

Chapter 2

Cholesterol and Its Role in Synaptic Transmission

Abstract Certain level of membrane cholesterol, which is an abundant constituent of eukaryotic membranes, is very important for normal functioning of a number of membrane proteins involved in synaptic transmission, such as ion channels, pumps, receptors, and transporters, while the alterations in cholesterol content change the property of membranes and the activity of these proteins. Moreover, cholesterol deficiency has been implicated in the pathogenesis of several neurodegenerative disorders.

Cholesterol is required to establish proper membrane permeability, fluidity, phase behavior, and thickness. Cholesterol stabilizes membranes and provides order to membranes, which are in the fluid phase, vice versa to membranes in the gel phase. Cholesterol regulates lipid chain order underlying many of the properties and functions of membrane including permeability to water and other molecules. Since cholesterol creates tighter packing of the membrane, it reduces movement of permeants (Kroes and Ostwald 1971; Crockett 1998). Many properties of biological membranes are particularly influenced by temperature. It is membrane order (increasing temperatures fluidize membranes, however decreasing temperatures order membranes) and also membrane phase transition, permeability, and thickness. Since cholesterol has pronounced effects on the physical properties of membranes, it may be used to minimize effects of temperature on membrane structure and function. Modulation of the cholesterol level could reverse, or at least ameliorate, temperature-induced perturbations in the properties of membranes (Crockett 1998).

In recent years, much attention has been focused on the role of membrane cholesterol in the regulation of the cellular functions (Subtil et al. 1999; Launikonis and Stephenson 2001; Mauch et al. 2001; Hill et al. 2002; Hering et al. 2003; Pfrieger 2003; Churchward et al. 2005; Lange et al. 2005; Rohrbough and Broadie 2005; Allen et al. 2007; Cho et al. 2007; Wasser et al. 2007). Membrane microdomains enriched with cholesterol are considered to serve as scaffolding regions, where the interface of different signal transduction pathways occurs (Martens et al. 2004).

The CNS, which is equal to 2 % of body mass, keeps a special place among other systems of the organism, because it contains approximately a quarter of total unesterified cholesterol (Dietschy and Turley 2001; Chattopadhyay and Paila 2007). It is necessary to underline that cholesterol is a prominent component of both partners of exocytotic process, i.e., the presynaptic plasma membrane and synaptic vesicles (Deutsch and Kelly 1981). The level of cholesterol determines the fluidity and curvature of the vesicle membranes (Cevc and Richardsen 1999; Zamir and Charlton 2006) and is important for the formation of the complex between synaptophysin, a major cholesterol-binding protein in brain synaptic vesicles, and synaptobrevin (Thiele et al. 2000), thereby affecting synaptic efficiency (Mitter et al. 2003).

Exocytosis, release mediated by transporter reversal, and unstimulated release and uptake of glutamate, which are tightly associated with membrane components of the cells, are not constant and subjected to regulation at multiple levels including modulation of lipid composition of the plasma membrane. Certain level of membrane cholesterol is very important for normal functioning of a number of membrane proteins involved in synaptic transmission, such as ion channels, pumps, receptors, and transporters, while the alterations in cholesterol content change the fusibility of membranes and the activity of these proteins (Fong and McNamee 1986; Jennings et al. 1999; Burger et al. 2000; Lang et al. 2001; Sooksawate and Simmonds 2001; Romanenko et al. 2002; Chou et al. 2003; Eroglu et al. 2003; Kato et al. 2003; Taverna et al. 2004; Butchbach et al. 2004; Xia et al. 2004, 2007; Dalskov et al. 2005; González et al. 2007; Zidovetzki and Levitan 2007). Localization and trafficking/internalization of neurotransmitter receptors (Fong and McNamee 1986; Burger et al. 2000; Sooksawate and Simmonds 2001; Eroglu et al. 2003) and activity of specific plasma membrane transporters (Butchbach et al. 2004; González et al. 2007) are greatly influenced by cholesterol depletion. Cholesterol is also important for the maintenance of synapse organization, processes of synaptogenesis, and synaptic vesicles recycling (Mauch et al. 2001; Hering et al. 2003; Pfrieger 2003; Salaun et al. 2004; Rohrbough and Broadie 2005; Wasser et al. 2007).

