTF in noradrenergic, serotonergic, cholinergic, and dopaminergic systems. There was some evidence for a synergistic effect of gangliosides on NGF-induced neuronal cell recovery and synaptogenesis. The sequence of events may include receptor–ligand interactions, ion channel modulation, or posttranslational modification of proteins. Following neurological injury, GM1 is thought to block calcium influx into neurons. It is likely that interactions between NGF and gangliosides occur as a consequence of their incorporation into cell membranes and enhancement of cell repair. Exogenously administered GM1 has been shown to enhance recovery from cerebral ischemia. Siagoside may stimulate or accelerate

the repair of neurons after CNS damage. Clinical applications are being explored as follows:

- Improved function has been reported in patients with Parkinson's disease and clinical trials are in progress.
- A randomized phase II trial of GM1 in patients with SCI showed enhanced recovery of motor function, and phase III trials are now in progress.
- Clinical trials in AD failed to show any benefit from GM1.
- The results of a few open trials during the past decade suggest some beneficial effect of GM1 in stroke patients. A randomized, placebo-controlled, double-blind, multicenter study examined the effect of a loading dose of GM1 within 5 h of onset of an ischemic stroke. The findings suggested that GM1 use was safe and that its efficacy in ischemic stroke was greater when given soon after the onset of stroke. Protocol-defined primary parameters, however, failed to show convincing evidence for the efficacy of GM1 treatment. There is no further exploration of GM1 for treatment for stroke.

Small-Molecule Activators of the Trk Receptors

Some studies have focused on the asterriquinone class of compounds (Webster and Pirrung 2008). The asterriquinones are naturally occurring bis-indolyl-dihydroxyquinones that were originally identified as activators of the insulin receptor. The molecules are small and readily cell permeable, and act directly on the receptor tyrosine kinase domain, although its mechanism of activation is not known. The original compound, demethylasterriquinone-B1 (DAQ-B1), was demonstrated to cross the BBB and activate hypothalamic signaling when given orally. Similar compounds have the very important additional advantage of targeting the kinase domain of TrkA and, therefore, not activating the p75NTR receptor. One of these, 5E5, is a potent activator of TrkA and a partial activator of TrkC, but does not appreciably stimulate TrkB or the insulin receptor. At low doses, it is able to potentiate the effect of NGF to activate TrkA and downstream signaling. 5E5 enhances neurite outgrowth and neuronal differentiation in the presence of a low dose of NGF, which alone is inefficient at promoting neurite outgrowth.

Peptidomimetics offer greater specificity, but are less readily delivered. Small-molecule neurotrophin mimetics are easily delivered, but less selective, and may have non-Trk-mediated side effects. In the end, molecules potentiating the effect of endogenous neurotrophins are particularly appealing as this may avoid the potential complications of systemic neurotrophin stimulation.

Nicotine and Nicotinic Receptor Agonists

Nicotine and nicotine-like compounds have been explored for therapeutic purposes on the assumption that they have beneficial effects on learning and memory. This was supported by the epidemiological studies, which showed that smokers had a lower

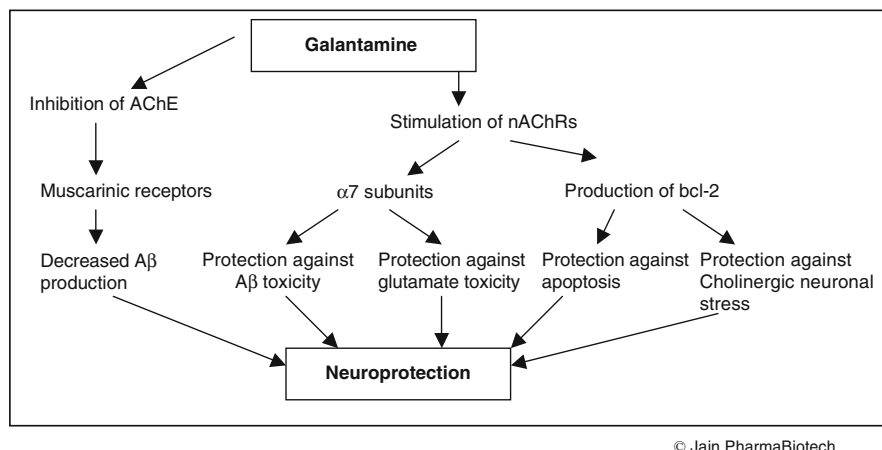


Fig. 2.2 Neuroprotective effect of galantamine

incidence of disorders such as AD and PD. Nicotine, however, is not a good candidate as it has undesirable effects on the body function and attempts have been directed to the study of nicotinic acid receptor agonists. Nicotine activates all neuronal nicotinic receptor types, including those that cause side effects. By designing novel nicotinic drugs that interact selectively with some, but not all, types of neuronal nicotinic receptors, it is possible to target only those receptors that are relevant to a given disease.

Nicotinic acetylcholine receptors (nAChRs) are located in neuromuscular junctions, autonomic ganglia, and the CNS. These ion channels are activated by nicotine with the influx of Na^+ and Ca^{2+} followed by depolarization. Those located in the CNS are relevant to the discussion of neuroprotection. At least 12 subunits, 8 α and 4 β , have been identified. There is 50% loss of these receptors with aging and even further loss in AD. Allosteric sensitization of nAChRs is considered to be a novel treatment strategy for AD. Galantamine, a ChE inhibitor approved for the treatment of AD, has a higher efficacy in improving cognitive impairment than other ChE inhibitors – donepezil and rivastigmine – as it is the only one of these drugs that acts as an allosterically potentiating ligand of the $\alpha 4/\beta 2$ nAChR. Preclinical studies show neuroprotective effect of galantamine in AD, especially in models related to glutamate and $\text{A}\beta$ toxicity in vitro and to cholinergic stress in vivo. These effects occur by upregulation of the protective protein bcl-2 and are mediated via $\alpha 7$ nAChRs as shown in Fig. 2.2.

A number of pharmaceutical companies have worked to discover and develop nicotine-like therapeutic compounds. However, no studies done so far have demonstrated that these compounds are effective in treating these diseases.

Nitric Oxide-Based Neuroprotection

The discovery that nitric oxide (NO) has powerful vasoactive properties identical to those of endothelium-derived relaxing factor spawned a vast body of research investigating the physiological actions of small gas molecules. In the nervous system,

NO can play different roles: as a neurotransmitter or an endogenous neuroprotective or a neurotoxin. Because NO differs from most conventional neurotransmitters in that it is not stored in synaptic vesicles, the synthesis of NO must be capable of rapid modulation. NO, which arises endogenously through the action of NOS enzymes, plays important roles in the regulation of vascular and immune function.

Nitric Oxide Synthase Inhibitors

The involvement of NO during stroke has been demonstrated in various animal models of stroke. During pathologic conditions such as stroke, calcium overload causes prolonged activation of NOS. This results in an excess amount of NO release, which causes neural damage. Stroke damage is reduced in neuronal NOS (nNOS) knock-out mice, i.e., mice that lack the gene to produce nNOS. Nonselective NOS inhibitors may worsen stroke damage, presumably through the inhibition of endothelial NOS (eNOS) which decreases cerebral blood flow. Selective inhibition of nNOS can be achieved by disrupting certain interactions between nNOS and nNOS-associated proteins – PIN-1 (Protein which Inhibits NOS) and CAPON (carboxyl-terminal PDZ ligand of nNOS) – which are required for nNOS activation and subsequent NO production. Selective nNOS inhibitors can be considered as neuroprotectives for neurological disorders in which NO and other neurotoxins play a significant part in eliciting neuronal death. These include (1) ischemic cerebrovascular disorders; (2) reperfusion injury; (3) traumatic brain and spinal cord injuries; and (4) neurodegenerative disorders.

Immunoreactive inducible NOS (iNOS) has been detected in brains of mice with AD-like disease resulting from transgenic expression of mutant human amyloid precursor protein (hAPP) and presenilin-1 (hPS1), indicating that iNOS contributes to AD pathogenesis (Nathan et al. 2005). Deficiency of iNOS substantially protected the AD-like mice from premature mortality, cerebral plaque formation, increased A β levels, protein tyrosine nitration, astrogliosis, and microgliosis. A β , a hallmark of AD, may serve as an irritant, “fooling” the body into thinking that an infection-like process is underway and spurring iNOS to begin producing NO. Thus, iNOS seems to be a major instigator of A β deposition and disease progression. Inhibition of iNOS may be a therapeutic option in AD. The findings are preliminary, but pharmaceutical companies have already developed agents that may safely inhibit iNOS as potential treatments for inflammatory disorders such as MS or rheumatoid arthritis.

Nitric Oxide Mimetics

NO mimetics have been investigated for neurological disorders. They have broader implications of cholinergic and noncholinergic neurotransmission, and bypass the synaptic receptors to activate directly the signal transduction cascades that are

triggered by these mechanisms. This approach targets an intracellular pathway that involves NO, soluble guanylyl cyclase (sGC), and cyclic guanosine monophosphate (cGMP).

Bilateral depletion of neocortical and hippocampal ACh produces deficits in a spatial learning task and in a delayed visual matching-to-sample task in rats with brain lesions similar to AD in humans. Oral administration of the novel nitrate, GT1061, and the AChE inhibitor, donepezil, reversed the cognitive deficits in both memory tasks in a dose-dependent manner (Bennett et al. 2007). GT1061 was superior in the delayed matching-to-sample task. GT1061 was absorbed rapidly after oral administration, crossed the BBB, and achieved brain concentrations that were slightly higher than those found in plasma. The activity of GT1061 was NO mimetic: sGC was activated, but selectivity was observed for sGC in the hippocampus relative to the vasculature; and hippocampal levels of phosphorylated ERK1/2, which is a postulated intermediary in the formation of long-term memory, were increased. The beneficial effect on visual and spatial memory task performance supports the concept that stimulating the NO/sGC/cGMP signal transduction system can provide new, effective treatments for cognitive disorders. This approach may be superior to that of currently available cholinesterase inhibitor drugs that attempt only to salvage the residual function of damaged cholinergic neurons. However, no further commercial clinical development has taken place.

