

Delineation of the Physiological Role of Kainate Receptors by Use of Subtype Selective Ligands

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1. INTRODUCTION

Ionotropic glutamate receptors mediate fast excitatory neurotransmission in practically all areas of the central nervous system (CNS). They are also critical for both the induction and expression of synaptic plasticity, and have been implicated in diverse pathological conditions, such as epilepsy, ischemic brain damage, anxiety, and addiction. There are three subtypes of ionotropic glutamate receptors that are named after their high-affinity agonists as α -amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), *N*-methyl-D-aspartate (NMDA), and kainate (KA) receptors (1).

Whereas the contribution of NMDA and AMPA-type of glutamate receptors to synaptic transmission is well-characterized, much less is known about the functions of kainate receptors. This is mainly owing to significant overlap in the pharmacological profile of the AMPA and KA types of glutamate receptors. Although many antagonists and agonists, including AMPA and KA, have different affinities towards AMPA and KA receptors, few of them are selective enough for pharmacological isolation or activation of one but not the other. Therefore, native receptors have often collectively been referred to as non-NMDA or AMPA/KA receptors, which in most brain areas display properties that are characteristic for AMPA receptors. Elucidation of the physiological functions of native kainate receptors in the CNS has become possible only recently, following the development of selective pharmacological tools as well as genetically engineered mice lacking KA receptor subunits (reviewed in 2,3).

2. MOLECULAR COMPOSITION AND EXPRESSION OF KAINATE RECEPTORS

Molecular cloning techniques have identified five KAR subunits, designated GLU_{K1}, GLU_{K2}, and GLU_{K5-7} (IUPHAR nomenclature [4]; subunits formerly known as KA1, KA2, GluR5-7, respectively [5]). Kainate receptor subunits are comprised of approx 900 amino acids and are thought to share the same transmembrane topology as, and assemble with similar subunit stoichiometry to, other ionotropic glutamate receptors. Thus, each subunit contains four hydrophobic regions within the sequence (designated

MI-IV), with three believed to form transmembrane domains (MI, III, and IV) with the fourth, MII, forming a reentrant membrane loop. These subunits are believed to assemble as a tetrameric receptor. Additional variation to the primary sequence is created by alternative splicing of GLU_{K5} and GLU_{K7} as well as by RNA editing of the codon that encodes the glutamine/arginine (Q/R) site in GLU_{K5} and GLU_{K6}. Furthermore, the latter can undergo editing at two additional sites, giving rise to further diversity (reviewed in refs. 6–9).

2.1. Recombinant Receptors

Functional properties of recombinant kainate receptors have been extensively studied in heterologous expression systems, including *Xenopus* oocytes as well as mammalian cells. GLU_{K5}, GLU_{K6}, and GLU_{K7} can form functional homomeric receptors, whereas GLU_{K1} and GLU_{K2} are only expressed as heteromeric combinations with the subunits GLU_{K5–7}. The various glutamate receptor subunits can be distinguished by their agonist affinity as well as desensitization in response to different agonists. For example, GLU_{K5–7} display lower affinity (50–100 nM) to kainate than the high-affinity subunits GLU_{K1} and GLU_{K2} (~ 5 nM), whereas the affinity of the AMPA receptor subunits GLU_{A1–4} to kainate is in the range of 10^{–3}–10^{–5} M. The potency of KA receptors for glutamate is similar to that of AMPA receptors (EC₅₀ ~ 0.3 mM), except for the KA receptors containing GLU_{K7} subunits (EC₅₀ ~ 6 mM) (10).

Both AMPA and KA receptors desensitize rapidly in the continued presence of glutamate. However, these receptors can be distinguished based on their desensitization in response to kainate. KA receptors typically mediate a rapidly desensitizing current in response to kainate, whereas kainate evokes nondesensitizing agonist responses in AMPA receptors. AMPA has a low activity at homomeric GLU_{K5} but not GLU_{K6} or GLU_{K7}, a phenomenon that appears to be determined by a single amino acid residue. These responses are largely nondesensitizing. In addition, heteromeric GLU_{K2/K6}, GLU_{K1/K7a} or GLU_{K2/K7a} (at least) are activated by AMPA (10), suggesting that the introduction of a high-affinity subunit confers sensitivity. In contrast to homomeric GLU_{K5}, AMPA currents in heteromeric GLU_{K1/K7a} and GLU_{K2/K7a} receptors desensitize. Whether the introduction of GLU_{K1} or GLU_{K2} always confers sensitivity to, and desensitization in the presence of, AMPA is not known (for reviews, see 2,3,6,7).

2.2. Native Receptors

Kainate receptors are widely expressed in the brain. Early studies using receptor autoradiography identified high affinity [³H]KA binding sites throughout the nervous system, and in particularly high levels in the stratum lucidum of area CA3 in hippocampus, corresponding to the mossy-fiber nerve terminal region (11–14). Analysis of the mRNA expression of the individual KA receptor subunits by *in situ* hybridization indicated that the expression pattern of the various subunits is distinct but overlapping, and changes during development (11,15,16). In the hippocampus, expression of mRNA for all the subunits has been detected. The GLU_{K1} mRNA occurs mainly in the CA3 field of the hippocampus and dentate gyrus, whereas the GLU_{K2} gene is widely expressed in pyramidal (CA1–CA3) and dentate granule cells. GLU_{K6} is abundantly expressed in pyramidal cells and dentate granule neurons, whereas GLU_{K5} is most prominently expressed in nonpyramidal cells. However, many neurons laying outside

the pyramidal cell layer also express $\text{GLU}_{\text{K}6}$, and there are some cells within the pyramidal cell layer containing $\text{GLU}_{\text{K}5}$ transcripts (15). $\text{GLU}_{\text{K}7}$ appears largely confined to the dentate and scattered interneurons of the stratum oriens. Most patterns of expression are established during late embryonic/early postnatal development. Exceptions include $\text{GLU}_{\text{K}6}$, which develops more slowly (postnatal day 12, i.e., P12), and a temporally restricted sharp peak in $\text{GLU}_{\text{K}5}$ expression (P0-5), which declines markedly (by P12) to significant but diminished adult levels (11).

The understanding of the subcellular localization of KA receptor subunits has been hampered by the lack of specific antibodies. By using antibodies recognizing $\text{GLU}_{\text{K}6/7}$ or $\text{GLU}_{\text{K}5/6/7}$, it has been shown that kainate receptor immunoreactivity is principally localized at the postsynaptic membranes (17–19). However, in area CA3 unmyelinated axons are also stained (19). Further, using immunogold electron microscopy, expression of $\text{GLU}_{\text{K}6/7}$ was detected in glutamatergic nerve terminals in the monkey striatum (20).

