PREFACE

The advent of the cloning of a large number of receptors and transporters for neurotransmitters and the simultaneous increase in the sophistication of tools available to produce specific mutations and chimeras of these proteins have provided scientists with the tools to understand the pharmacological and functional properties of such receptors and transporters at an hitherto unattained level. When this knowledge is combined with expertise in medicinal and computational chemistry, the basis for understanding the interactions between ligands and their corresponding macromolecules is greatly facilitated.

Molecular Neuropharmacology: Strategies and Methods is intended to bridge the gap between molecular biology and advanced chemistry. In addition, it attempts to include information about x-ray crystallographic analyses whenever available. This book discusses interdisciplinary interactions for monoamine transporters, amino acid transporters, ionotropic receptors, metabotropic glutamate receptors, GABA receptors, and other G protein-coupled receptors.

Arne Schousboe, ***PhD, DSc***
Hans Bräuner-Osborne, ***PhD, DSc***

2

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

Sari E. Lauri, Vernon R. J. Clarke, and Graham L. Collingridge

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

From: *Molecular Neuropharmacology: Strategies and Methods*
Edited by: A. Schousboe and H. Bräuner-Osborne © Humana Press Inc., Totowa, NJ

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 n*M*) to kainate than the high-affinity subunits GLU_{K1} and GLU_{K2} (~ 5 n*M*), 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 (EC_{50} ~ 0.3 m*M*), except for the KA receptors containing GLU_{K7} subunits (EC_{50} ~ 6 m*M*) *(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 [^{3}H]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 GLU_{K6}, and there are some cells within the pyramidal cell layer containing GLU_{K5} transcripts *(15)*. GLU_{K7} appears largely confined to the dentate and scattered interneurones of the stratum oriens. Most patterns of expression are established during late embryonic/early postnatal development. Exceptions include GLU_{K6}, which develops more slowly (postnatal day 12, i.e., *P12*), and a temporally restricted sharp peak in GLU_{K5} 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 $GLU_{K6/7}$ or $GLU_{K5/6/7}$, 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 $GLU_{K6/7}$ 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 GLU_{K5} *(26)*, and that large amounts of GLU_{K5} 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 *Digenea 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 GLU_{K7} receptors, which display a reduced agonist sensitivity and altered order of potency. Indeed, domoate has no agonist activity at homomeric GLU_{K7} even at 100 μ*M* (*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 GLU_{K5} >> $GLU_{A1–4}$, GLU_{K2} and GLU_{K7} >> GLU_{K6}, GLU_{K6}/GLU_{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} (EC_{50} ~ 2.1 μ*M*) and in acutely isolated DRG neurones (EC_{50} ~ 0.6 μ*M*) *(31)* and as a partial agonist at heteromeric $GLU_{K5/6}$ (EC_{50} ~ 1.1 μ*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 μ*M*–10 m*M* *(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 μ*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 threefold) for AMPA over KA receptors *(45)*, and has been used in low concentrations (1 μ*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*).

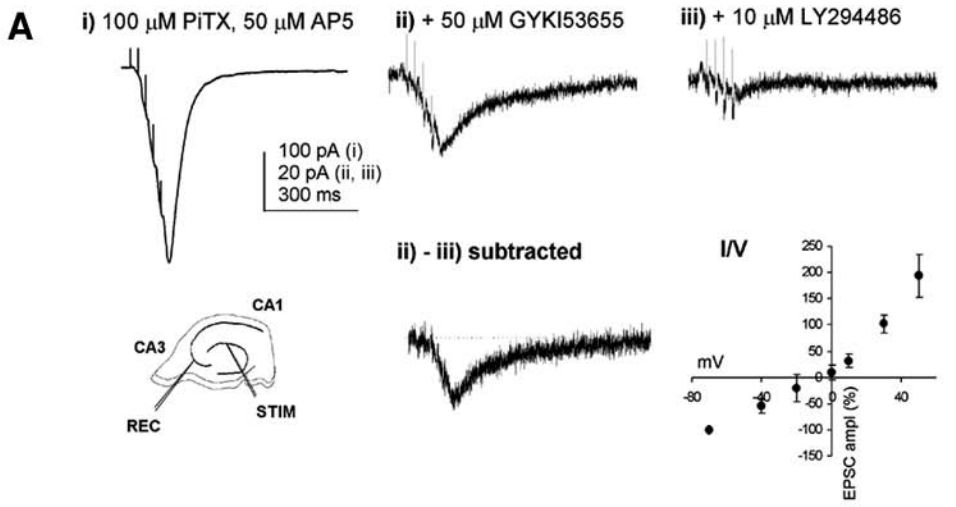
2,3-Benzodiazepines, 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine) (GYKI 52466) and 1-(4-aminophenyl)-3-methylcarbamy-4-methyl-7,8-methylenedioxy-3,4-dihydro-5*H*-2,3-benzodiazepine (GYKI 53655 or LY300168), act as negative allosteric modulators of AMPA receptors *(22,58–60)*. 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)*.