It was shown recently that cholesterol depletion differently influenced transporter-mediated glutamate uptake in mouse brain plasma membrane vesicles, primary cortical cultures, and in hippocampal astrocytes. The latter was analyzed by Tsai et al. (2006), who demonstrated that cholesterol deprivation increased uptake of a stable analogue of glutamate, D-[³H]aspartate, by glutamate transporters in hippocampal astrocyte cultures. In contrast, Butchbach et al. (2004) revealed a significant decrease in glutamate transporter activity in cholesterol-depleted primary cortical cultures as well as mouse brain plasma membrane vesicles. It was suggested that after extraction of membrane cholesterol the alterations in the functioning of glutamate transporters, which are organized in a clustered manner on the plasma membrane of neurons, might be caused by the reduction of cluster number and cluster size and also could originate from changed cell surface expression of glutamate transporters. Thus, in nerve terminals, the effect of cholesterol depletion on glutamate uptake mediated by glutamate transporters was opposite to that observed in astrocytes. Glutamate transporters of both cells more or less contribute to the maintenance of the low level of ambient glutamate (Danbolt 2001; Brasnjo and Otis 2001;

Cavelier and Attwell 2005; Beurrier et al. 2009), so the alteration in membrane cholesterol content may cause diverse changes in this level in neurons and astrocytes. To uncover different underlying mechanisms, the effects of cholesterol depletion should be examined independently in each type of the cells. Despite recent intense research and numerous experimental data on the diverse effects of cholesterol, new regulatory mechanisms are continuously uncovering and the exact role of this steroid in neuronal function and development is further elucidating (Subtil et al. 1999; Launikonis and Stephenson 2001; Mauch et al. 2001; Hill et al. 2002; Hering et al. 2003; Pfrieger 2003; Salaun et al. 2004; Rohrbough and Broadie 2005; Lange et al. 2005; Churchward et al. 2005; Salaun et al. 2005; Allen et al. 2007; Chattopadhyay and Paila 2007; Cho et al. 2007; Wasser et al. 2007).

Cholesterol-depleted brain synaptosomes, plasma membrane vesicles, rat primary cortical cultures, and cell lines are routinely used to evaluate the physiological role of cholesterol in neuronal function. The treatment with cyclodextrins, which are a family of cyclic oligosaccharides composed of a lipophilic cavity and hydrophilic outer surface, is the common methodological approach in this research. Methyl- β -cyclodextrin (M β CD), which contains seven α -(1,4) linked glycosyl units, is known as an effective cholesterol-depleting agent (Yancey et al. 1996; Rodal et al. 1999; Jadot et al. 2001; Steck et al. 2002; Singh et al. 2002; Barnes et al. 2004). It should be noted that the measurements were carried out in the presence of the acceptor in the incubation media (Yancey et al. 1996; Jadot et al. 2001; Butchbach et al. 2004; Zamir and Charlton 2006) as well as after washing of M β CD (Levitan et al. 2000; Lange et al. 2001; Taverna et al. 2004; Wasser et al. 2007). M β CD evokes cholesterol from the plasma membrane with the half-time of cholesterol transfer across the cell bilayer equaled to approximately 1 s at 37 °C (Steck et al. 2002). Biexponential kinetics of cyclodextrin-mediated cholesterol efflux was shown by Yancey et al. (1996) indicating the existence of two different pools of cholesterol in cells: a fast and slow pool with a half-time of 19–23 s and 15–30 min, respectively. It should be underlined that the fast pool of cholesterol was completely restored after 40-min recovery period (Yancey et al. 1996). Experimental data showed that the treatment of cells with M β CD not only resulted in cholesterol removal from the plasma membrane but also induced more dramatic changes in the level of cholesterol in intracellular membranes, such as lysosomal membrane and membrane of endoplasmic reticulum (Jadot et al. 2001; Lange et al. 2005; Zidovetzki and Levitan 2007). In neurochemical studies, it should be kept in mind that cholesterol is a prominent component of synaptic vesicle membrane (Deutsch and Kelly 1981). Since M β CD-mediated cholesterol extraction from the plasma membrane was a complicated multistep process followed by the redistribution and restoration of steroid (Yancey et al. 1996; Jadot et al. 2001; Steck et al. 2002; Lange et al. 2004; Zidovetzki and Levitan 2007), it may be suggested that the cellular mechanisms underlying the changes in the key processes of synaptic transmission could differ in dependence of the manner of the acceptor application. It is still unclear exactly what intracellular processes occur just after the addition of M β CD to the cells, i.e., during acute depletion of membrane cholesterol.