3. KAINATE RECEPTOR PHARMACOLOGY

Characterization of the physiological roles of kainate receptors in central neurons has been closely linked to the development of selective pharmacological tools for AMPA vs kainate receptor antagonism (21–23). In most central neurons, the response to kainate is dominated by activation of AMPA receptors, and therefore, identification of kainate receptor function is often performed following selective antagonism of the AMPA receptors. In contrast, agonist responses similar to that described for recombinant kainate receptors have been identified in the peripheral nervous system in the dorsal root fibers (24) and in the trigeminal ganglia (25). Dorsal root ganglion (DRG) neurons have been widely used as a model for native KA receptors, given that the pharmacological profile of the kainate currents expressed in these neurons closely matches that of homomeric $\text{GLU}_{\text{K}5}$ (26), and that large amounts of $\text{GLU}_{\text{K}5}$ mRNA are expressed in neuronal cell bodies (27).

There are several recent reviews that contain detailed descriptions of the development of AMPA- and KA-selective compounds (2,28–30). Here, only an overview of the compounds that have been important in advancing the understanding the physiological functions of the KA receptors is included.

3.1. Competitive Agonists and Antagonists

The “classic” competitive agonists for kainate receptors include kainate and domoate toxins, originally isolated from the red algae *Digenia simplex* and *Chondria armata*, respectively. Most KA receptor-mediated effects show a rank order of agonist potency of domoate > kainate > L-glutamate. The exception within the kainate receptor family appear to be homomeric $\text{GLU}_{\text{K}7}$ receptors, which display a reduced agonist sensitivity and altered order of potency. Indeed, domoate has no agonist activity at homomeric $\text{GLU}_{\text{K}7}$ even at 100 μM (see ref. 10). Kainate and domoate also act on AMPA receptors at higher concentrations. More selective agonists for KA receptors have been developed, and include the AMPA analog (RS)-2-amino-3-(3-hydroxy-5-tert-butylisoxazol-4-yl)propanoic acid (ATPA) (31), 5-iodowillardine (5-IW), 5-iodo-6-azawillardine (32), and (2S,4R)-4-methylglutamate (SYM2081) (33). Of these, ATPA has been widely used in the hippocampus to study the functional consequences of activating native kainate receptors (31,34–40).

The kainate receptor subunits differ in their responses to a variety of agonists, despite their relatively high primary sequence homology. ATPA and 5-IW are highly active at GLU_{K5} subunits, with practically no binding to GLU_{K6} , and show good selectivity over AMPA receptors (31,32,41). Radioligand studies demonstrate a rank order of binding for ATPA of $\text{GLU}_{\text{K5}} \gg \text{GLU}_{\text{A1-4}}, \text{GLU}_{\text{K2}} \text{ and } \text{GLU}_{\text{K7}} \gg \text{GLU}_{\text{K6}}, \text{GLU}_{\text{K6}}/\text{GLU}_{\text{K2}}$ (31). EC_{50} values obtained from electrophysiological studies where kainate receptor subunits are expressed in isolated cells or primary cultures confirm that ATPA shows good selectivity towards kainate receptors containing, though not necessarily comprising, solely of the GLU_{K5} subunit (31,15). Thus, ATPA acts as a full agonist at homomeric GLU_{K5} ($\text{EC}_{50} \sim 2.1 \mu\text{M}$) and in acutely isolated DRG neurones ($\text{EC}_{50} \sim 0.6 \mu\text{M}$) (31) and as a partial agonist at heteromeric $\text{GLU}_{\text{K5/6}}$ ($\text{EC}_{50} \sim 1.1 \mu\text{M}$) (15). These values were obtained in the presence of concanavalin A, avoiding the complicating issue as to whether this lectin, routinely used to reduce desensitization of kainate receptors (26,27; see Subheading 3.2.), increases the affinity of kainate receptors to agonist (42) or not (26,43). Although showing no activity at homomeric GLU_{K6} at concentrations of $100 \mu\text{M}$ – 10 mM (15,31,44), co-expression of either GLU_{K6} , GLU_{K7} , or GLU_{K2} with GLU_{K5} (15,44) does confer sensitivity to ATPA. Perhaps more surprising, ATPA acts as a partial agonist on heteromeric assemblies of GLU_{K2} and GLU_{K6} . The resulting currents are nondesensitizing, differentiating them from those at GLU_{K5} -containing receptors (15). Curiously, this activity is observed despite ATPA having an apparent $K_i > 100 \mu\text{M}$ at these receptors (31).

The quinoxalinedione derivatives 6-cyano-7-nitroquinoxaline (CNQX), 6,7-dinitroquinoxaline-2,3-dione (DNQX), and 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulphonamide (NBQX) are potent competitive antagonists of AMPA and kainate receptors. Of these, NBQX shows the most functional selectivity (approximately three-fold) for AMPA over KA receptors (45), and has been used in low concentrations ($1 \mu\text{M}$) to isolate kainate currents in hippocampal interneurons (46).

Recently, decahydroisoquinoxaline derivatives have been identified as glutamate receptor ligands that display selectivity for GLU_{K5} over the other kainate receptor subunits. Of these, LY293558 shows little or no selectivity between homomeric AMPA receptor and GLU_{K5} receptors (47). LY294486 and its active enantiomer LY377770 (48) also inhibit AMPA receptors, but show moderate selectivity toward GLU_{K5} (31). Both have been used to study the physiological functions of kainate receptors in the presence of AMPA receptor selective antagonists (31,34,35). The more recently developed LY382884, although less potent on GLU_{K5} -containing receptors expressed in cloned cell lines than LY294486, is the first antagonist that is selective enough to allow the study of the modulation of AMPA-mediated synaptic transmission by GLU_{K5} containing kainate receptors (39,49,50).

3.2. Allosteric Modulators

The first allosteric modulators discovered to act on AMPA/KA receptors were the plant lectins, including concanavalin A (ConA), that block receptor desensitization, probably through binding to *N*-linked oligosaccharides (51,52). It was soon discovered that certain benzothiazides, such as diazoxide and cyclothiazide, also act on the same allosteric site controlling non-NMDA receptor desensitization (53–55). Interestingly, ConA and cyclothiazide show high selectivity for KA and AMPA receptors, respec-

tively (27). This allowed, for the first time, assessment of the contribution made by each receptor type to currents evoked by native receptors (e.g., 26,43,56,57).