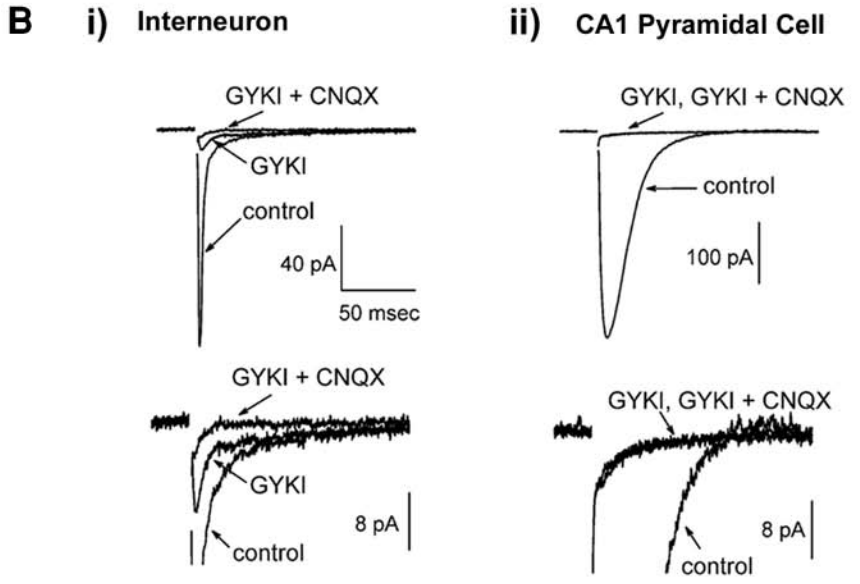
4. PHARMACOLOGICAL ISOLATION OF KAINATE RECEPTOR-MEDIATED EPSCS

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


A
i) 100 μM PiTX, 50 μM AP5
ii) + 50 μM GYKI53655
iii) + 10 μM LY294486
100 pA (i)
20 pA (ii, iii)
300 ms
ii) - iii) subtracted
I/V
CA1
CA3
REC
STIM
mV
EPSC ampl (%)


B
i) Interneuron
ii) CA1 Pyramidal Cell
GYKI + CNQX
GYKI
control
40 pA
50 msec
GYKI, GYKI + CNQX
100 pA
8 pA

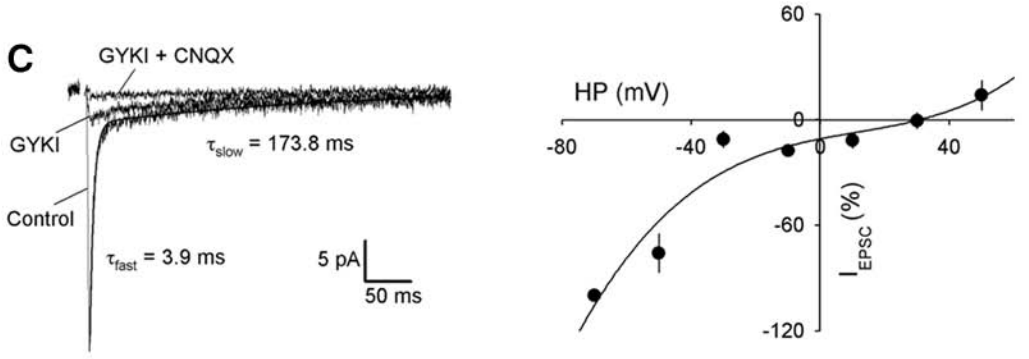

C
GYKI + CNQX
GYKI
Control
τslow = 173.8 ms
τfast = 3.9 ms
5 pA
50 ms
HP (mV)
IEPSC (%)