Nootropics

The term nootropic was coined from the Greek words “noos” (mind) and “tropos” (turn toward). The primary effect of these drugs is enhancement of the cognitive function and the main site of action is the telencephalon. These drugs act as metabolic enhancers of the CNS and facilitate the utilization of glucose, transport of oxygen, and turnover of energy (ADP/ATP balance). The term is sometimes extended to include other actions such as neuroprotection. The ideal nootropic should have the following properties:

1. Improvement of cerebral metabolism demonstrable in humans.
2. Cell protection against hypoxia.
3. No disturbing influence on the basic EEG rhythm.
4. Minimal side effects and good tolerance by the elderly population.
5. No stimulating, sedative, or tranquilizing effects.
6. No primary vasoactive action.

The best known nootropics belong to the pyrrolidone family of chemicals and have been the subject of research for more than three decades. The lack of other generally adverse psychopharmacological actions (e.g., sedation, analgesia, or motor or behavioral changes) distinguishes the pyrrolidones from other psychoactive drug classes. The mechanisms of action of these drugs are still not fully established; indeed, different compounds in this class may have different modes of action.

Experimental and clinical work first focused on their nootropic effects and later on the possibilities for neuroprotection after stroke and use as antiepileptic agents. Piracetam was the first compound of this class. A related compound, levetiracetam, is a major new AED.

Piracetam

Piracetam has been a subject of recent interest because of its anti-myoclonic action and effects after stroke and in mild cognitive impairment. Piracetam, a cyclical derivative of GABA, has the following pharmacological effects:

1. Increases cerebral energy metabolism.
2. Accelerates hippocampal ACh turnover.
3. Increases the cAMP concentration in the brain.
4. Protects against the effects of cerebral ischemia/hypoxia.
5. Improves microcirculation.

Piracetam has a special affinity for the brain and crosses BBB easily. The drug is approved for use in several countries but not in the USA. Clinical trials in AD failed to show its efficacy and phase III clinical trials for stroke are still ongoing.

Nutraceuticals and Food Constituents

The dividing line between foods and drugs is becoming increasingly blurred, but the term “nutraceuticals” usually refers to nutritional agents used for therapeutic purposes. Several dietary supplements and other substances of natural origin are available at the counter with claims of protecting the brain or enhancing brainpower. Some of the vitamins and other agents have been incorporated to pharmaceutical products and developed as such. Some examples are (1) creatine produced naturally in the body and also used as a food supplement, (2) nicotinamide, (3) curcumin found in the curry, (4) green tea, and (5) resveratrol found in grapes and red wine.

Creatine

Creatine (creatine monohydrate) is an amino acid produced naturally in the body from the proteins arginine, glycine, and methionine. Creatine is converted (phosphorylated) to phosphocreatine (PCr) by the enzyme creatine kinase. PCr in turn buffers the ATP concentration in cells, the basic energy source for living cells. ATP is formed when creatine combines with ADP. When the energy in ATP is used, it

becomes ADP again and needs to be regenerated back to ATP by phosphocreatine. PCr regenerates ADP by transferring a phosphate group to it. Creatine supplementation increases PCr levels, which in turn increase ATP levels that nourish the brain. Creatine breaks down into creatinine, a waste product processed by the kidneys and excreted in the urine.

Creatine kinase and its substrates creatine and phosphocreatine constitute an intricate cellular energy buffering and transport system connecting sites of energy production (mitochondria) with sites of energy consumption, and creatine administration stabilizes the mitochondrial creatine kinase and inhibits the opening of the mitochondrial transition pore. Creatine, one of the most common food supplements used by individuals at almost every level of athleticism, promotes gains in performance, strength, and fat-free mass. Recent experimental findings have demonstrated that creatine affords significant neuroprotection against ischemic and oxidative insults.

In experimental studies, creatine significantly attenuated striatal excitotoxic lesions produced by NMDA, but had no effect on lesions produced by AMPA or kainic acid. Nicotinamide combined with creatine produced significantly better neuroprotection than creatine alone against malonate-induced lesions. Creatine can, therefore, produce significant neuroprotective effects against NMDA-mediated excitotoxic lesions *in vivo*, and the combination of nicotinamide with creatine exerts additive neuroprotective effects. Creatine mediates remarkable neuroprotection in experimental models of neurodegenerative disorders. The Avicena Group is conducting phase III clinical trials of creatine for AD, PD, and ALS. Use of creatine is also being explored for treatment of cognitive impairment. Its use in TBI is described in Chap. 4.

Curcumin/Curry

Curcumin is a constituent of turmeric, a spice used in making curry. Interest in the potential neuroprotective properties of curcumin started after studies found very low levels of neurological diseases, such as AD, in elderly Indian populations. Added to this were studies confirming curcumin as a potent anti-inflammatory agent and a potent polyphenolic antioxidant.

Curcumin as a Neuroprotectant in Alzheimer's Disease

Curcumin has been reported to slow the progression of AD in mice (Lim et al. 2001). The authors tested low and high doses of dietary curcumin on inflammation, oxidative damage, and plaque pathology in the APPSw (Tg2576) mouse model of AD. Low and high doses of curcumin significantly lowered oxidized proteins and IL-1 β , a proinflammatory cytokine elevated in the brains of these mice.

With low-dose but not high-dose curcumin treatment, the astrocytic marker glial fibrillary acidic protein (GFAP) was reduced, and insoluble A β , soluble A β , and plaque burden were significantly decreased by 43–50%. However, level of amyloid precursor protein in the membrane fraction was not reduced. Microgliosis was also suppressed in neuronal layers but not adjacent to plaques.

Curcumin as a Neuroprotectant in Stroke

Curcumin has been shown to protect the brain from damage caused by MCAO in rats. This effect may be mediated through upregulation of the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), which coordinates expression of genes required for free radical scavenging, detoxification of xenobiotics, and maintenance of redox potential (Yang et al. 2009).

In another study on the rat stroke model, curcumin treatment significantly reduced infarct volume and improved neurological scores at different time points compared with the vehicle-treated group (Zhao et al. 2010). Curcumin treatment decreased malondialdehyde levels, cytochrome *c*, and cleaved caspase-3 expression and increased mitochondrial Bcl-2 expression. Inhibition of oxidative stress with curcumin treatment improves outcomes after focal cerebral ischemia. This neuroprotective effect is likely exerted by anti-apoptotic mechanisms.

Curcumin as a Neuroprotectant in Multiple Sclerosis

In Asian countries, such as India and China, where people eat more spicy foods and more yellow compounds such as curcumin, reports of MS are very rare. Preliminary studies in mice models of experimental encephalomyelitis suggest that curcumin may block the progression of MS. Mice injected with curcumin showed little or no disease symptoms, while untreated animals developed severe paralysis. Mode of action of curcumin in preventing the progression of demyelination remains unclear. It may interrupt the production of IL-12, which plays a key role in signaling immune cells to attack the myelin sheath. In view of its efficacy and apparent low toxicity, curcumin shows promise as a neuroprotective. Further studies, both experimental and clinical, are needed to prove the effectiveness in neurodegenerative disorders.

Mechanism of Neuroprotective Effect of Curcumin

Curcumin application has been shown to promote the viability of cultured rodent cortical neurons (Wang et al. 2010a). When neurons were pretreated with tyrosine kinase B (TrkB) antibody, known to inhibit the activity of BDNF, the protective effect of curcumin was blocked. Additionally, treatment with curcumin increased

BDNF and phosphor-TrkB, and both these enhancements could be suppressed by ERK and PI3K inhibitors. The administration of curcumin led to increased levels of phosphor-ERK and AKT, which were each blocked by MAPK and PI3K inhibitors. Furthermore, the curcumin-induced increase in phosphorylated cAMP-response element-binding protein (CREB) was prevented by MAPK and PI3K inhibitors. These findings led to the hypothesis that neuroprotection by curcumin might be mediated via BDNF/TrkB-MAPK/PI3K-CREB signaling pathway.

Glyceryltriacetate

Glyceryltriacetate (GTA, triacetin), an FDA approved food and drug additive, can be used as a dietary supplement to increase acetate levels in the brain several fold. It is considered to be safe for oral intake. It is an immediate source of metabolic energy for the mitochondria in the form of an acetate precursor. Supplementation with GTA has been proposed as a possible therapy for Canavan disease, based on the hypothesis that an acetate deficiency in oligodendrocytes is responsible for this fatal genetic neurodegenerative disorder caused by mutations in the gene for aspartoacylase. It is under investigation as a neuroprotective agent.

Green Tea

The active constituents in green tea are polyphenols, with an antioxidant called epigallocatechin-3-gallate, which is 25–100 times more potent than vitamins C and E. One cup of green tea may provide 10–40 mg of polyphenols and has antioxidant effects that are greater than a serving of broccoli, spinach, carrots, or strawberries. Tea flavonoids (catechins) have been reported to possess divalent metal chelating, antioxidant, and anti-inflammatory activities, to penetrate the BBB and to protect neuronal death in a wide array of cellular and animal models of neurological diseases. Animal studies have shown that green tea extract limits the size of stroke lesions in a dose-dependent manner when administered immediately after an ischemic episode, leading to the suggestion that green tea may have promise in the acute treatment of ischemic stroke. Intranasal administration of gallotannin, a green tea compound, has been shown to protect the brains of rats with induced strokes.

Green tea catechins as brain-permeable natural iron chelators-antioxidants have been considered as neuroprotective for the treatment of neurodegenerative disorders (Mandel et al. 2006). Green tea inhibits the activity of the enzymes AChE, BuChE, and β -secretase. Research is in progress to find out exactly which components of green tea have this beneficial effect with the aim of developing a therapeutic for AD.

Nicotinamide

Nicotinamide, an amide of vitamin B3, is the precursor for the coenzyme β -nicotinamide adenine dinucleotide (NAD⁺) and is considered to be necessary for cellular function and metabolism. Recently, interest in nicotinamide has shifted from its role as a nutrient to that as a novel neuroprotective agent. Treatment with nicotinamide enhances neuronal survival during a variety of insults such as free radical exposure and oxidative stress. The mechanism of nicotinamide-mediated neuroprotection in vitro may be in part due to the inhibition of caspase-3 and release of cytochrome *c* from mitochondria during oxygen-glucose deprivation. NAD⁺ and NADH play important roles in multiple biological processes in the brain such as neurotransmission, learning, and memory. NAD⁺ and NADH may also mediate brain aging and tissue damage in various brain illnesses. Several studies have suggested that NADH can be transported across the plasma membranes of astrocytes, and that NAD⁺ administration can markedly decrease ischemic brain injury (Ying 2007).