2,3-Benzodiazepines, 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (GYKI 52466) and 1-(4-aminophenyl)-3-methylcarbamyl-4-methyl-7,8-methylenedioxy-3,4-dihydro-5*H*-2,3-benzodiazepine (GYKI 53655 or LY300168), act as negative allosteric modulators of AMPA receptors (22,58–60). These 2,3-benzodiazepines antagonize kainate receptors only in concentrations well in excess of that required for functional AMPA receptor antagonism, and thus, have enabled pharmacological isolation of currents mediated by KA receptors (21–23). The best selectivity for AMPA over kainate receptor antagonism is provided by GYKI 53655 (and its active isomer LY303070) (57,61).

4. PHARMACOLOGICAL ISOLATION OF KAINATE RECEPTOR-MEDIATED EPSCS

The development of 2,3-benzodiazepines was a key step leading to understanding of the functions of native kainate receptors. GYKI 53655 (10 μ M) inhibits currents elicited by AMPA preferring receptors on cortical neurons with at least 200-fold greater potency, compared with those at kainate-preferring receptors on DRG cells (57). This highly selective antagonism of AMPA receptors allowed isolation of native kainate receptor mediated currents in embryonic hippocampal neurons, where application of GYKI 53655 unmasks a small desensitizing response to kainate (22,62).

Synaptic kainate receptor-mediated currents were first described in CA3 pyramidal neurons in response to high frequency stimulation of mossy-fibers (21,23). Application of GYKI53655 in the presence of antagonists to block currents mediated by NMDA, GABA_A, and GABA_B receptors inhibited practically all synaptic responses evoked by single pulse stimulation. However, high-frequency mossy-fiber stimulation produced a slow, small amplitude inward current that has a linear I-V relationship and is blocked by AMPA/kainate receptor antagonists NBQX and CNQX, and the GLU_{K5} selective antagonist LY294486 (*see* Fig. 1).

Since their definitive demonstration at the mossy fiber-CA3 synapse (21,23), KA receptor-mediated postsynaptic currents have been described at several synapses in the brain, including the Schaffer collateral-interneuron synapse in the hippocampus (34,63), thalamocortical synapse in the barrel cortex (64), parallel fiber-Golgi cell synapse in the cerebellum (65), external capsule-basolateral amygdala neuron synapse in the amygdala (66), and cone photoreceptors-‘Off’ bipolar cell synapse in the retina (67). The slow kinetics of the KA receptor-mediated synaptic current differs considerably from the fast onset and rapidly desensitizing response characterized for recombinant kainate receptors. One explanation is that these receptors are located extrasynaptically and their activation depends on glutamate spillover. However, the decay of KA EPSC does not depend on manipulations that modulate glutamate uptake or diffusion in the synaptic cleft at this (21,23) or other synapses (65,68). Furthermore, in most synapses described, and also in the mossy fiber-CA3 synapse under certain conditions (69), KA receptor-mediated EPSCs can be evoked by single shock stimulation, arguing against the idea that its kinetic properties are owing to glutamate build-up. In contrast, the functional properties of KA receptors can be altered by their interactions with various cytosolic proteins and by phosphorylation (70–74). Expression of a

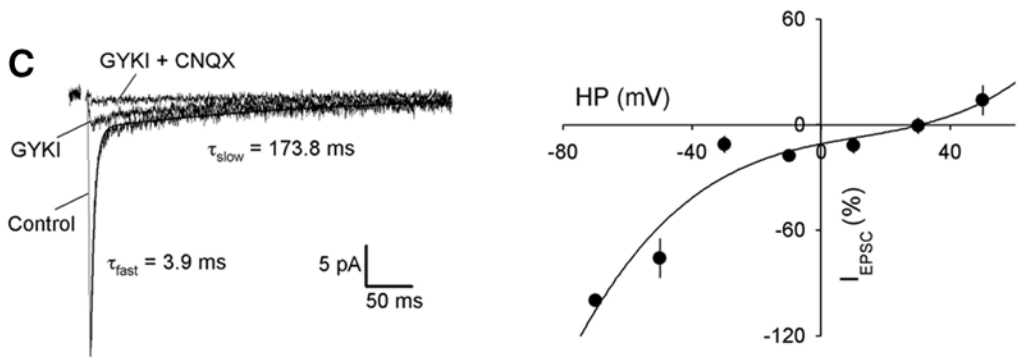
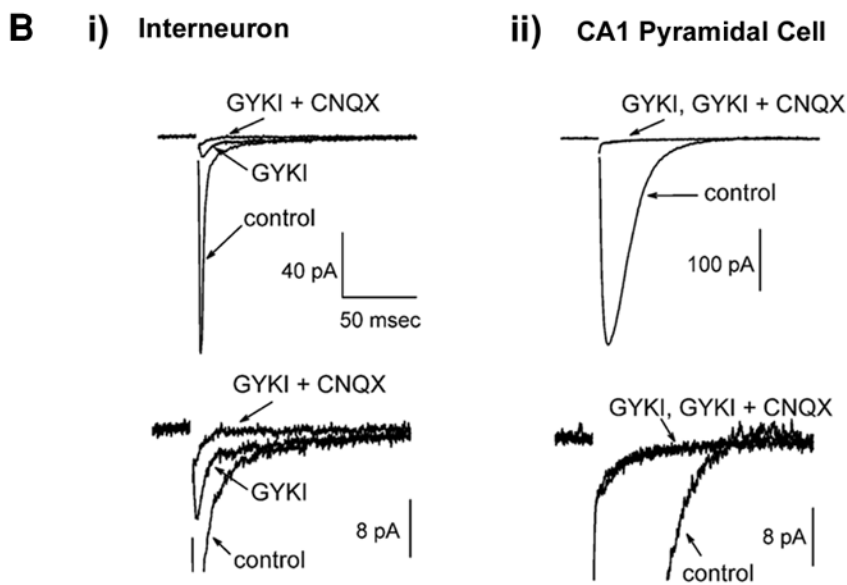
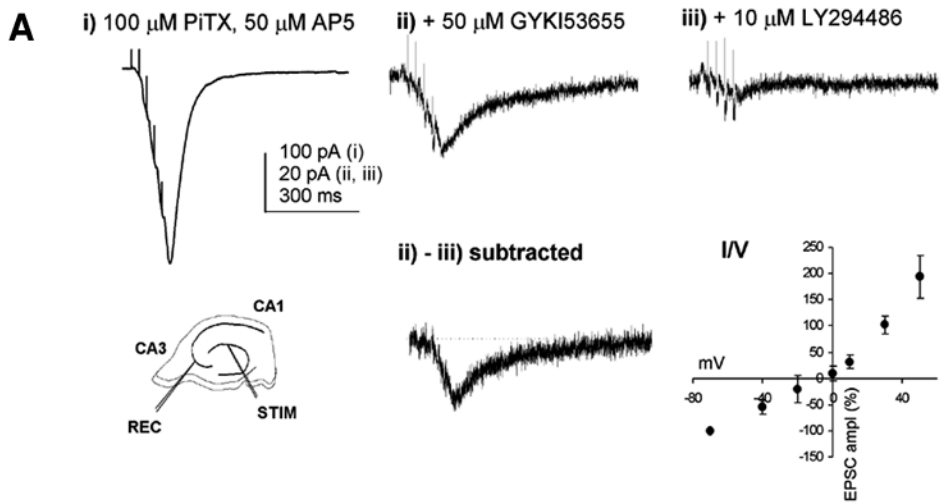