In aqueous solutions, cyclodextrin molecules self-assemble and form nano-sized aggregates. Based on experimental data, Miyajima et al. (1983, 1986) showed the spontaneous formation of aggregates of cyclodextrins in aqueous medium. The anomalously low solubility of β -cyclodextrins in aqueous solutions may be explained by intensive formation of aggregates, which became notable at concentrations of higher than 3 mM (Bonini et al. 2006; Messner et al. 2010). This fact creates the potential to develop sophisticated drug delivery systems with cyclodextrins (Messner et al. 2010). It was demonstrated that the aggregate formation was concentration-dependent process, which was increased with the enhancement of cyclodextrin concentration. The aggregation of cyclodextrin complexes can be driven by hydrophobic guests, especially by those guests that tend to self-aggregate themselves. Formation of guest inclusion complexes can increase significantly the tendency of cyclodextrin molecules to form aggregates. This is especially critical during drug formulation studies where concentrations of both drug and cyclodextrins are relatively high (Messner et al. 2010).

Taking into account the above data, it is suggested that cholesterol can be a potent endogenous modulator of glutamate transport in the brain. It is important because there are no substances used clinically to modulate functioning of glutamate transporters in the brain. The main questions should be asked, “How altered cholesterol composition of neuronal membrane could mediate pathogenic mechanisms responsible for the neurodegeneration and whether reduced cholesterol content of neuronal membrane can modulate pathogenic mechanisms underlying the development of neurotoxicity?”

References

- Allen JA, Halverson-Tamboli RA, Rasenick MM (2007) Lipid rafts microdomains and neurotransmitter signaling. *Nat Rev Neurosci* 8:128–140
- Barnes K, Ingram JC, Bennett MDM et al (2004) Methyl-beta-cyclodextrin stimulates glucose uptake in Clone 9 cells: a possible role for lipid rafts. *Biochem J* 378:343–351
- Beurrier C, Bonvento G, Kerkerian-Le Goff L, Gubellini P (2009) Role of glutamate transporters in corticostriatal synaptic transmission. *Neuroscience* 158:1608–1615
- Bonini M, Rossi S, Karlsson G, Almgren M, Lo Nostro P, Baglioni P (2006) Self-assembly of beta-cyclodextrin in water. Part 1: Cryo-TEM and dynamic and static light scattering. *Langmuir* 22:1478–1484
- Brasnjó G, Otis T (2001) Neuronal glutamate transporters control activation of postsynaptic metabotropic glutamate receptor and influence cerebellar long-term depression. *Neuron* 31:607–616
- Burger K, Gimpl G, Fahrenholz F (2000) Regulation of receptor function by cholesterol. *Cell Mol Life Sci* 57:1577–1592
- Butchbach M, Tian G, Guo H, Lin CG (2004) Association of excitatory amino acid transporters, especially EAAT2, with cholesterol-rich lipid raft microdomains. *J Biol Chem* 279:34388–34396
- Cavelier P, Attwell D (2005) Tonic release of glutamate by a DIDS-sensitive mechanism in rat hippocampal slices. *J Physiol* 564:397–410
- Cevc G, Richardsen H (1999) Lipid vesicles and membrane fusion. *Adv Drug Deliv Rev* 38:207–232