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 n*M*) 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} knockout 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 LY3777770, the active enantiomer of LY294486, and LY382884 show similar potencies at presumed native GLU_{K5}-mediated responses in DRG cells (IC_{50} ~ 1 μ*M* *(49,50)*, LY3777770 shows an approx 10-fold greater potency compared with LY382884 at both recombinant homomeric GLU_{K5} and heteromeric GLU_{K5}/GLU_{K6} (compare IC_{50}s 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 m*M*). At 2 m*M* 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 interneurones. 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 interneurones 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 interneurones 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

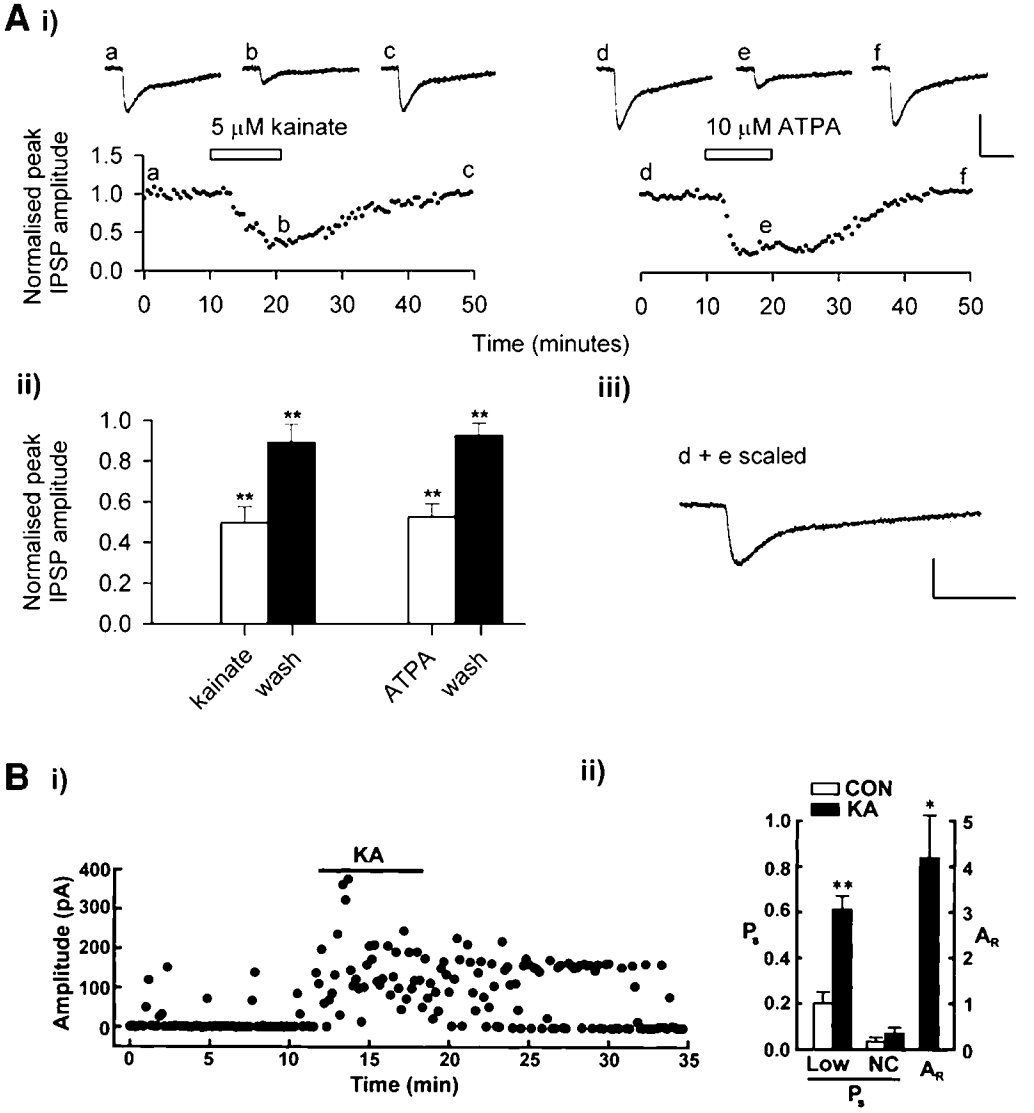


Fig. 2. Effects of presynaptic kainate receptors on GABAergic transmission in CA1. **(A)** Activation of GluR5 depresses inhibitory synaptic transmission. **(i)** Single example and **(ii)** pooled data illustrates the depression of monosynaptic IPSPs, in CA1 pyramidal neurons by kainate (5 μ*M*) and 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 interneurones 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 n*M*) 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 n*M*) 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)*.