Fig. 1. Pharmacological isolation of kainate receptor-mediated synaptic currents in the central nervous system. **(A)** Synaptic activation of kainate receptors in mossy-fiber synapses in CA3. The traces show EPSCs recorded in the presence of D-AP5 (100 μ M) and picrotoxin (100 μ M) before **(i)** and after **(ii)** addition of the AMPA-receptor selective antagonist GYKI53655 (50 μ M) in response to high-frequency (5 shocks, 100 Hz) stimulation of mossy fibers, and the effect of kainate-receptor selective antagonist LY294486 **(iii)**. Although the early phase of the EPSC is mostly blocked by GYKI53655, the late phase is only slightly affected, and is blocked by the AMPA/KA receptor antagonist LY294486. Subtraction of the traces in III–II gives the pure KAR-mediated component of the EPSC which has an approximately linear I–V relationship and a reversal potential close to 0 mV. **(B)** EPSCs in interneurons, but not in pyramidal cells in CA1 have a KA receptor-mediated component. **(i)** Averaged EPSCs recorded from an interneuron, shown at a low (top) and high (bottom) gain, in control conditions, after bath application of 70 μ M GYKI 53655, and after addition of 100 μ M CNQX. **(ii)** The same experiment done here in pyramidal cells. In these cells, no KAR mediated EPSC could be found. (Reprinted from ref. (63) with permission from Nature Publishing Group.) (<http://www.nature.com/>) **(C)** KAR-mediated synaptic transmission at developing thalamocortical synapses. Superimposed EPSCs in: ‘Control’ (D-AP5 [100 μ M], picrotoxin [50 μ M]); ‘GYKI’ (+GYKI 53655 (25 μ M active isomer)); ‘GYKI + CNQX’ (+ CNQX [100 μ M]), and Summary I–V analysis tail of dual-component EPSCs. Continuous lines represent linear regressions. (Reprinted from ref (64) with permission from Nature Publishing Group.) (<http://www.nature.com/>)

different selection of interacting proteins that regulate KA receptor kinetics and targeting might thus provide one explanation for the different functional properties observed in native vs recombinant receptors.

The GLU_{K5} selective antagonists, LY293558 and LY294486, block both postsynaptic currents induced by kainate (200 nM) and synaptically released glutamate in CA3 pyramidal neurons (75). The observation that the CA3 region shows a reduced sensitivity to kainate and that synaptically evoked kainate currents are absent in GLU_{K6} knock-out mice (76), together with the pharmacological profile of these receptors, suggests that they are probably heteromeric assemblies of GLU_{K5}/GLU_{K6}. However, there are contrasting data that question the contribution of the GLU_{K5} subunit to the EPSCs in CA3. First, expression of GLU_{K5} mRNA in CA3 pyramidal cells is low or is not detected (11,15). Second, ATPA does not depolarize CA3 pyramidal neurons under conditions where large inward currents are induced by nanomolar concentrations of kainate (35). Third, another GLU_{K5} selective antagonist 10 μ M LY382884 has little effect on kainate-evoked currents in CA3 (77). However, whereas LY377770, the active enantiomer of LY294486, and LY382884 show similar potencies at presumed native GLU_{K5}-mediated responses in DRG cells (IC₅₀ ~ 1 μ M (49,50), LY377770 shows an approx 10-fold greater potency compared with LY382884 at both recombinant homomeric GLU_{K5} and heteromeric GLU_{K5}/GLU_{K6} (compare IC₅₀s of 0.69 μ M and 7.25 μ M for LY377770 and LY382884, respectively, at GLU_{K5} and 0.35 μ M and 3.61 μ M at GLU_{K5}/GLU_{K6}) (50). Thus, the absence of effect of LY382884 on KA receptor-mediated postsynaptic currents in the CA3 (77) is not inconsistent with a role for either homomeric GLU_{K5} or heteromeric GLU_{K5}/GLU_{K6}. Finally, the action of LY382884, LY293558, and LY294486 on various heteromeric combinations of kainate receptor subunits and on the N-terminal splice variants of GLU_{K5} receptors is not

known. This may significantly complicate the interpretation of the pharmacological results in terms of subunit composition of native KA receptors.

On the other hand, the results obtained from the knockout mice are complicated by possible functional compensation between KA receptor subunits. In a recent study (78), the effects of ATPA, LY382884, and LY293558 on kainate currents were studied in dorsal horn neurons of wild-type, GLU_{K5} , and GLU_{K6} knockout mice. LY382884 and LY293558 were effective in antagonizing kainate currents in wild-type and $GLU_{K6-/-}$, but not $GLU_{K5-/-}$, mice (78), thus confirming the selectivity of these compounds on native kainate receptors. Interestingly, the current density in $GLU_{K5-/-}$ mice and wild-types was similar. This shows that GLU_{K5} -dependent functions in wild-type mice can be compensated for in $GLU_{K5-/-}$ mice. Assuming that postsynaptic kainate receptors in CA3 are heteromeric assemblies containing at least GLU_{K5} and GLU_{K6} subunits, in GLU_{K5} knockouts the other kainate receptor subunits would still assemble into functional receptors (79). Moreover, the effects of ATPA and LY382884 in the dorsal horn neurons were greater in $GLU_{K6-/-}$ neurons than in wild-types (78), suggesting that GLU_{K6} deletion increases the GLU_{K5} stoichiometry at the level of individual receptors. This finding further suggests that the stoichiometry of GLU_{K5} influences the antagonism by subunit selective compounds.