Resveratrol

Epidemiological studies of French red wine drinkers have shown that despite their fatty diets, red wine drinkers were protected from cardiovascular disease, a finding known among researchers as the “French paradox.” More recent epidemiological studies have linked red wine consumption with protection from aging-related disorders such as AD. Antioxidant compounds in red wine might help to protect the brain cells from apoptosis of neurodegenerative disorders. One of these, resveratrol (trans-3,4',5-trihydroxystilbene), a naturally occurring polyphenol mainly found in grapes and red wine, markedly lowers the levels of secreted and intracellular A β as it promotes intracellular degradation of A β via a mechanism that involves the proteasome. While grapes are one good source of resveratrol, eating them may not be an effective treatment for AD. Resveratrol in grapes may never reach the concentrations required to obtain the effect observed in experimental studies. It must be noted that the immense amounts of resveratrol used in animal experimental studies cannot be obtained from drinking red wine, and a pharmaceutical preparation is now available. Grapes and wine contain over 600 different components, including well-characterized antioxidant molecules. Therefore, one cannot exclude the possibility that several compounds work in synergy with small amounts of resveratrol to slow down the progression of the neurodegenerative processes in humans. Some analogs of resveratrol are 20 times more potent than the original natural compound. Research is in progress to find more stable analogs and to test them in vivo in animal models of AD.

The many putative beneficial effects of the polyphenol resveratrol include the ability to modulate NO synthesis and promote vasodilation, which thereby improves CBF. In a randomized, double-blind, placebo-controlled, crossover study on healthy adults, resveratrol administration resulted in dose-dependent increases in

CBF during task performance, as indexed by total concentrations of hemoglobin and an increase in deoxyhemoglobin, which suggested enhanced oxygen extraction (Kennedy et al. 2010).

A study has revealed that resveratrol selectively induces HO1 in a dose- and time-dependent manner in cultured mouse cortical neuronal cells and provides neuroprotection from free-radical or excitotoxicity damage (Sakata et al. 2010). This protection was lost when cells were treated with a protein synthesis or HO1 inhibitor, suggesting that HO1 induction is at least partially required for resveratrol's prophylactic properties. Furthermore, resveratrol pretreatment dose dependently protected mice subjected to an optimized ischemic-reperfusion stroke model. Mice in which HO1 was selectively deleted lost most, if not all, of the beneficial effects. Together, the data suggest a potential intracellular pathway by which resveratrol can provide neuroprotection.

Osmotic Diuretics

Osmotic diuretics are used to lower acutely raised intracranial pressure (ICP) and thus protect the brain. Two commonly used agents in neurology and neurosurgery are mannitol and frusemide. Mannitol, an osmotic diuretic, is used more frequently for this purpose. Its other use is in renal failure to preserve renal function.

Mannitol

Mannitol is used as 25% intravenous infusion to reduce raised ICP due to swelling (edema) of the brain. It acts mainly by establishing an osmotic gradient between the plasma and the brain, and thus its action depends on an intact BBB. If the BBB is damaged, administration of mannitol can produce a rebound rise in ICP. There is also evidence that mannitol reduces the water content of the swollen brain and not of the normal brain even if BBB is disrupted.

Mannitol is used for control of raised ICP of various origins including severe TBI, stroke, and hepatic encephalopathy associated with liver failure. Mannitol is sometimes dramatically effective in reversing acute brain swelling, but its effectiveness in the on-going management of severe TBI remains open to question. There is evidence that, in prolonged dosage, mannitol may pass from the blood into the brain, where it might cause reverse osmotic shifts that increase ICP. There is currently not enough evidence to decide whether routine use of mannitol in acute stroke would result in any beneficial or harmful effect. The routine use of mannitol in all patients with acute stroke is not supported by any evidence from randomized controlled clinical trials.

Several studies have demonstrated that mannitol provides a significant degree of neuroprotection when administered either before or immediately after vessel occlusion

in animal models of cerebral ischemia, which is attributed to mannitol's action in improving cerebral microcirculation and scavenging free radicals. Mannitol reduces ischemic neuronal injury most effectively when administered after, rather than before, the production of ischemia. This is consistent with a possible beneficial influence on the development of delayed cerebral edema.

Osteopontin

Osteopontin (OPN) is a secreted extracellular phosphoprotein involved in diverse biological functions, including inflammation, cell migration, and anti-apoptotic processes. Incubation of cortical neuron cultures with OPN protects against cell death from oxygen and glucose deprivation (Meller et al. 2005). The effect of OPN depends on the Arg-Gly-Asp (RGD)-containing motif as the protective effect of OPN in vitro was blocked by an RGD-containing hexapeptide, which prevents integrin receptors binding to their ligands. Osteopontin treatment of cortical neuron cultures caused an increase in Akt and p42/p44 MAPK phosphorylation, which is consistent with OPN-inducing neuroprotection via the activation of these protein kinases. Indeed, the protective effect of OPN was reduced by inhibiting the activation of Akt and p42/p44 MAPK using LY294002 and U0126, respectively. The protective effect of OPN was also blocked by the protein synthesis inhibitor cycloheximide, suggesting that the neuroprotective effect of OPN required new protein synthesis. Finally, intracerebral ventricular administration of OPN caused a marked reduction in infarct size after transient MCAO in a murine stroke model. These data suggest that OPN is a potent neuroprotectant against ischemic injury.

Oxygen Therapeutics

Oxygen, the most essential element of life on earth, is widely used therapeutically. The human body has only limited reserves of oxygen. The rational basis of therapeutic use of oxygen is hypoxia, with broad applications in respiratory and cardiovascular disorders. Physiology of oxygen and its therapeutic uses have been discussed in detail elsewhere (Jain 1989). The brain consumes about 20–25% of the total oxygen uptake of the human body even though it makes up only 2–3% of the body weight. The brain is very susceptible to the effects of hypoxia, which is the underlying pathology in many acute insults to the brain. Oxygen inhalation is used in several neurological disorders.

Oxygen is a highly neuroprotective molecule in transient focal cerebral ischemia in rats, when applied early and at high doses (Eschenfelder et al. 2008). Reducing the time to treatment enhances the degree of neuroprotection. Hyperoxia reduced the infarct volume, but inhalation of heliox (helium–oxygen mixture) further diminished the infarct volume and improved 24-h neurological deficits in a rat model of

focal ischemia. This suggests that a greater benefit may accrue from heliox therapy (Pan et al. 2007).

There is some concern, based mostly on animal experiments, that hyperoxia may add to the oxidative stress of acute ischemic and traumatic lesions. In practice, however, correction of hypoxia reduces the oxidative stress, and supplementation with antioxidant therapy further protects against the accumulation of free radicals.

Oxygen Carriers

The aim of oxygen carriers is to improve oxygen delivery to the brain and protect it from ischemia/hypoxia. Two main categories of pharmaceutical agents have been used for delivery of oxygen to the tissues: perfluorocarbons (PFCs) and hemoglobin-based oxygen carriers (HBOCs). Both are primarily blood substitutes. They can be used as oxygen carriers to the ischemic brain in stroke or help in the resuscitation of shock/trauma patients who have hemorrhagic shock and suffer from hypoxia/ischemia of the brain. Theoretically, cell-free carriers should transfer oxygen to the tissues more efficiently than red cells but so far, this has not been readily demonstrable. A third category is liposome-encapsulated hemoglobin where hemoglobin is entrapped in a bilayer of phospholipid. A modifier of heme function is also enclosed to adjust the oxygen-carrying capacity of hemoglobin. Half-life of this product can be increased by coating the liposome surface with sugar.

Hemoglobin-Based Oxygen Carriers

HBOCs have been in development for the longest period of time, dating from the early part of this century. The US Army, in its search for blood substitutes, has been the source of most of the early research in this area and only during the last decade has the industry surpassed this investment. Preparation of HBOCs involves removing the cellular capsule of hemoglobin (Hb) and modifying the structure of Hb to prevent it from degradation, to enable it to function in simple electrolyte solutions and avoid toxic effects in the body. An extensive purification process is required to remove cell fragments so that the preparation can be administered as an acellular intravenous solution, which can carry more oxygen than standard electrolyte solutions. The primary function is to replace blood loss but it can also be used to increase the transport of oxygen to tissues lacking adequate blood flow or perfusion. This is its potential application in acute ischemic stroke. HBOCs have several advantages over blood transfusions as it eliminates the risk of viral contamination and transfusion reactions (antigens are located in the cell membrane which is removed). Major applications are in shock/trauma and surgery. The best-known

commercial product in this category is diaspirin cross-linked hemoglobin (Baxter's DCLHb). Some of the limitations of these products are as follows:

- The production of Hb solutions is expensive.
- This is a new class of therapeutics and is subject to intense regulatory scrutiny.
- The duration of effect is limited, ranging from 4 to 48 h depending on the preparation, whereas the half-life of transfused blood is 34 days.

DCLHb has been shown to reduce neurological damage in animal stroke models. Hemodilution with DCLHb improves cerebral perfusion pressure and ICP without decrease in lesion volume. Results of a phase II trial in acute ischemic stroke showed that there were more deaths and adverse events in the treatment group than that in controls. Further development of the product for this indication was discontinued.

Perfluorocarbons as Oxygen Carriers

Perfluorocarbons (PFCs) are inert organic substances produced by substituting fluorine for hydrogen in specific positions within highly stable carbon chains. They are not metabolized by the body and are excreted via the lungs, urine, and feces, although a small amount may be stored in the reticuloendothelial system. The two major mechanisms underlying the efficacy of PFCs for oxygen transport and delivery are (1) a high solubility coefficient for oxygen and CO₂ and (2) PFCs release O₂ to the tissues more effectively because their O₂ binding constant is negligible. They have been investigated intensively over the past 30 years. PFC-based oxygen carriers, while more recent than HBOCs, have some advantages over HBOCs. They are synthetic, chemically inert, and inexpensive. PFCs have a high affinity for oxygen and carbon dioxide. PFCs can substitute for red blood cells as they transport oxygen as a dissolved gas rather than as chemically bound as in the case of HBOCs. This makes a difference in the way oxygen is presented to the tissues. It is not yet certain if PFCs can provide more oxygen to the tissues than simple oxygen inhalation. Other drawbacks are adverse characteristics such as instability, short intravascular half-life, and uncertainty concerning toxic effects.