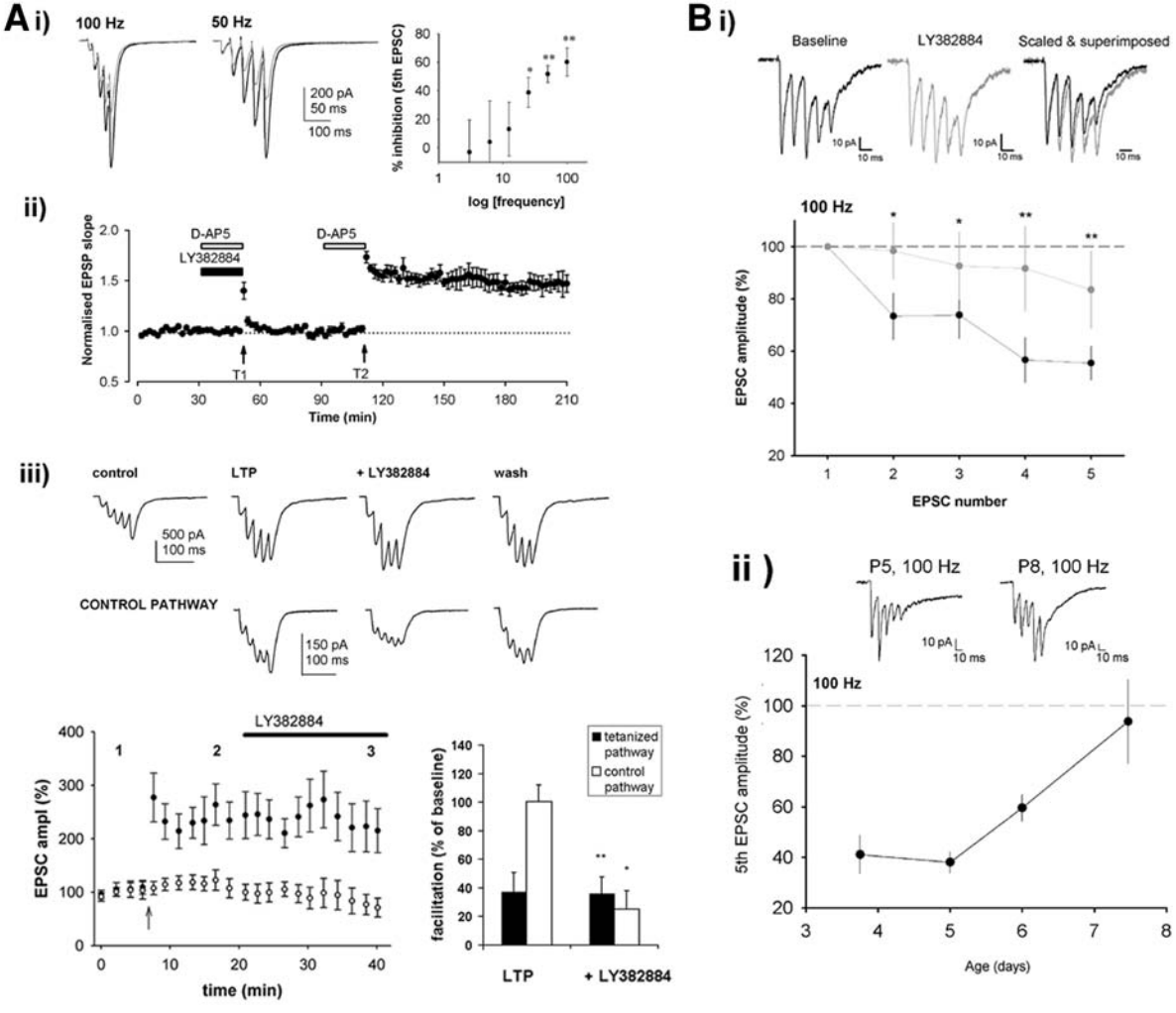
GABAergic inhibition between interneurones can also be enhanced by glutamate spillover from neighboring excitatory synapses acting on kainate receptors *(38)*. In CA1 interneurones, an increase in spontaneous action potential discharge and consequent increase in spontaneous IPSC frequency is observed 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.)