5. EVIDENCE FOR PRESYNAPTIC KAINATE RECEPTORS AS MODULATORS OF NEUROTRANSMITTER RELEASE

Presynaptic receptors for neurotransmitters are widespread in the CNS, and can have a crucial role in modulating synaptic transmission (80). Before the synaptic activation of postsynaptic kainate receptors was identified (21,23), a presynaptic locus for the effects of kainate on synaptic transmission in the hippocampus had already been proposed (81–83), supported by biochemical and histological evidence for presynaptic high-affinity KA binding sites (14). It appears that kainate receptors, located presynaptically to the site of recording, modulate transmission at both glutamatergic and GABAergic synapses (reviewed in ref. 84). Finally, growing evidence supports a role for kainate autoreceptors in the regulation of short- and long-term synaptic plasticity (49,77,85–87).

5.1. Inhibitory Presynaptic Kainate Receptors

Kainate receptors mediate a depression of evoked excitatory synaptic transmission in areas CA1 (40,88–90) and CA3 (35,37,91,92) of the hippocampus. There is strong evidence that in area CA1 the locus of this effect is presynaptic. Thus, activation of kainate receptors depresses release of L-glutamate from synaptosomes (88) and depresses both NMDA and AMPA receptor-mediated components of the evoked EPSC in parallel (88,90). Furthermore, the effects of kainate receptor activation on excitatory synaptic transmission in CA1 are associated with changes in presynaptic Ca^{2+} (89), an increase in paired-pulse facilitation (35,88,89), and a reduction in quantal content, as assessed using $1/CV^2$, but no change in mEPSC amplitude (90).

There is growing evidence to suggest that, within the hippocampus, kainate and ATPA may depress fast excitatory transmission by distinct mechanisms. For example, in area CA3, kainate exerts its effects via an action on presynaptic mossy-fiber excitability (37,92), whereas ATPA is suggested to act indirectly via an increase in

interneuronal excitability and the subsequent heterosynaptic action of GABA at presynaptic GABA_B receptors on mossy-fiber terminals (37). Notably, these experiments were done in the presence of elevated extracellular calcium concentration (4 mM). At 2 mM calcium, GABA_B receptor antagonists have no effect on ATPA-induced depression in area CA3 (39). Similarly, in area CA1, the effects of ATPA are independent of GABA release (40,88). However, in area CA1, depression of glutamatergic transmission in response to kainate and ATPA can be differentiated based on their sensitivity to LY382884 as well as on extracellular calcium concentration (40).

Kainate and ATPA also mediate a depression of evoked GABAergic synaptic transmission in area CA1 (31,93) (Fig. 2). The effects of either ligand on GABAergic transmission can be antagonized using the GLU_{K5} receptor antagonist LY294486 (31), implying that, unlike the effects on evoked excitatory transmission in CA1, the effects of either agonist are on the same receptor subtype. The ability of KA receptor agonists to depress both the GABA_A and GABA_B components of the evoked IPSP in parallel and by similar magnitudes suggests that the effect is owing to a presynaptic decrease in GABA release (31). This depression is thought to involve a metabotropic kainate receptor (36,94), an argument lent more weight by the demonstration that kainate receptors can couple to G(i)/G(o) proteins (95).

The simplest explanation for the effect of KA receptor agonists on evoked GABAergic synaptic transmission would be a direct regulation of GABA release probability by kainate receptors located at the axonal presynaptic terminals of interneurons. In support, a modest reduction in mini IPSC frequency in CA1 pyramidal cells has been reported following kainate application (34,93), whereas other studies have observed no effect (63,65,96). The direct depolarization of interneurons via the activation of dendritic or somatic kainate receptors (34,63) results in an increase in spontaneous action potential discharge and consequent increase in spontaneous IPSC frequency observed to kainate and ATPA application (34,36,63,65,83,96). Such increases in spontaneous GABA release may underlie alternative indirect mechanisms for the depression of evoked transmission. In support, by mimicking the increase in firing frequency of interneurons seen on kainate application, an analogous depression of the evoked IPSC occurs (36,46,63). Furthermore, raising the threshold for interneurone action potential generation by increasing external divalents abolished the depression of the evoked IPSC by kainate (63). It has been proposed that the increase in GABA concentration as a result of spontaneous interneurone activity leads to a direct reduction of GABA release following the activation of presynaptic GABA_B receptors and a passive shunting of the postsynaptic GABAergic response via the activation of postsynaptic GABA_A receptors (97). However, such an explanation appears insufficient to account for the observed effects of kainate receptor activation on GABAergic transmission in area CA1. Thus, (1) an earlier study observed no effect of kainate on iontophoretically applied GABA (83), arguing against a passive shunt contribution. (2) The magnitude of depression of the evoked IPSP_B was unaltered following pharmacological antagonism of GABA_A receptors (31). (3) The two effects, namely interneurone depolarization and depression of evoked GABAergic transmission, can be dissociated pharmacologically (34,36) and couple to separate signaling systems (36). For example, low doses of ATPA (1 μ M) have profound effects on interneurone excitability, while having no significant effect on evoked GABAergic responses or postsynaptic properties (34). (4) Previous