- Chattopadhyay A, Paila YD (2007) Lipid-protein interactions, regulation and dysfunction of brain cholesterol. *Biochem Biophys Res Commun* 354:627–633
- Cho WJ, Jeremic A, Jin H et al (2007) Neuronal fusion pore assembly requires membrane cholesterol. *Cell Biol Int* 31:1301–1308
- Chou YC, Lin SB, Tsai LH, Tsai HI, Lin CM (2003) Cholesterol deficiency increases the vulnerability of hippocampal glia in primary culture to glutamate-induced excitotoxicity. *Neurochem Int* 43:197–209
- Churchward MA, Rogasevskaja T, Hofgen J et al (2005) Cholesterol facilitates the native mechanism of Ca^{2+} -triggered membrane fusion. *J Cell Sci* 118:4833–4848
- Crockett EL (1998) Cholesterol function in plasma membranes from ectotherms: membrane-specific roles in adaptation to temperature. *Am Zool* 38:291–304
- Danbolt NC (2001) Glutamate uptake. *Prog Neurobiol* 65:1–105
- Dalskov SM, Immerdal L, Niels-Christiansen LL, Hansen GH, Schousboe A, Danielsen EM (2005) Lipid raft localization of GABA A receptor and Na^+ , K^+ -ATPase in discrete microdomain clusters in rat cerebellar granule cells. *Neurochem Int* 46:489–499
- Deutsch JW, Kelly RB (1981) Lipids of synaptic vesicles: relevance to the mechanism of membrane fusion. *Biochemistry* 20:378–385
- Dietschy JM, Turley SD (2001) Cholesterol metabolism in the brain. *Curr Opin Lipidol* 12:105–112
- Eroglu C, Bruger B, Wieland F et al (2003) Glutamate-binding affinity of *Drosophila* metabotropic glutamate receptor is modulated by association with lipid rafts. *Proc Natl Acad Sci USA* 100:10219–10224
- Fong TM, McNamee MG (1986) Correlation between acetylcholine receptor function and structural properties of membranes. *Biochemistry* 25:830–840
- González MI, Susarla BT, Fournier KM (2007) Constitutive endocytosis and recycling of the neuronal glutamate transporter, excitatory amino acid carrier 1. *J Neurochem* 103:1917–1931
- Hering H, Lin CC, Sheng M (2003) Lipid rafts in the maintenance of synapses, dendritic spines, and surface AMPA receptor stability. *J Neurosci* 23:3262–3271
- Hill W, An B, Johnson J (2002) Endogenously expressed epithelial sodium channel is present in lipid rafts in A6 cells. *J Biol Chem* 277:33541–33544
- Jadot M, Andrianavo F, Dubois F, Wattiaux R (2001) Effects of methylcyclodextrin on lysosomes. *Eur J Biochem* 268:1392–1399
- Jennings LJ, Xu QW, Firth TA (1999) Cholesterol inhibits spontaneous action potentials and calcium currents in guinea pig gallbladder smooth muscle. *Am J Physiol* 277:1017–1026
- Kato N, Nakanishi M, Hirashima N (2003) Cholesterol depletion inhibits store-operated calcium currents and exocytotic membrane fusion in RBL-2H3 cells. *Biochemistry* 42:11808–11814
- Kroes J, Ostwald R (1971) Erythrocyte membranes—effect of increased cholesterol content on permeability. *Biochim Biophys Acta* 249:647–650
- Lang T, Bruns D, Wenzel D et al (2001) SNAREs are concentrated in cholesterol-dependent clusters that define docking and fusion sites for exocytosis. *EMBO J* 20:2202–2213
- Lange Y, Ye J, Steck TL (2004) How cholesterol homeostasis is regulated by plasma membrane cholesterol in excess of phospholipids. *Proc Natl Acad Sci USA* 101(32):11664–11667
- Lange Y, Ye J, Steck TL (2005) Activation of membrane cholesterol by displacement from phospholipids. *J Biol Chem* 280:36126–36131
- Launikonis BS, Stephenson DG (2001) Effects of membrane cholesterol manipulation on excitation-contraction coupling in skeletal muscle of the toad. *J Physiol* 534:71–85
- Levitani I, Christian AE, Tulenko TN, Rothblat GH (2000) Membrane cholesterol content modulates activation of volume-regulated anion current in bovine endothelial cells. *J Gen Physiol* 115:405–416
- Martens J, O'Connell K, Tamkun M (2004) Targeting of ion channels to membrane microdomains: localization of Kv channels to lipid rafts. *Trends Pharmacol Sci* 25:16–21
- Mauch DH, Nädler K, Schumacher S et al (2001) CNS synaptogenesis promoted by glia-derived cholesterol. *Science* 294:1354–1357
- Messner M, Kurkov SV, Jansook P, Loftsson T (2010) Self-assembled cyclodextrin aggregates and nanoparticles. *Int J Pharm* 387:199–208