A i)
100 Hz
50 Hz
200 pA
50 ms
100 ms
% inhibition (5th EPSC)
log [frequency]
ii)
Normalised EPSP slope
D-AP5
LY382884
D-AP5
T1
T2
Time (min)
iii)
control
LTP
+ LY382884
wash
500 pA
100 ms
CONTROL PATHWAY
150 pA
100 ms
LY382884
EPSC ampl (%)
time (min)
facilitation (% of baseline)
tetanized pathway
control pathway
LTP
+ LY382884
B i)
Baseline
LY382884
Scaled & superimposed
10 pA
10 ms
100 Hz
EPSC amplitude (%)
EPSC number
ii)
P5, 100 Hz
P8, 100 Hz
10 pA
10 ms
100 Hz
5th EPSC amplitude (%)
Age (days)

LY382884 at 10 μ*M* 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 Ca^{2+} 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 (< l0 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)*.

REFERENCES

1. Watkins, J. C. and Evans, R. H. (1981) Excitatory amino acid transmitters. *Annu. Rev. Pharmacol. Toxicol.* **21,** 165–204.
2. Bleakman, D. and Lodge, D. (1998) Neuropharmacology of AMPA and kainate receptors. *Neuropharmacology* **37,** 1187–1204.
3. Lerma, J., Paternain, A. V., Rodriguez-Moreno, A., and Lopez-Garcia, J. C. (2001) Molecular physiology of kainate receptors. *Physiol. Rev.* **81,** 971–998.
4. Lodge, D. and Dingledine, R. (2000) *The IUPHAR Compendium of Receptor Characterization and Classification*, 2nd ed., IUPHAR Media, London, pp. 189–194.
5. Hollmann, M. and Heinemann, S. (1994) Cloned glutamate receptors. *Annu. Rev. Neurosci.* **17,** 31–108.
6. Bettler, B. and Mulle, C. (1995) Review: neurotransmitter receptors. II. AMPA and kainate receptors. *Neuropharmacology* **34,** 123–139.
7. Chittajallu, R., Braithwaite, S. P., Clarke, V. R. J., and Henley, J. M. (1999) Kainate receptors: subunits, synaptic localization and function. *Trends Pharmacol. Sci.* **20,** 26–35.
8. Dingledine, R., Borges, K., Bowie, D., and Traynelis, S. F. (1999) The glutamate receptor ion channels. *Pharmacol. Rev.* **51,** 7–61.
9. Ozawa, S., Kamiya, H., and Tsuzuki, K. (1998) Glutamate receptors in the mammalian central nervous system. *Prog. Neurobiol.* **54,** 581–618.
10. Schiffer, H. H., Swanson, G. T., and Heinemann, S. F. (1997) Rat GluR7 and a carboxy-terminal splice variant, GluR7b, are functional kainate receptor subunits with a low sensitivity to glutamate. *Neuron* **19,** 1141–1146.