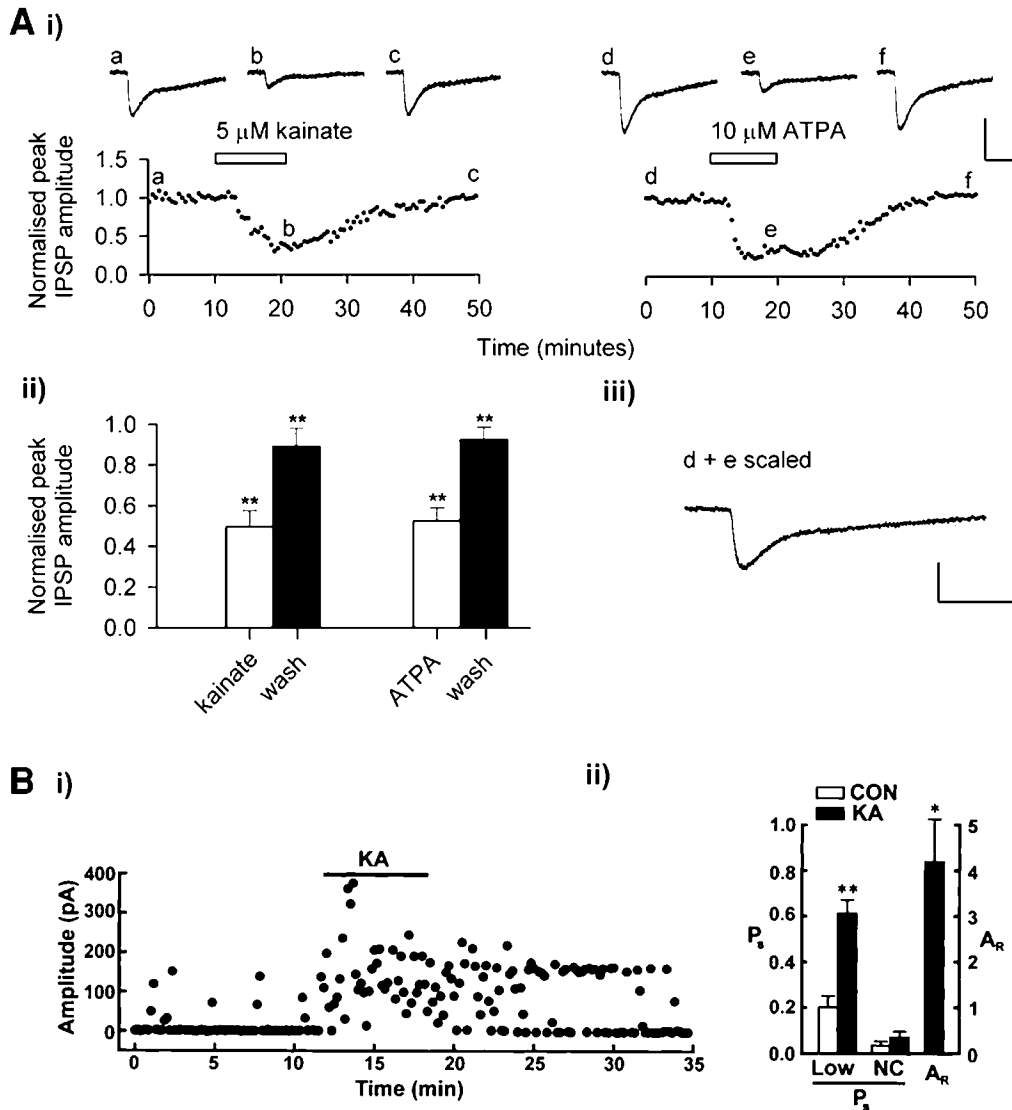


Fig. 2. Effects of presynaptic kainate receptors on GABAergic transmission in CA1. **(A)** Activation of GluR5 depresses inhibitory synaptic transmission. **(i)** Single example and **(ii)** pooled data illustrates the depression of monosynaptic IPSPs, in CA1 pyramidal neurons by kainate (5 μ M) and ATPA (10 μ M). **(iii)** The maximally depressed response, in the presence of ATPA, is scaled and superimposed with a control response. The lack of change in IPSP shape shows parallel inhibition of GABA_A and GABA_B receptor-mediated IPSPs. Scale bars, 5 mV, 100 ms. (Reprinted from ref. (31) with permission from Nature Publishing Group.) (<http://www.nature.com/>) **(B)** Low concentrations of KAR agonists potentiate GABAergic synapses in CA1. **(i)** Dual patch-clamp recordings from a representative low-release probability pair showing the effect of 300 nM KA on uIPSC amplitude against the time. Note the increase in successful events. **(ii)** Pooled data showing that KA induced a significant increase in the P_s and the average amplitude (A_R) of uIPSCs in low- P_s pairs. Coincidental sIPSCs in noncoupled pairs (NC) were not significantly increased by 300 nM KA. (Reprinted from ref. (86) with permission from Elsevier Science.)

studies have found no effect of GABA_B receptor antagonists on the kainate-induced depression of GABAergic transmission (31,63).

Regardless of whether the depolarization of interneurons and subsequent increase in IPSC frequency and the depression of evoked transmission are manifestations of the same phenomenon, knockout studies involving mice lacking either GLU_{K5} or GLU_{K6} or both (i.e., double knockouts) suggest that all these effects are absent only in mice lacking both subunits, but are comparable to wild-type control in the absence of either GLU_{K5}-/- or GLU_{K6}-/- mice (46,98). Two separate populations of receptor may exist, with one compensating for the loss of the other, as observed recently in the dorsal horn (78). However, these studies, together with the observations that the effects of ATPA and kainate on interneurone excitability (34) and depression of evoked IPSPs (31) are antagonized by LY293558 and LY294486, respectively, implies that the native KA receptors responsible for these effects are most likely a heteromeric receptor comprised of both GLU_{K5} and GLU_{K6}.

5.2. Facilitatory Presynaptic Kainate Receptors

Facilitatory actions of presynaptic kainate receptors (82,88) were long overshadowed by the pronounced inhibitory effects of kainate receptor agonists on both glutamatergic and GABAergic transmission. Recently, facilitation of synaptic transmission via activation of a presynaptic kainate receptors has been described in the GABAergic synapses in area CA1 (86), in the spinal cord (99), and probably most thoroughly, in the mossy-fiber synapse in area CA3 (39,77,100).

Kainate (200 nM) renders the mossy-fiber axons more excitable, as evidenced by an increase in the presynaptic fiber volley as well as lowered threshold for antidromic action potentials. At the same time, a kainate-induced suppression of synaptic transmission and depression of presynaptic calcium influx was observed (92). In contrast, application of very low concentrations of kainate (50 nM) facilitates synaptic transmission at the mossy fibers (77,100). The kainate-induced facilitation is blocked by LY382884, suggesting a role for GLU_{K5}-containing receptors (77). However, only depression of transmission has been observed with ATPA (35). Thus, the facilitatory and depressory effects of kainate could be mediated by different receptor populations that have distinct pharmacological properties.

In contrast to the metabotropic effects described for presynaptic kainate receptors in CA1 (90,94), the effects of kainate in CA3 appear to be mediated by direct depolarization of the presynaptic terminals. The kainate-induced facilitation is not sensitive to antagonists of other receptors (e.g., GABA_B), and can be mimicked by elevating the extracellular potassium concentration (77,100). It has been proposed that the facilitation is owing to increased calcium influx that is induced by modest depolarization of the terminals by kainate receptors, whereas a strong depolarization, in response to activation of a larger receptor population, causes the sodium channels to inactivate and thereby depresses transmission (77,84,88,100–102).