Only one product of this category, Fluosol-DA, was approved by the FDA for use in coronary angioplasty but was subsequently withdrawn by the manufacturer because of the disappointing results in efficacy trials. Second-generation PFCs incorporate well-tolerated emulsifiers (egg-yolk phospholipids). Potential therapeutic applications include neuroprotection.

Oxygenated fluorocarbon nutrient solutions have been used for ventriculo-subarachnoid perfusion in animal models of cerebral ischemia. This system functions as an alternate vascular tree and enables the perfusate to accomplish many of the functions of blood. It has also been shown that cerebral infarction can be reduced in experimental animals by ventriculocisternal perfusion with oxygenated

perfluorooctylbromide. Ventriculocisternal perfusion with oxygenated fluorochemical emulsion following cerebral ischemia also offers the possibility of reducing elevated ICP associated with cerebral infarction in stroke models.

Hemodilution with PFCs can combine the advantages of reduced viscosity while maintaining oxygen-carrying capacity and osmotic pressure at the pre-hemodilution level. Fluoromethyloadamantane is a PFC with small particle size, low viscosity and high PFC content. Used as a hemodiluent, it increases oxygen in the diluted blood and reduces the area of infarcted tissue in experimental models of cerebral ischemia.

Another preparation is a lecithin emulsion of bis-perfluorobutyl ethylene suspended in a continuous aqueous phase that simulates CSF with albumin added as an osmotic agent. It is delivered to the patients after equilibration with a mixture of carbon dioxide and oxygen so as to obtain a physiologic pH and an elevated oxygen tension (650–680 torr). A phase I clinical trial using this product was conducted some years ago but this did not develop any further.

Oxycyte® (Oxygen Biotherapeutics Inc.), a second-generation PFC, is an oxygen-carrying intravenous emulsion that can carry five times more oxygen than hemoglobin, making it an effective means of transporting oxygen to tissues and carbon dioxide to the lungs for disposal. A study on a rat model of focal cerebral ischemia shows that Oxycyte®, administered immediately after the onset of MCAO, may exert neuroprotective effects in the early phase of brain ischemia (Woitzik et al. 2005). Cerebral oxygen tension following Oxycyte® administration was significantly increased in patients in a phase II proof-of-concept study of use of Oxycyte in TBI. It is in phase IIb clinical trials now.

Hyperbaric Oxygen Therapy

Hyperbaric oxygen (HBO) therapy is therapeutic use of oxygen under greater than atmospheric pressure. Role of HBO therapy in the management of stroke has been discussed elsewhere (Jain 2009). Rationale for the neuroprotective effect of HBO is that it relieves hypoxia, improves microcirculation, relieves cerebral edema, and protects the partially damaged tissue and progression of secondary effects of infarction. Applications in stroke and TBI will be discussed further in Chaps. 3 and 4, respectively.

Peptides

The following peptides have been investigated for their potential use as neuroprotective agents.

C3-Derived Peptide for Neuroprotection and Neuroregeneration

Anaphylatoxin C3a is a third complement component (C3)-derived peptide, the multiple functions of which include neuroprotection. C3a is generated through the activation of the complement system, and is a group of proteins in the blood that is essential for the body's immune defense. Signaling through C3a receptor positively regulates *in vivo* neurogenesis in adult mouse brain. An *in vitro* study shows that the whole brain-derived neural progenitor cells (NPCs) bind C3a in a specific and reversible manner and that C3a stimulates neuronal differentiation of NPCs (Shinjo et al. 2009). Furthermore, C3a stimulates the migration of NPCs induced by low concentrations of stromal cell-derived factor (SDF)-1, whereas it inhibits NPC migration at high concentration of SDF-1. In the same manner, C3a modulates SDF-1-induced extracellular signal-regulated kinases 1 and 2 (ERK1/2) phosphorylation in these cells. In addition, C3a has an inhibitory effect on SDF-1-induced neuronal differentiation of NPCs. These data show that C3a modulates SDF-1-induced differentiation and migration of these cells, conceivably through the regulation of ERK1/2 phosphorylation. The results provide the first evidence that the complement system also plays an important role in the repair and regeneration of the brain and that C3a regulates neurogenesis by directly affecting the fate and properties of NPCs. Although the research has been carried out using mice and cultured cells, it could lead to a new medicine for human beings, which could be given to patients who have had a stroke or other disorders that damage or destroy the nerve cells. It could be possible to use molecules that are similar to the peptide C3a to boost the formation of nerve cells and stimulate the replacement of nerve cells lost due to injury or illness.

Corticotropin-Releasing Hormone

Corticotropin-releasing hormone (CRH) is the central regulator of the hypothalamic–pituitary–adrenal stress response system and is implicated in various stress-related conditions. The role of CRH, a 41-amino acid peptide, remains controversial. Detailed investigations on the mechanisms mediating this neuroprotective effect show that activation of the adenylate cyclase pathway and induction of MAP kinase phosphorylation mediate the CRH action. CRH can afford neuroprotection against neurotoxicity up to 12 h following the insult, suggesting that CRH acts at a late stage in the neuronal death cycle, and this might be important in the development of novel neuroprotective agents in order to improve neuronal survival following the insult.

Thyrotropin-Releasing Hormone

Thyrotropin-releasing hormone (TRH, protirelin), an endogenous CNS neuropeptide, as well as its analogs, can be used to treat neurodegenerative disorders, TBI, and

intractable seizures – conditions in which excess excitatory amino acid release is involved in disease pathways. The mechanism of neuroprotection by TRH is poorly understood. However, therapy utilizing TRH and its analogs has been limited due to the difficulty of delivering the drugs to the appropriate sites in the brain in adequate amounts. Low doses of TRH and its analogs are sufficient for inhibiting glutamate release, but higher doses delivered by nanoconstructs could effectively inhibit both glutamate and aspartate release. Long-term intranasal delivery of TRH in therapeutic doses through defined nasal pathways would not produce CNS and/or endocrine side effects because of three factors:

1. Continuous TRH doses are well below those known to induce endocrine effects.
2. Significant dilution of the TRH nanoconstructs in two large volume compartments (CSF and blood).
3. Continuous metabolism of small quantities of peptide released over time in both compartments.

TRH analogs are in preclinical development as neuroprotectives in neurodegenerative diseases and TBI.

Vasoactive Intestinal Peptide

Vasoactive intestinal peptide (VIP) has been shown to prevent neuronal cell death produced by five variants of HIV-1 envelope protein (gp120). Neuroprotective action of VIP is linked in part to its release of MIP-1 α . This may be relevant to drug strategies for the treatment of AIDS dementia.

The peptide epitope of VIP that is shared with the envelope protein of HIV-1 (gp120) has been suggested as a neuroprotective intranasally delivered drug termed peptide T. A stable VIP analog, stearyl-Nle 17-VIP (SNV) interacts with specific GTP-insensitive VIP-binding sites that are abundant in the olfactory bulb and thalamus and is recognized by VIP receptors. SNV is 100-fold more potent than VIP in providing neuroprotection and could access the brain by intranasal delivery and enhance spatial memory in cholinergically deficient rats. VIP or SNV (28 amino acids) can be shortened to an active neuroprotective core of four amino acids VIP (stearyl-VIP 20–23). SNV and stearyl-VIP 20–23 might represent future neuroprotective drug candidates.

To provide neuroprotection, VIP requires the presence of glial cells. Several VIP-responsive proteins that are expressed in glial cells have been identified and termed activity-dependent neurotrophic factor (ADNF) and activity-dependent neuroprotective protein (ADNP). Short, highly potent peptides, derived from ADNF and ADNP – ADNF-14, ADNF-9, and neutrophil-activating peptide (NAP) – have potent neuroprotective and neurotrophic effects. These peptides represent a new concept in neuroprotective drug design. One mechanism by which NAP could exert

its neuroprotective actions is the activation of MAPK/extracellular signal-regulated protein kinase, the phosphatidylinositol-3-kinase (PI3K)/Akt pathways, and the transcription factor CREB signaling pathways that are important in neuronal growth and differentiation (Pascual and Guerri 2007).

Pharmacological Preconditioning

Some drugs can trigger lasting neuroprotective mechanisms that enable neurons to resist a subsequent severe insult. This pharmacological preconditioning has far-reaching implications for conditions in which blood flow to the brain is interrupted. Preconditioning with the AMPA receptor antagonist GYKI 52466 induces tolerance to kainic acid (KA)-induced toxicity in hippocampus. This effect persists well after washout of the drug and may be mediated via inverse agonism of GPCRs. Pharmacological preconditioning with low-dose GYKI imparts a significant protection against KA-induced seizures in vivo (Goulton et al. 2010). GYKI, administered at a dose of 3 mg/kg subcutaneously, 90–180 min prior to the administration of high-dose KA, markedly reduced seizure scores, virtually abolished all level 3 and level 4 seizures, and completely suppressed KA-induced hippocampal c-FOS expression. Adverse behaviors often associated with higher doses of GYKI were not evident during preconditioning. The fact that GYKI is effective at doses well below and at pre-administration intervals well beyond those in previous studies suggests that a classical blockade of ionotropic AMPA receptors does not underlie anticonvulsant effects. Low-dose GYKI preconditioning may represent a novel, prophylactic strategy for neuroprotection in a field almost completely devoid of effective pharmaceuticals. GYKI has been investigated as a neuroprotective in ALS (see Chap. 10).

Diazoxide, a mito K_{ATP} channel opener, dose dependently prolonged the latency to hypoxic depolarization. Preconditioning with the K_{ATP} channel opener diazoxide may offer effective neuroprotection during hypothermic circulatory arrest.