- Mitter D, Reisinger C, Hinz B (2003) The synaptophysin/synaptobrevin interaction critically depends on the cholesterol content. *J Neurochem* 84:35–42
- Miyajima K, Sawada M, Nakagaki M (1983) Viscosity B-coefficients, apparent molar volumes, and activity-coefficients for alpha-cyclodextrin and gamma-cyclodextrin in aqueous-solutions. *Bull Chem Soc Jpn* 56:3556–3560
- Miyajima K, Mukai T, Nakagaki M, Otagiri M, Uekama K (1986) Activity-coefficients of dimethyl-beta-cyclodextrin in aqueous-solutions. *Bull Chem Soc Jpn* 59:643–644
- Pfriege FW (2003) Cholesterol homeostasis and function in neurons of the central nervous system. *Cell Mol Life Sci* 60:1158–1171
- Rodal SK, Skretting G, Garred O, Vilhardt F, van Deurs B, Sandvig K (1999) Extraction of cholesterol with methyl-beta-cyclodextrin perturbs formation of clathrin-coated endocytic vesicles. *Mol Biol Cell* 10:961–974
- Rohrbough J, Broadie K (2005) Lipid regulation of the synaptic vesicle cycle. *Nat Rev Neurosci* 6:139–150
- Romanenko VG, Rothblat GH, Levitan I (2002) Modulation of endothelial inward-rectifier K⁺ current by optical isomers of cholesterol. *Biophys J* 83:3211–3222
- Salaun C, James DJ, Chamberlain LH (2004) Lipid rafts and the regulation of exocytosis. *Traffic* 5:1–10
- Salaun C, Gould GW, Chamberlain LH (2005) The SNARE proteins SNAP-25 and SNAP-23 display different affinities for lipid rafts in PC12 cells. Regulation by distinct cysteine-rich domains. *J Biol Chem* 280(2):1236–1240
- Singh M, Sherma R, Banerjee U (2002) Biotechnological application of cyclodextrins. *Biotechnol Adv* 20:341–359
- Sooksawate T, Simmonds MA (2001) Effects of membrane cholesterol on the sensitivity of the GABA(A) receptor to GABA in acutely dissociated rat hippocampal neurones. *Neuropharmacology* 40:178–184
- Steck TL, Ye J, Lange Y (2002) Probing red cell membrane cholesterol movement with cyclopextrin. *Biophys J* 83:2118–2125
- Subtil A, Gaidarov I, Kobylarz K et al (1999) Acute cholesterol depletion inhibits clathrin-coated pit budding. *Proc Natl Acad Sci USA* 96:6775–6780
- Taverna E, Saba E, Rowe J et al (2004) Role of lipid microdomains in P/Q-type calcium channel (Cav2.1) clustering and function in presynaptic membranes. *J Biol Chem* 279:5127–5134
- Thiele C, Hannah MJ, Fahrenholz F, Huttner WB (2000) Cholesterol binds to synaptophysin and is required for biogenesis of synaptic vesicles. *Nat Cell Biol* 2:42–49
- Tsai HI, Tsai LH, Chen MY et al (2006) Cholesterol deficiency perturbs actin signaling and glutamate homeostasis in hippocampal astrocytes. *Brain Res* 1104:27–38
- Wasser CR, Ertunc M, Liu X et al (2007) Cholesterol-dependent balance between evoked and spontaneous vesicle recycling. *J Physiol* 579(2):413–429
- Xia F, Gao X, Kwan E et al (2004) Disruption of pancreatic β -cells lipid rafts modifies Kv2.1 channel gating and insulin exocytosis. *J Biol Chem* 279:24685–24691
- Xia F, Leung YM, Gaisano G et al (2007) Targeting of Kv4, Cav1.2 and SNARE proteins to cholesterol-rich lipid rafts in pancreatic α -cells: effects on glucagons stimulus-secretion coupling. *Endocrinology* 148:2157–2167
- Yancey PG, Rodriguez WV, Kilsdonk EP et al (1996) Cellular cholesterol efflux mediated by cyclodextrins. *J Biol Chem* 271:16026–16034
- Zamir O, Charlton MP (2006) Cholesterol and synaptic transmitter release at crayfish neuromuscular junctions. *J Physiol* 571:83–99
- Zidovetzki R, Levitan I (2007) Use of cyclodextrins to manipulate plasma membrane cholesterol content: evidence, misconceptions and control strategies. *Biochim Biophys Acta* 1768:1311–1324

<http://www.springer.com/978-1-4614-7758-7>

Cholesterol and Presynaptic Glutamate Transport in the
Brain

Borisova, T.

2013, XVI, 75 p. 32 illus., 11 illus. in color., Softcover

ISBN: 978-1-4614-7758-7