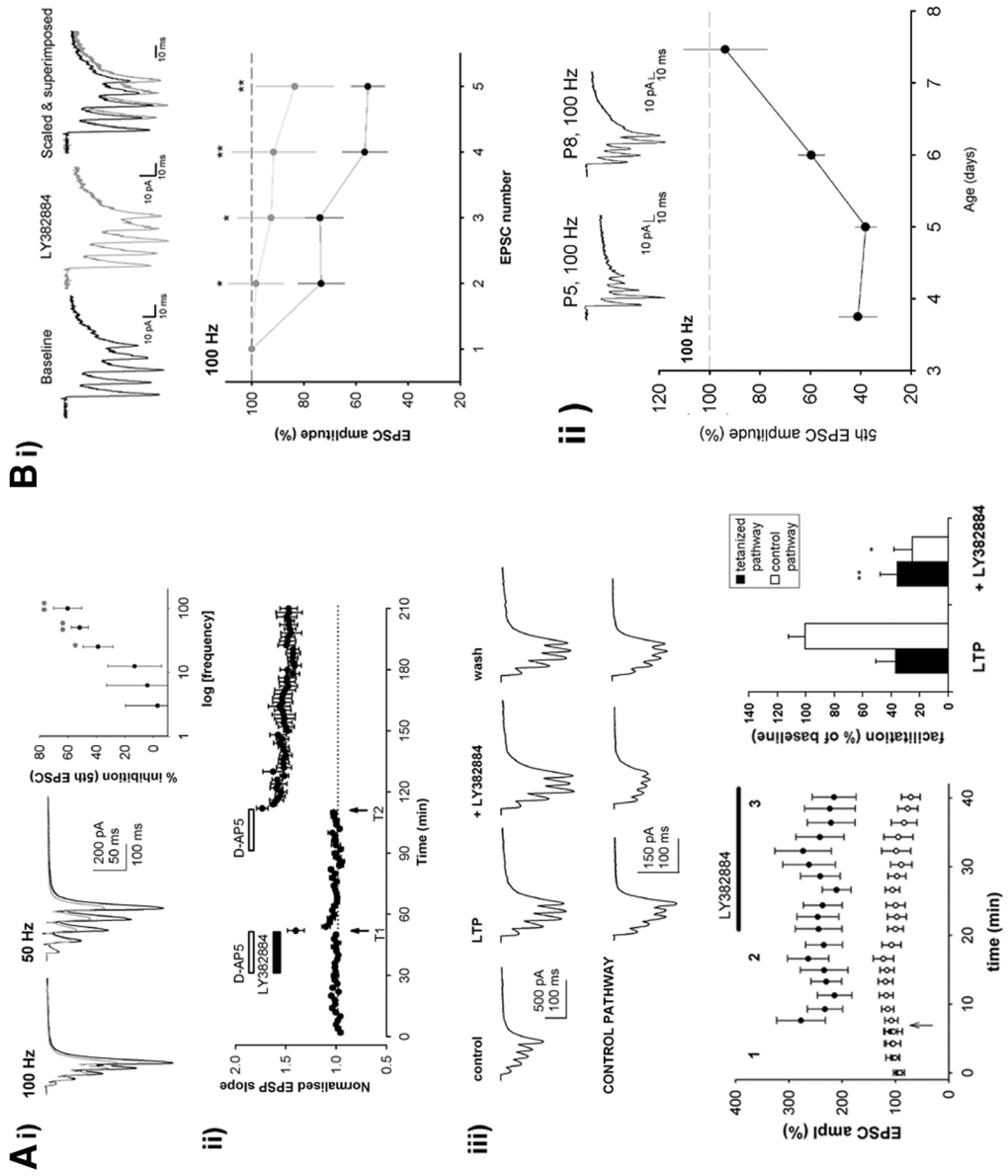
GABAergic inhibition between interneurons can also be enhanced by glutamate spillover from neighboring excitatory synapses acting on kainate receptors (38). In CA1 interneurons, an increase in spontaneous action potential discharge and consequent increase in spontaneous IPSC frequency is observed to kainate and ATPA application (34,36,46,63,83,96,98,103). These effects are in part owing to direct depolarization of

interneurones via somatic and dendritic KA receptors (34,63; *see* Subheading 5.1.), but also an increase in the efficacy of GABA release is thought to contribute. Activation of presynaptic kainate receptors is proposed to increase the probability of GABA release at interneuron-interneuron synapses, evidenced by kainate receptor mediated increase in the mini IPSC frequency and a decrease in the failure rate of the evoked IPSC in CA1 interneurones (38,46, but *see* 103). Although GLU_k5-containing receptors are clearly involved in the postsynaptic activation of interneurones and consequent increase in excitability as demonstrated pharmacologically (34) and using knockout mice (46,98), increases in efficacy are not mimicked by ATPA and appear absent in GLU_k6^{-/-} mice only. Finally, in a manner analogous to that seen at mossy fibers, kainate has also been shown to decrease the threshold of action potential firing by directly depolarizing the interneuronal axons (103). Recently, a biphasic effect of kainate on unitary IPSCs has been described at CA1 pyramidal neurons (86). KA receptor agonists increased the success rate of uIPSCs, whereas higher concentrations depressed GABAergic transmission, preferentially on synapses with a high release probability (86). Although the mechanism behind these effects is unclear, there is evidence suggesting that the KA receptors increase calcium-dependent GABA release via nonmetabotropic mechanisms (86,103).

6. ROLE OF KA RECEPTORS IN SYNAPTIC PLASTICITY

The recently developed GLU_k5 selective compound LY382884 (47) is the first antagonist that is selective enough for kainate receptors over AMPA receptors to be used to study the functions of native KA receptors in the presence of intact AMPA receptor-mediated transmission. The use of LY382884 has uncovered a role for kainate receptors in the regulation of short- and long-term synaptic plasticity in the mossy-fiber pathway (49,77) as well as at thalamocortical synapses (87) (Fig. 3)