PPARs as Drug Targets for Neuroprotection

Peroxisome-proliferator-activated receptors (PPARs) are ligand-activated transcriptional factor receptors belonging to the so-called nuclear receptor family. The three isoforms of PPAR (alpha, beta/delta and gamma) are involved in regulation of lipid or glucose metabolism. Beyond metabolic effects, PPAR- α and PPAR- γ activation also induces anti-inflammatory and antioxidant effects in different organs. These effects explain why PPAR- α or PPAR- γ activation has been tested as a neuroprotective agent in cerebral ischemia (Bordet et al. 2006). Fibrates and other non-fibrate PPAR- α activators as well as thiazolidinediones and other non-thiazolidinedione

PPAR- γ agonists have been demonstrated to induce both preventive and acute neuroprotection. This neuroprotective effect involves both cerebral and vascular mechanisms. PPAR activation induces a decrease in neuronal death by prevention of oxidative or inflammatory mechanisms implicated in cerebral injury. PPAR- α activation also induces a vascular protection as demonstrated by the prevention of postischemic endothelial dysfunction. These vascular effects result from a decrease in oxidative stress and prevention of adhesion proteins, such as vascular cell-adhesion molecule 1 or intercellular cell-adhesion molecule 1. Moreover, PPAR activation might be able to induce neural repair and endothelium regeneration. Beyond neuroprotection in cerebral ischemia, PPARs are also pharmacological targets to induce neuroprotection in chronic neurodegenerative diseases.

Riluzole

Riluzole was approved for the treatment of ALS. In addition to its anti-excitotoxic action, riluzole protects against nonexcitotoxic oxidative neuronal injury. In addition, it appears possible that PKC inhibition may be able to explain some of its well-known channel inhibitory and neuroprotective effects (Jain 2010j). It also blocks Na⁺ channels and is neuroprotective in models of TBI or SCI. Treatment with riluzole can also increase the tolerance of spinal cord motoneurons to a period of normothermic ischemia in experimental animals. Riluzole is reported to have neuroprotective effects in animal models of PD, HD, and brain ischemia. Riluzole might exert neuroprotective effects, at least in part, via stimulation of neurotrophic factors; it stimulates synthesis of NGF, BDNF, and GDNF in cultured astrocytes. Riluzole's neuroprotective action might be partly mediated by enhancing transporter-mediated glutamate uptake (Fumagalli et al. 2008). Clinical trials for patients with PD and related diseases, comprising mainly progressive supranuclear palsy and multiple system atrophy, did not show any efficacy of riluzole.

Role of RNA Interference in Neuroprotection

RNAi or gene silencing, a refinement of antisense approach, is a cellular mechanism to regulate the expression of genes and the replication of viruses. RNAi involves the use of a double-stranded RNA (dsRNA). Once in the cell, the dsRNAs are processed into short, 21–23 nucleotide dsRNAs termed small interfering RNAs (siRNAs) that are used in a sequence-specific manner to recognize and destroy complementary RNAs. Use of RNAi is under investigation for the treatment of neurodegenerative disorders such as PD, HD, and ALS, and this will be described in sections dealing with these diseases.

Sigma Receptor Agonists as Neuroprotective Agents

The sigma receptor consists of at least two subtypes: sigma-1 and sigma-2. Sigma-1 receptor, found in the endoplasmic reticulum (ER) of cells, plays an important role in many diseases such as depression, AD, and stroke. The most prominent action of sigma-1 receptors in biological systems including cell lines, primary cultures, and animals is the regulation and modulation of voltage-regulated and ligand-gated ion channels, including Ca^{2+} , K^+ , Na^+ , and Cl^- , as well as NMDA and IP₃ receptors. Sigma-1 receptors function as “receptor chaperones.” In the steady state, sigma-1 receptors form a complex with another molecular chaperone, BiP, which prevents their activation. A decrease in Ca^{2+} in the ER causes the sigma-1 receptor to detach from BiP complex and thus becomes an activated chaperone. The activated sigma-1 receptor then binds to the inositol 1-4-5-triphosphate (IP₃) receptor, which is a very unstable protein and is easily degraded by proteasomes, but the binding of sigma-1 receptor stabilizes it. As a result of this stabilization, Ca^{2+} flows into the mitochondria via voltage-dependent anion channels, leading to the activation of the intramitochondrial tricarboxylic acid cycle, which induces cell hypermetabolization, and ultimately results in neuroprotection and neurite outgrowth. Thus, sigma-1 receptors are assumed to serve as a regulator of adenosine triphosphate (ATP) production and bioenergetics within the cell. The ability to detach BiP from sigma-1 receptors distinguishes sigma-1 agonists from antagonists. Sigma-1 receptor agonists inhibit voltage-gated ion channels, while they potentiate ligand-gated channels. The inhibition or potentiation induced by agonists is blocked by sigma-1 receptor antagonists.

Sigma-1 and sigma-2 receptors represent potential fruitful targets for therapeutic developments in combating many human diseases. Sigma-1 agonists include fluvoxamine, igmesine, pregnenolone-S, dehydroepiandrosterone (DHEA), and donepezil. Such neuroprotection and neurite outgrowth can contribute to the improvement of various neuropsychiatric diseases (Hashimoto 2009).

SIRT Group of Proteins

Human sirtuins are a family of seven conserved proteins (SIRT1–7). Protein SIRT1 delays the breakdown of axons in nerve cells mechanically cut off from the cell body or exposed to a chemotherapeutic agent. Axonal degeneration may be an active self-destructive process that the neuron activates under certain conditions. Increased activation of SIRT1 appears to block some or all of those self-destructive processes.

The neuroprotective role of reticuloendothelial system (RES) has been examined in a cellular model of oxidative stress, a common feature of neurodegeneration. RES prevents toxicity triggered by hydrogen peroxide or 6-hydroxydopamine and

this action is likely mediated by SIRT1 activation, as the protection is lost in the presence of the SIRT1 inhibitor sirtinol and when SIRT1 expression is downregulated by siRNA approach. SIRT1 activation by RES can prevent the deleterious effects triggered by oxidative stress or α -synuclein aggregation in a neuroblastoma model, while RES displays a SIRT1-independent protective action against A β 42 (Albani et al. 2009).

If nerve cells are exposed to Sirtinol, a drug that shuts down the activity of SRT proteins, the protective effect disappears even if extra NAD (which has neuroprotective effect) is applied to the nerve cells several hours before they are injured. The protective effect seems to be most strongly associated with SIRT1. The next step is to find out what genes SIRT1 is turning on and off that protect axons when the nerve cell is injured. It is possible that gene therapy approaches that increase these protective effects can delay disease in mouse models of human neurodegenerative disorders. Activation of SIRT might open the door to new ways to treat a wide range of neurodegenerative disorders, including PD, AD, ALS, various kinds of neuropathy, and MS. Nerve cell death in these disorders is often preceded by the degeneration and loss of axons. If this mechanism for delaying or preventing axonal degeneration after an injury can be activated via genetic or pharmaceutical treatments, it may delay or inhibit nerve cell death in neurodegenerative diseases.

Statins

The beneficial effect of beta-hydroxy-beta-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) in primary prevention of coronary artery disease in those with hypercholesterolemia and in secondary prevention in those with established coronary vascular disease is now well known. Statins improve blood flow, reduce coagulation, modulate the immune system, and reduce oxidative damage (van der Most et al. 2009). Statins induce several potential neuroprotective effects including an increase in cerebral perfusion, activation of survival signals, synthesis of HSP27, and increases in angiogenesis and neurogenesis (Salat et al. 2009). Neuroprotective properties of statins likely attenuate the effects of ischemia on the brain vasculature as well as parenchyma and ameliorate reperfusion injury. Additionally, statins have been shown to reduce infarct size in experimental animal models of stroke. Mechanisms of neuroprotective effects of statins are as follows:

1. Statins both upregulate endothelial NOS (eNOS) and inhibit iNOS, effects that are potentially neuroprotective. The preservation of eNOS activity in cerebral vasculature, particularly in the ischemic penumbra, may be especially important in preserving blood flow and limiting neurological loss.
2. Statins may also attenuate the inflammatory cytokine responses that accompany cerebral ischemia.
3. Statins possess antioxidant properties that likely ameliorate ischemic oxidative stress in the brain.

4. Lovastatin protects cortical neurons in a concentration-dependent manner against glutamate-mediated excitotoxicity. Lovastatin with or without glutamate and/or TNF- α increases TNF receptor 2 (TNF-R2) expression in cortical neurons. Activation of TNF-R2 signaling, which includes phosphorylation of protein kinase B (PKB)/Akt and activation of NF- κ B, protects neurons against ischemic or excitotoxic insults. It has been shown that lovastatin is neuroprotective in TNF-R1(-/-) neurons, while protection is completely absent in TNF-R2(-/-) neurons (Dolga et al. 2008). Furthermore, lovastatin-mediated neuroprotection leads to an increase in PKB/Akt and NF- κ B phosphorylation, whereas inhibition of PKB/Akt activation entirely abolishes lovastatin-induced neuroprotection. Thus, lovastatin induces neuroprotection against glutamate excitotoxicity via activation of TNF-R2-signaling pathways.

Statins have also been shown to have neuroprotective effect in acute ischemic stroke, TBI, AD, HD, and MS. These actions are described in sections dealing with these diseases.

Steroids

Steroids are synthesized in peripheral glands or centrally in the brain. The latter, named neurosteroids, exerts an important role as modulators of the neuronal activity by interacting with different receptors or ion channels. In addition to the modulation of GABA_A, NMDA, or cholinergic receptors, neuroactive steroids interact with an atypical intracellular receptor, the sigma1 protein. This receptor has been cloned in several species, and highly selective synthetic ligands are available. At the cellular level, sigma1 agonists modulate intracellular calcium mobilization and extracellular calcium influx, NMDA-mediated responses, and acetylcholine release, and alter monoaminergic systems. At the behavioral level, the sigma1 receptor is involved in learning and memory processes, the response to stress, depression, neuroprotection, and pharmacodependence. Pregnenolone, dehydroepiandrosterone (DHEA), and their sulfate esters behave as sigma1 agonists, while progesterone is a potent antagonist.