Fig. 3. Presynaptic kainate receptors in synaptic plasticity. **(A)** Effects of LY382884 on short- and long-term synaptic plasticity at the mossy fibers. **(i)** LY382884 inhibits facilitation of mossy-fiber EPSCs at frequencies of 25 Hz and higher. Traces from recordings in CA3 pyramidal cells in the presence of D-AP5 (100 μ M) and picrotoxin (100 μ M) show a response to high-frequency (100 and 50 Hz) 5-pulse stimulation of mossy fibers in before (black) and after washing of the kainate receptor antagonist LY382884 (gray). Right, a pooled data showing the frequency dependent inhibition of 5th EPSC by LY382884. **(ii)** LY382884 specifically blocks the induction of the NMDA-receptor-independent mossy-fiber LTP. (Reprinted from ref. (49) with permission from Nature Publishing Group.) (<http://www.nature.com/>) **(iii)** Mossy-fiber LTP occludes the action of the presynaptic facilitatory kainate receptor. Representative traces showing the responses to five shocks at 50 Hz before and after the induction of LTP, and the lack of effect of LY382884 (10 μ M). The lower traces are from a control (i.e., nontetanized) input, showing the typical effect of LY382884 on frequency facilitation. The histogram shows pooled data on the amount of frequency facilitation expressed as a percentage of the frequency facilitation during baseline. (Reprinted from ref. (77) with permission from Elsevier Science.) **(B)** The synaptic activation of the presynaptic kainate receptor in developing thalamocortical synapses causes depression during high-frequency transmission. **(i)** EPSC amplitude (percentage of first EPSC in the train) during a 100Hz train and the effect of LY382884 (gray). **(ii)** Developmental regulation of the presynaptic kainate receptor; fifth EPSC amplitude (percentage of the first EPSC in the train) vs age for 100 Hz trains. Inset, examples of responses to trains of stimuli at 100 Hz for P5 and P8. (Reprinted from ref. (87) with permission from Elsevier Science.)



LY382884 at 10 μM has little effect on AMPA receptor-mediated EPSPs, and affected neither monosynaptic GABA_A and GABA_B receptor-mediated synaptic transmission nor passive membrane properties of pyramidal neurons in area CA1 (49). In area CA3, where kainate receptors are expressed in high levels, LY382884 has no effect on low-frequency AMPA-receptor mediated transmission (39), but inhibits pharmacologically isolated kainate EPSCs, induced by brief high-frequency stimulation of mossy fibers by about 40% (49). This effect is not owing to antagonism of the postsynaptic kainate receptor, because LY382884 had little or no effect on the kainate currents in CA3 pyramidal neurons. In contrast, LY382884 significantly inhibited kainate currents at dentate granule cells (77). Together, these data suggests that LY382884 acts as a selective antagonist of presynaptic kainate receptors at mossy fibers (49,77).

Mossy-fiber synaptic transmission is characterized by a large frequency-dependent facilitation, which is mediated by presynaptic mechanisms. The lack of effect of LY382884 on baseline AMPA responses suggested that presynaptic kainate receptors do not contribute to low-frequency synaptic transmission (49). However, LY382884 caused a substantial reduction in the facilitation of AMPA receptor mediated EPSCs at frequencies of 25 Hz or higher (39,77). LY382884 as well as the mixed AMPA/KA antagonist NBQX also blocks facilitation of NMDA EPSCs, induced by a brief 100Hz train and measured as facilitation of a single test pulse 200–1000 ms after the train (39,100). These data suggest that synaptically released glutamate acts on a presynaptic kainate autoreceptor to facilitate mossy-fiber synaptic transmission during high-frequency transmission (39,77,100). This autoreceptor mechanism is thought to act by an ionotropic mechanism, which facilitates presynaptic calcium influx and thereby glutamate release by depolarizing the terminals (39,77,100). In addition, there is evidence suggesting that presynaptic GLU_K5-containing kainate receptors on mossy-fiber terminals are directly permeable to Ca²⁺ and linked to calcium release from intracellular stores (104). In support, activation of presynaptic kainate receptors has been shown to amplify presynaptic calcium signals at mossy-fiber terminals (101). Both the inhibition of synaptic facilitation and presynaptic calcium signals by kainate receptor antagonism is evident already on the second stimulus, showing that the kainate autoreceptors can be activated extremely rapidly (< 10 ms) by only a single preceding stimulus (39,77,101). In addition to the homosynaptic facilitatory autoreceptor mechanism, heterosynaptic glutamate release from the associational-commissural fibers has been reported to depress mossy-fiber transmission via activation of a presynaptic kainate receptors (37). These two actions might reflect the concentration-dependent effects (e.g., facilitation vs depression) of glutamate on mossy-fiber transmission.

Kainate receptors have recently been implicated in the induction of LTP in the mossy fibers (49,85). Unlike LTP in the area CA1, induction of mossy-fiber LTP is independent of NMDA-receptor activation and involves presynaptic mechanisms (105). Synaptic activation of the facilitatory presynaptic receptor can account for the role of KA receptors in the induction of mossy-fiber LTP by maintaining a high level of release during high-frequency transmission (77). Furthermore, following induction of LTP, the presynaptic kainate receptor-mediated facilitation of synaptic transmission is lost, suggesting that the mechanism by which presynaptic kainate receptors facilitate

transmission is utilized for the expression of mossy-fiber LTP (77). Consistently, LTP also reduces the sensitivity of mossy-fiber transmission to depolarization (77,106).

A role for kainate receptors in the regulation of synaptic plasticity is also emerging in other brain areas (64,79,86,87,107). In developing thalamocortical synapses, the contribution of postsynaptic kainate receptors to transmission decreases during the critical period of development, an effect that can be mimicked by induction of LTP (64). At the same time, kainate autoreceptors mediate short-term depression of synaptic transmission at high frequencies (87). Also this effect is lost after the critical period of experience dependent plasticity and is thought to reflect maturation of the sensory processing network. In the basolateral amygdala, induction of enduring synaptic enhancement by low-frequency stimulation requires activation of GLU_K5 containing KA receptors acting through a calcium-dependent mechanism (107). The induction of this form of heterosynaptic plasticity was blocked by selective antagonists of GLU_K5 kainate receptors and mimicked by the GLU_K5 agonist ATPA. Interestingly, similar to mossy fibers, synaptic plasticity in amygdala is independent of NMDA receptor activation. Whether the requirement of kainate receptors for NMDA-independent forms of synaptic plasticity is a more general principle awaits further studies.

7. CONCLUSIONS

The development of selective pharmacological tools has been critical in advancing the understanding of the physiological functions of kainate receptors. A role for kainate receptors is emerging in both the mediation and modulation of synaptic transmission in several areas of the nervous system. In particular, the recruitment of GLU_K5 containing receptors during high-frequency activity might be important in the control of network excitability, and thus provide an important target for antiepileptic drugs (50).

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Molecular Neuropharmacology

Strategies and Methods

Schousboe, A.; Brauner-Osborne, H. (Eds.)

2004, XI, 271 p., Hardcover

ISBN: 978-1-58829-143-1

A product of Humana Press