Dehydroepiandrosterone

DHEA is a fast-acting, broad-spectrum neurosteroid. In humans, DHEA is synthesized in several organs – brain, gonads, and adrenals. The human adrenal cortex produces three major classes of steroid – glucocorticoids, mineralocorticoids, and androgens – through metabolic conversion of dietary cholesterol and under ACTH regulation. It is generally believed that 7 α - and 7 β -OHDHEA, the main metabolites formed in the rat hippocampus, are responsible for the protection of neurons by hippocampal astrocytes against excitatory amino acid-induced toxicity. The potential

neuroprotective action of DHEA might be extended to include the treatment of other neurodegenerative diseases not directly linked to excitotoxicity. DHEA prevents the $A\beta_{25-35}$ -impaired survival and dendritic growth of newborn neurons through a sigma-1 receptor-mediated modulation of PI3K-Akt-mTOR-p70S6k signaling (Li et al. 2010a).

HF0220

HF0220 (Newron) is a neurosteroid that acts by activation of endogenous 7-hydroxysteroid-driven neuroprotection pathways. It protects against oxidative stress, which contributes to brain damage in ischemic stroke, TBI, and global ischemia resulting from cardio-respiratory arrest. It has a potential neuroprotective effect in chronic neurodegenerative disorders such as AD, where $A\beta$ -induced oxidative stress plays a role in the pathogenesis of the disease.

HF0220 has been shown to be a highly potent neuroprotective/cytoprotective agent within a range of accepted in vitro (hypoxia/reperfusion-induced, $A\beta$ peptide-induced, and superoxide-induced hippocampal cell death) and in vivo (focal and global ischemia-induced neuronal cell death, $A\beta$ -induced neurotoxicity, and regional ischemia-induced neurodegeneration and loss of function) models. Indeed, HF0220 was found to be effective against MCAO-induced brain damage when administration was delayed for 6 h post-occlusion, even at extremely low concentrations. This unique window of protection may be explained by the ability of exogenous 7β -hydroxysteroids to induce protective genes in potentially viable cells within the ischemic penumbra before they enter into programmed cell death. Similarly, treatment with HF0220 might reduce myocardial tissue damage in patients with pending myocardial infarction in patients with coronary heart disease.

Preclinical safety studies show that HF0220 is a well-tolerated compound. Since HF0220 is an “end product” of steroid metabolism, it does not undergo significant metabolism prior to elimination. It is considered to be a novel pharmacological intervention strategy to treat both acute and chronic neurodegenerative disorders, as well as cardiac ischemia through the activation of an (endogenous) 7-hydroxysteroid-driven cytoprotection mechanism. It is in phase II clinical trials for AD.

Sulforaphane

Sulforaphane, a naturally occurring compound, has neuroprotective effect as it stimulates the expression of cytoprotective genes in the brain. Binding of the transcription factor nuclear factor E2-related factor 2 (Nrf2) to the antioxidant response element (ARE) in neural cells results in the induction of a battery of genes that can coordinate a protective response against a variety of oxidative stressors (Kraft et al. 2004).

Nrf2-dependent genetic changes alter neuron–glia interactions, resulting in neuroprotection. Sulforaphane is an activator of this pathway. Enhancing the expression of Nrf2-driven genes also protects the BBB following brain injury (Zhao et al. 2007). Sulforaphane enhances aquaporin-4 expression and decreases cerebral edema following TBI (Zhao et al. 2005).

Tauroursodeoxycholic Acid

Tauroursodeoxycholic acid (TUDCA), a hydrophilic bile acid that is normally produced endogenously in humans at very low levels, prevents the production of reactive oxygen species and thus acts as an antioxidant. Additionally, TUDCA mitigates mitochondrial insufficiency and toxicity, and prevents apoptosis in hepatocytes and, hence, is used for the treatment of chronic cholestatic liver diseases. TUDCA reduces cytotoxicity in neurons through similar mechanisms and has been tested for neuroprotective action in experimental hemorrhagic stroke (see Chap. 3) and Huntington's disease (see Chap. 9). It is in clinical trials for ALS (see Chap. 10). Advantages of TUDCA are that it is able to cross the BBB and that it is less likely to cause side effects when given as a drug because it is an intrinsic substance. Orally administered ursodeoxycholic acid, the parent molecule, is already approved by the FDA for the treatment of primary biliary cirrhosis.

Tetanus Toxin as a Neuroprotective Agent

Tetanus toxin is a neurotoxin belonging to the same family as botulinum neurotoxins, which cause botulism. Molecules derived from tetanus toxin could also be used for therapeutic purposes. The tetanus toxin molecule has two distinct parts: one is the cause of the toxic effects and the tetanus symptoms; and the other, however, is harmless and is able to penetrate and affect the nervous system. This harmless part, called the carboxy-terminal domain, has been reproduced in large quantities in the laboratory so that tests can be carried out on its effects on the nervous system of rats. Carboxy-terminal domain of the heavy chain of tetanus toxin has been shown to act as a neuroprotector in a model of MPP^+ -triggered apoptosis (Chaïb-Oukadour et al. 2009). It inhibits serotonin from being transported through the synaptic membranes that connect the neurons. The molecule is as effective as the selective inhibitors currently used, but is more powerful, lasts longer, and is more specific. This inhibitory effect converts the molecule into what could be a drug with many uses. It would be more effective than any drug that works by selectively inhibiting the transportation of serotonin in the nerve endings. Thus tetanus toxin could be extremely useful in sublethal doses to slow the progress of neurodegenerative disorders such as PD.

Thrombolytic Agents as Neuroprotective Agents

Thrombolytic agents are used in stroke to dissolve a blood clot to restore blood circulation in cerebral arteries. Some of these agents have a neuroprotective action as well.

The best-known function of the serine protease tissue plasminogen activator (tPA) is as a thrombolytic enzyme (Jain 2010). However, it is also found in structures of the brain that are highly vulnerable to hypoxia-induced cell death, where its association with neuronal survival is poorly understood. A study has demonstrated that hippocampal areas of the mouse brain lacking tPA activity are more vulnerable to neuronal death following an ischemic insult (Echeverry et al. 2010). Sublethal hypoxia, which elicits tolerance to subsequent lethal hypoxic/ischemic injury in a natural process known as ischemic preconditioning (IPC), induces a rapid release of neuronal tPA. Treatment of hippocampal neurons with tPA induced tolerance against a lethal hypoxic insult applied either immediately following insult (early IPC) or 24 h later (delayed IPC). tPA-induced early IPC was independent of the proteolytic activity of tPA and required the engagement of a member of the LDL receptor family. In contrast, tPA-induced delayed IPC required the proteolytic activity of tPA and was mediated by plasmin, the NMDA receptor, and PKB phosphorylation. The study also found that IPC *in vivo* increased tPA activity in the cornu ammonis area 1 (CA1) layer and Akt phosphorylation in the hippocampus, as well as ischemic tolerance in wild-type but not tPA- or plasminogen-deficient mice. These data show that tPA can act as an endogenous neuroprotectant in the murine hippocampus.

Uncoupling Protein 2

Studies using genomic methods – hybridization, cloning techniques, and cDNA array analysis – have identified that increased expression of uncoupling protein 2 (UCP-2) correlates with neuronal survival (Mattiasson et al. 2003). In mice overexpressing human UCP-2, brain damage was diminished after experimental stroke and TBI, and neurological recovery was enhanced. In cultured cortical neurons, UCP-2 reduced cell death and inhibited caspase-3 activation induced by oxygen and glucose deprivation. Mild mitochondrial uncoupling by 2,4-dinitrophenol (DNP) reduced neuronal death, and UCP-2 activity was enhanced by palmitic acid in isolated mitochondria. Also in isolated mitochondria, UCP-2 shifted the release of reactive oxygen species from the mitochondrial matrix to the extramitochondrial space. This study concluded that UCP-2 is an inducible protein that is neuroprotective by activating cellular redox signaling or by inducing mild mitochondrial uncoupling that prevents the release of apoptogenic proteins.

Vaccines as Neuroprotectives

Vaccines for neurological disorders are developed not only for the prevention of infectious diseases but also for the treatment of autoimmune disorders such as MS, CNS trauma, and degenerative disorders such as AD (Jain 2010m). These disorders have important inflammatory and immune components and may be amenable to treatment by anti-inflammatory and immunotherapeutic approaches. Immunological approaches to neuroprotection are based on the concept that T cells can play an important role without causing autoimmune disorders. This could be accomplished by vaccination with a universal weak T cell-reactive antigen. T cells that home to the damaged area in the brain may modulate local microglial response and protect against neurodegeneration (Schwartz and Kipnis 2005). This concept is supported by experimental studies, but there is no product in development based on it. Vaccines for TBI are described in Chap. 4, for AD in Chap. 8, for ALS in Chap. 10, and for MS in Chap. 11.

Vitamins as Neuroprotective Agents

Vitamins play an important role in brain development and functioning (Bourre 2006). For instance, to produce energy, the use of glucose by nervous tissue implies the presence of vitamin B1; this vitamin modulates cognitive performance, especially in the elderly population. Vitamin B9 preserves brain during its development and memory during aging. Vitamins B6 and B12, among others, are directly involved in the synthesis of some neurotransmitters. Adolescents who have a borderline level of vitamin B12 develop signs of cognitive changes. In the brain, the nerve endings contain the highest concentrations of vitamin C in the human body (after the suprarenal glands). Vitamin D (or certain of its analogs) could be of interest in the prevention of various aspects of neurodegenerative or neuroimmune diseases. Vitamin E, already described in the section on antioxidants, is directly involved in the protection of nervous membranes (Jain 2010n). Vitamin B12 is described here.

Vitamin B12

Elevated homocysteine has been associated with cognitive decline and dementia onset in a number of studies, and is related to insufficient levels of vitamin B12 and folate. There is an association between decreased levels of vitamin B12 and a decline in brain volume or brain atrophy, which has been associated with AD, and it is used as a marker of the disease's progression. In Oxford Project to Investigate Memory and Aging (OPTIMA) Blood, plasma samples collected upon enrollment

and yearly thereafter were analyzed for folate, B12, homocysteine, and the vitamin B12 markers holotranscobalamin, transcobalamin saturation, and methylmalonic acid, in subjects who did not have cognitive impairment (Vogiatzoglou et al. 2008). MRI scans of the brain, cognitive assessments, and physical examinations were conducted at the beginning of the study and annually over a 5-year period. Comparison of MRI images obtained at the beginning of the study with those obtained after 5 years found a greater amount of brain volume loss among participants with low plasma levels of vitamin B12. Subjects whose plasma B12 levels at less than 308 pmol per liter were among the lowest one-third of participants had a six times greater adjusted risk of increased brain volume loss than those whose vitamin B12 levels were in the top two-thirds. Similar patterns were observed for holotranscobalamin and transcobalamin saturation. Low vitamin B12 status should be further investigated as a modifiable cause of brain atrophy and of likely subsequent cognitive impairment in the elderly population. Supplementation of dietary B12 may have neuroprotective in those with low levels of this vitamin.

Methylcobalamin (mecobalamin) is a form of vitamin B12 and differs from cyanocobalamin in that the cyanide is replaced by a methyl group. It is used in the treatment of peripheral neuropathy and is in clinical trials for amyotrophic lateral sclerosis.

Nonpharmacological Approaches to Neuroprotection

Nonpharmacological approaches to neuroprotection include environmental enrichment, physical exercise, hypothermia, hibernation induced by hydrogen sulfide, ketogenic diet, preconditioning-induced neuroprotection, and transcranial magnetic stimulation.

Environmental Enrichment

Environmental enrichment means mental stimulation by interaction with the environment including social activation. Cerebral activation by environmental manipulation in rats can lead to an increase in the thickness of the cerebral cortex and neuronal size compared with that in animals reared in impoverished environments. Environmental stimulation is well known as an influence on dendritic growth, not only during development but also in the adult brain. Mature rats, when placed in activated environments, have been shown to have a greater and more ramified dendritic tree in the cerebral cortex than the control group of rats without any external activating influence. Stimulation of the brain by social interaction may be due to increased metabolism in neurons and existing synapses, and it is also possible that some new synapses may be formed. The complexed enriched environment combined with cerebral plasticity thus provides protection against cerebral insults by stimulating new cells' birth and preventing cell death.

Mental Training

Improvement of cerebral blood flow, cerebral metabolism, and mental function in parallel with structural changes in the brain (dendritic proliferation) has been shown to result from cerebral exercise. Various systems of mental exercises (brain jogging) are available for use in the prevention of decline of mental function. It is suggested that the onset of manifestation of AD may be delayed in persons used to intensive mental activity.

Physical Exercise

It is generally believed that decline in cognitive function can be reduced by physical activity. A systematic meta-analysis of all the available prospective studies investigated the association between physical activity and risk of cognitive decline in nondemented subjects (Sofi et al. 2011). Results showed that subjects who performed a high level of physical activity were significantly protected (−38%) against cognitive decline during the follow-up and even low-to-moderate level exercise showed a significant protection (−35%). Thus there is a significant and consistent protection for all levels of physical activity against the occurrence of cognitive decline.

One study demonstrated that long-term physical activity enhanced the learning ability in a transgenic mouse model of AD and decreased the level of A β protein fragments, and could delay, or even prevent, development of AD-like pathology (Adlard et al. 2005). Compared to the sedentary animals, mice that had exercised for 5 months on the running wheels had significantly fewer plaques and fewer A β peptides in the cerebral cortex and hippocampus, approximately by 50%. Additional studies of exercised animals at 10 weeks old showed that the mechanism underlying this difference began within the first month of exercise. These results suggest that exercise in these mice may bring about a change in the way that APP is metabolized. This study shows that development of those deposits can be reduced and possibly eliminated through exercise, at least in this mouse model. Further research will help us to understand those mechanisms, to learn how much and what kind of exercise is best, and to see if these same effects occur in humans.

Hypothermia

Hypothermia is therapeutic cooling of the human body to reduce the metabolism, and its use for neuroprotection is well established. There is evidence that mild hypothermia can have neuroprotective effects by reducing the inflammatory reaction in the brain (Prandini et al. 2005). Mild hypothermia (32–33°C) for a few hours is usually used in adults as neuroprotection. Profound hypothermia (5–8°C) is

usually used for prolonged surgical procedures where total circulation to the brain is interrupted for more than a few minutes. It carries severe metabolic and cardiac complications and is rarely used. Various techniques are used for cooling, the most common of which is the use of a cooling blanket. Specialized techniques of regional cooling of the brain involve regional perfusion with cold fluids to bring down the temperature of the brain, which is difficult to achieve in practice. Hypothermia is used for neuroprotection in stroke (Chap. 3), head injury (Chap. 4), and miscellaneous hypoxic/ischemic encephalopathies and during surgery involving interruption of cerebral circulation (Chap. 11). In patients who have been fully resuscitated after cardiac arrest due to ventricular fibrillation, therapeutic mild hypothermia increases the rate of a favorable neurologic outcome and mortality. Potential applications of hypothermia include the following:

(a) Therapeutic hypothermia:

- Acute ischemic stroke
- TBI
- Hemorrhagic stroke

(b) General temperature management for neuroprotection in surgery and critical care:

- Cardiovascular surgery
- Neurological surgery

Limitations of Hypothermia

One concern is that additional stress imposed on the body during hypothermia seems to reduce the beneficial effect. The stress can be eliminated by using a proper technique and avoiding sudden and marked drops in temperature.

Many ongoing clinical trials try to reduce brain temperatures through cooling units incorporated into hats or other devices that surround the head. However, in most patients, such techniques will be unable to defeat the natural temperature regulation built into the brain via the blood system. The problem has been that the core temperature of the human brain is not known and there is no way to measure it short of surgery, which is just not the same as measuring temperature in an intact brain. The first detailed measurement of the brain-temperature profile shows that it is exponential, defined by a characteristic temperature shielding length, with cooler peripheral areas and a warmer brain core approaching body temperature (Zhu et al. 2006). Direct cerebral blood flow (CBF) measurements with microspheres show that the characteristic temperature shielding length is inversely proportional to the square root of CBF, which is in excellent agreement with a theoretical model. This “temperature shielding effect” quantifies the means by which CBF prevents “extracranial cold” from penetrating deep brain structures. Attempts to use hypothermia for brain injury treatment in rats were encouraging because rats have faster metabolism and higher blood flow than humans, making their characteristic shielding

length proportionally smaller. But the rat brain is already so much smaller that this still leaves room for cooling to penetrate throughout its brain.

There is still room for fine-tuning attempts to use hypothermia to reduce brain injury. Other approaches to induce hypothermia currently under consideration include cooling the entire body all at once and inserting cooling devices into the arteries that supply the brain with blood.

Hypothermic Neuroprotection in Hypoxia-Ischemia

Severe hypoxia-ischemia does not necessarily cause immediate cell death, but can precipitate a complex biochemical cascade, leading to delayed neuronal loss. This provides an opportunity for the application of hypothermia during or after resuscitation from asphyxia at birth or cardiac arrest in adults to reduce evolving damage. Clinically and experimentally, the key phases of asphyxic injury include a latent phase after reperfusion, with initial recovery of cerebral energy metabolism followed by a secondary phase characterized by accumulation of cytotoxins, seizures, cytotoxic edema, and failure of cerebral oxidative metabolism starting 6–15 h post-insult (Gunn and Thoresen 2006). Studies have shown that moderate cerebral hypothermia initiated as early as possible before the onset of secondary deterioration, and continued for a sufficient duration in relation to the severity of the cerebral injury, is associated with potent, long-lasting neuroprotection in both adult and infants. Two large controlled trials, one of head cooling with mild hypothermia (Inder et al. 2004) and one of moderate whole-body cooling (Gluckman et al. 2005), have demonstrated that post-resuscitation cooling is generally safe in intensive care, and reduces death or disability at 18 months of age after neonatal encephalopathy. The long-term effects, at school age and later, are not yet known. The challenge for the future is to find ways of improving the effectiveness of treatment.

Hibernation Induced by Hydrogen Sulfide

Hydrogen sulfide (H_2S) gas is an environmental toxin but small amounts are produced in the body naturally, which helps regulate normal body temperature by adjusting the amount of oxygen that cells burn to produce energy. Scientists at the University of Washington (Seattle, WA) have reported that H_2S can induce a suspended animation-like state in a nonhibernating species, the house mouse (Blackstone et al. 2005). They showed that a small amount of H_2S can force the mice into a state of hibernation for 6 h. Within minutes of inhaling the gas, the mice became unconscious and their body temperature dropped from the normal down to $15^\circ C$ and their respiratory rate slowed to fewer than 10 per minute, down from a normal of 120 breaths a minute. Overall, their metabolic rate dropped by 90%, i.e., the normal cellular activity slowed to almost a standstill, thus reducing the need for oxygen. Fresh air revived the mice, and testing uncovered no differences in behavior or functional ability

between the treated and untreated mice. The next step is to see whether this hibernating state can be induced in large animals and if doing so while an animal is ill actually helps. This approach has potential for neuroprotection.

Ketogenic Diet

The ketogenic diet has been in clinical use for over 80 years, primarily for the symptomatic treatment of epilepsy. There is evidence from uncontrolled clinical trials and studies in animal models that the ketogenic diet can provide symptomatic and disease-modifying activity in a broad range of neurodegenerative disorders and may also be neuroprotective in TBI and stroke. These observations are supported by studies in animal models and isolated cells that show that ketone bodies, especially beta-hydroxybutyrate, confer neuroprotection against diverse types of cellular injury (Gasior et al. 2006). Although the mechanism by which the diet protects against seizures is unknown, there is evidence that it causes effects on intermediary metabolism that influence the dynamics of the major inhibitory and excitatory neurotransmitter systems in brain (Hartman et al. 2007). During consumption of the ketogenic diet, marked alterations in brain energy metabolism occur, with ketone bodies partly replacing glucose as fuel. It is plausible that neuroprotection results from enhanced neuronal energy reserves, which improve the ability of neurons to resist metabolic challenges, and possibly through other actions including antioxidant and anti-inflammatory effects. As the underlying mechanisms become better understood, it will be possible to develop alternative strategies that produce similar or even improved therapeutic effects without the need for exposure to ketogenic diet.

Ketone bodies can also reverse the edema associated with brain injuries. This has multiple benefits including decreased ICP and improved oxygenation of brain cells. D- β -hydroxybutyrate (8 D- β -OHB), a ketone body, has been shown to decrease cell death in models of stroke, AD and PD. Thus a number of neurologic disorders, genetic as well as acquired, might benefit by ketosis. Other beneficial effects from D- β -OHB include an increased energy of ATP hydrolysis and its linked ionic gradients. This may be significant in drug-resistant epilepsy and in injury and anoxic states. The ability of D- β -OHB to oxidize coenzyme Q and reduce NADP⁺ may also be important in decreasing free radical damage. Because of the safety record and its ability to penetrate the BBB, D- β -OHB may be a promising neuroprotective therapy for various neurological disorders.

Nonpharmacological Preconditioning for Neuroprotection

Pharmacological preconditioning was described earlier in this chapter. Various methods of neuroprotection based on nonpharmacological preconditioning are shown in Table 2.8.

Table 2.8 Methods for neuroprotection based on nonpharmacological preconditioning*Manipulation of oxygenation*

Hypoxia

Intermittent exposure to hyperbaric oxygen

Manipulation of cerebral circulation

Ischemic preconditioning

Manipulation of free radicals

Moderate stimulation of reactive oxygen species

Preconditioning by exposure to toxic chemicals

3-Nitropropionic acid, a neurotoxin in subtoxic doses

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Short and moderate episodes of sublethal stimuli such as ischemia, hypoxia, and exposure to toxic agents have been described to induce protection against subsequent severe damage. Preconditioning studies have only been conducted in animals. Decrease of O₂ availability (hypoxia) may constitute an early signal inducing tolerance. An experimental study showed that hypoxia induces tolerance against focal permanent ischemia in adult mice. DOR activation is protective during hypoxic injury. Many adaptive responses occur during exposure to hypoxia to facilitate survival. It is possible that increased activity of the DOR system is one such adaptation.

In experimental studies, preconditioning associated with stimulation of reactive oxygen species (ROS) has been performed by incubating mixed cultures of neurons and astrocytes from neonatal rat hippocampus with xanthine/xanthine oxidase, which protects the neurons against subsequent staurosporine-induced apoptosis. This involves the activation of NF- κ B and an increase in the protein level of Mn-superoxide dismutase.

As cell death activators such as ROS generation and decreased ATP can initiate tolerance, scientists have tested whether other cellular elements normally associated with neuronal injury could add to this process. Widespread caspase-3 cleavage without cell death has been observed in an in vivo model of preconditioned ischemia. In models of excitotoxic tolerance, antioxidants and caspase inhibitors block ischemia-induced protection against NMDA toxicity.

Chemical preconditioning with low dose of 3-nitropropionic acid (3-NPA) prolongs the latency to hypoxic depolarization, which triggers cell death, and also restores the synaptic transmission, which disappears during hypoxia. These neuroprotective effects are mediated by the activation of K_{ATP} channels. The extent of the chemical preconditioning with 3-NPA is the same as that achieved by pharmaceutical preconditioning with the mito K_{ATP} channel opener diazoxide.

Transcranial Magnetic Stimulation

Transcranial magnetic (TCM) stimulation applies to noninvasive stimulation of the cerebral cortex using externally applied magnetic fields. The effect of magnetic

energy on the nervous system has been investigated extensively and the physiological effects of TCM stimulation are well documented. TCM stimulation may be applied as single pulse or paired pulse. Repeated rhythmic application of TCM is called repetitive TCM (rTCM). If the stimulation occurs faster than once per second (1 Hz), it is referred to as fast rTCM. Changes may be induced in the electrochemical properties of the neurons by rTCM; these persist for some time after the termination of the stimulation. Cortical excitability increases with higher frequency pulses and decreases with lower frequency pulses. With the ability to increase or decrease cerebral activity, rTCM can partially correct overactivity or underactivity and has the potential to “tune” the cortex. Therapeutic rTCM can be used for several neurological disorders including epilepsy, PD, and MS, and in stroke rehabilitation (Jain 2005). Results of a randomized clinical trial of rTCM in patients with refractory epilepsy show that it reduces the seizure frequency and may be an alternative treatment for drug-resistant patients with clearly identifiable seizure foci in the cortical convexity such as arteriovenous malformations and who are not eligible for surgical treatment (Fregni et al. 2006). TMS is considered to be safe. Several studies suggest that TCM might be a suitable method to combine with physiotherapy and improve recovery of useful limb function in stroke patients, but further investigations are required to determine the best stimulation parameters and selection of patients who are likely to respond to this treatment (Profice et al. 2007).

In vitro studies have shown that magnetic stimulation analogous to rTMS increased the overall viability of mouse monoclonal hippocampal HT22 cells and had a neuroprotective effect against oxidative stressors, e.g., A β and glutamate. The treatment increased the release of secreted amyloid precursor protein (sAPP) into the supernatant of HT22 cells and into CSF from rats. HT22 cells preincubated with CSF from rTMS-treated rats were found to be protected against A β . These findings suggest that neurochemical effects induced by rTMS do not lead to reduced neuronal viability, and may even reduce the detrimental effects of oxidative stress in neurons. Long-term rTMS enhances the expression of BDNF, which may contribute to the neuroprotective effects of rTMS.

Electrical Fields for Improvement of Cerebral Function in Neurodegeneration

Recent studies have demonstrated that enhancing brain oscillations with applied electric fields can improve memory formation and may have application in AD. There is considerable evidence that the long-term consolidation of new memories occurs during sleep. This function of sleep has been linked to slow (<1 Hz) potential oscillations, which predominantly arise from the prefrontal neocortex and characterize slow wave sleep. Inducing slow oscillation-like potential fields by transcranial application of oscillating potentials (0.75 Hz) during early nocturnal nonrapid-eye-movement sleep, that is, a period of emerging slow wave sleep, enhances the retention of hippocampus-dependent declarative memories in healthy

humans (Marshall et al. 2006). The slowly oscillating potential stimulation induced an immediate increase in slow wave sleep, endogenous cortical slow oscillations, and slow spindle activity in the frontal cortex. These findings indicate that endogenous slow potential oscillations have a causal role in the sleep-associated consolidation of memory, and that this role is enhanced by field effects in cortical extracellular space. This finding may herald an era where external devices for electrical stimulation of the brain may be used to treat neurodegenerative diseases. Methods to enhance neuronal plasticity might help compensate for neurons lost to aging or disease. Since aging is associated with a reduction in slow wave sleep, this might be reversed with applied electric fields.

Patients with AD or other neurodegenerative disorders show remarkable fluctuations in neurological functions, even during the same day. These fluctuations cannot be caused by sudden loss or gain of nerve cells. Instead, it is likely that they reflect variations in the activity of neural networks and, perhaps, chronic intoxication by abnormal proteins that the brain is temporarily able to overcome (Palop et al. 2006). These ideas have far-reaching therapeutic implications. Imaging studies have revealed that the distribution of changes in neuronal activity in diseased brains far exceeds the regions where neurons have degenerated. The abnormal patterns of neuronal activity and network interactions, which are susceptible to pharmacological manipulation, may also be susceptible to modulation with applied electric fields and open up new opportunities to treat neurodegenerative diseases.

Neuroprotective Effect of Exercise

Various investigations in humans supported by studies on animal models suggest that exercise could have benefits for overall health and cognitive function. The neurobiological bases of these benefits include the exercise-induced increase in levels of BDNF and other growth factors, stimulation of neurogenesis, increase in resistance to brain insult, and improvement of learning and mental performance. Use of high-density oligonucleotide microarray analysis has demonstrated that, in addition to increasing levels of BDNF, exercise mobilizes gene expression profiles that are predicted to benefit brain plasticity processes and afford neuroprotection.

In an experimental study, voluntary training on running wheels or exercise on a treadmill apparatus for 3 weeks, respectively, reduced cerebral infarct size and functional deficits, improved endothelium-dependent vasorelaxation, and augmented CBF in wild-type mice (Endres et al. 2003). The neuroprotective effects of physical training were completely absent in eNOS-deficient mice, indicating that the enhanced eNOS activity by physical training was the predominant mechanism by which this modality protects against cerebral injury. These results suggest that physical activity not only decreases stroke risk, but also provides a prophylactic treatment strategy for increasing blood flow and reducing brain injury during cerebral ischemia. There is some evidence that those regularly engaged in physical exercise are less likely to show clinical manifestations of neurodegenerative diseases such as PD and AD.

Hibernation and Neuroprotection

Hibernation protects animals from starving to death. The compound that triggers hibernation in ground squirrels, delta opioid peptide [D-Ala²,D-Leu⁵]enkephalin (DADLE), also protects neurons under other adverse conditions. In one study, adult rats pretreated with DADLE prior to unilateral occlusion of the middle cerebral artery did not show any significant behavioral dysfunctions compared with saline-treated ischemic animals (Borlongan et al. 2009). Histological examination of brains from animals treated with DADLE showed almost completely intact striata in contrast to significant infarction in the lateral striatum of brains from animals that received saline. This protective effect was accompanied by significant increments in levels of GDNF in the striatum of DADLE-treated ischemic animals. These results indicate that DADLE protected against apoptotic cell death associated with ischemia-reperfusion injury. It also shows that delta opioids are crucially involved in stroke, suggesting that the opioid system is important in the study of brain injury and neuroprotection.

Suspended Animation and Neuroprotection

Oxygen deprivation is a major cause of cellular damage and death. *Caenorhabditis elegans* embryos can survive in both anoxia (<0.001 kPa O₂), by entering into suspended animation, and mild hypoxia (0.25–1 kPa O₂), through a HIF-1-mediated response, and cannot survive in intermediate concentrations of oxygen, between 0.01 and 0.1 kPa O₂ (Nystul and Roth 2004). Moreover, carbon monoxide (CO) can protect *C. elegans* embryos against hypoxic damage in this sensitive range. CO can also rescue the hypoxia-sensitive mutant hif-1(ia04) from lethality in hypoxia. This work defines the oxygen tensions over which hypoxic damage occurs in *C. elegans* embryos and demonstrates that CO can prevent this damage by inducing suspended animation. Likewise, in anoxia it may be that, although energy availability is drastically reduced, other effects of mitochondrial inefficiency, such as the formation of damaging free radicals, are also reduced because mitochondrial activity is at a minimum. These findings indicate that the brain would be protected during suspended animation.

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