11. Bahn, S., Volk, B., and Wisden, W. (1994) Kainate receptor gene expression in the developing rat brain. *J. Neurosci.* **14,** 5525–5547.
12. Foster, A. C., Mena, E. E., Monaghan, D. T., and Cotman, C. W. (1981) Synaptic localization of kainic acid binding sites. *Nature* **289,** 73–75.
13. Monaghan, D. T. and Cotman, C. W. (1982) The distribution of [3H]kainic acid binding sites in rat CNS as determined by autoradiography. *Brain Res.* **252,** 91–100.
14. Represa, A., Tremblay, E., and Ben Ari, Y. (1987) Kainate binding sites in the hippocampal mossy fibers: localization and plasticity. *Neuroscience* **20,** 739–748.
15. Paternain, A. V., Herrera, M. T., Nieto, M. A., and Lerma, J. (2000) GluR5 and GluR6 kainate receptor subunits coexist in hippocampal neurons and coassemble to form functional receptors. *J. Neurosci.* **20,** 196–205.
16. Wisden, W. and Seeburg, P. H. (1993) A complex mosaic of high-affinity kainate receptors in rat brain. *J. Neurosci.* **13,** 3582–3598.
17. Huntley, G. W., Rogers, S. W., Moran, T., Janssen, W., Archin, N., Vickers, J. C., et al. (1993) Selective distribution of kainate receptor subunit immunoreactivity in monkey neocortex revealed by a monoclonal antibody that recognizes glutamate receptor subunits GluR5/6/7. *J. Neurosci.* **13,** 2965–2981.
18. Petralia, R. S., Wang, Y. X., and Wenthold, R. J. (1994) Histological and ultrastructural localization of the kainate receptor subunits, KA2 and GluR6/7, in the rat nervous system using selective antipeptide antibodies. *J. Comp. Neurol.* **349,** 85–110.
19. Siegel, S. J., Janssen, W. G., Tullai, J. W., Rogers, S. W., Moran, T., Heinemann, S. F., and Morrison, J. H. (1995) Distribution of the excitatory amino acid receptor subunits GluR2(4) in monkey hippocampus and colocalization with subunits GluR5-7 and NMDAR1. *J. Neurosci.* **15,** 2707–2719.
20. Charara, A., Blankstein, E., and Smith, Y. (1999) Presynaptic kainate receptors in the monkey striatum. *Neuroscience* **91,** 1195–1200.
21. Castillo, P. E., Malenka, R. C., and Nicoll, R. A. (1997) Kainate receptors mediate a slow postsynaptic current in hippocampal CA3 neurons. *Nature* **388,** 182–186.
22. Paternain, A. V., Morales, M., and Lerma, J. (1995) Selective antagonism of AMPA receptors unmasks kainate receptor-mediated responses in hippocampal neurons. *Neuron* **14,** 185–189.
23. Vignes, M. and Collingridge, G. L. (1997) The synaptic activation of kainate receptors. *Nature* **388,** 179–182.
24. Agrawal, S. G. and Evans, R. H. (1986) The primary afferent depolarizing action of kainate in the rat. *Br. J. Pharmacol.* **87,** 345–355.
25. Sahara, Y., Noro, N., Iida, Y., Soma, K., and Nakamura, Y. (1997) Glutamate receptor subunits GluR5 and KA-2 are coexpressed in rat trigeminal ganglion neurons. *J. Neurosci.* **17,** 6611–6620.
26. Huettner, J. E. (1990) Glutamate receptor channels in rat DRG neurons: activation by kainate and quisqualate and blockade of desensitization by Con A. *Neuron* **5,** 255–266.
27. Partin, K. M., Patneau, D. K., Winters, C. A., Mayer, M. L., and Buonanno, A. (1993) Selective modulation of desensitization at AMPA versus kainate receptors by cyclothiazide and concanavalin A. *Neuron* **11,** 1069–1082.
28. Bleakman, D., Gates, M. R., Ogden, A. M., and Mackowiak, M. (2002) Kainate receptor agonists, antagonists and allosteric modulators. *Curr. Pharm. Des.* **8,** 873–885.
29. Fletcher, E. J. and Lodge, D. (1996) New developments in the molecular pharmacology of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate and kainate receptors. *Pharmacol. Ther.* **70,** 65–89.
30. Lees, G. J. (2000) Pharmacology of AMPA/kainate receptor ligands and their therapeutic potential in neurological and psychiatric disorders. *Drugs* **59,** 33–78.
31. Clarke, V. R. J., Ballyk, B. A., Hoo, K. H., Mandelzys, A., Pellizzari, A., Bath, C. P., et al. (1997) A hippocampal GluR5 kainate receptor regulating inhibitory synaptic transmission. *Nature* **389,** 599–603.

32. Jane, D. E., Hoo, K., Kamboj, R., Deverill, M., Bleakman, D., and Mandelzys, A. (1997) Synthesis of willardiine and 6-azawillardiine analogs: pharmacological characterization on cloned homomeric human AMPA and kainate receptor subtypes. *J. Med. Chem.* **40,** 3645–3650.
33. Gu, Y. P. and Huang, L. Y. (1991) Block of kainate receptor channels by Ca2+ in isolated spinal trigeminal neurons of rat. *Neuron* **6,** 777–784
34. Cossart, R., Esclapez, M., Hirsch, J. C., Bernard, C., and Ben Ari, Y. (1998) GluR5 kainate receptor activation in interneurons increases tonic inhibition of pyramidal cells. *Nat. Neurosci.* **1,** 470–478.
35. Vignes, M., Clarke, V. R. J., Parry, M. J., Bleakman, D., Lodge, D., Ornstein, P. L., and Collingridge, G. L.(1998) The GluR5 subtype of kainate receptor regulates excitatory synaptic transmission in areas CA1 and CA3 of the rat hippocampus. *Neuropharmacology* **37,** 1269–1277.
36. Rodriguez-Moreno, A., Lopez-Garcia, J. C., and Lerma, J. (2000) Two populations of kainate receptors with separate signaling mechanisms in hippocampal interneurons. *Proc. Natl. Acad. Sci. USA* **97,** 1293–1298.
37. Schmitz, D., Frerking, M., and Nicoll, R. A. (2000) Synaptic activation of presynaptic kainate receptors on hippocampal mossy fiber synapses. *Neuron* **27,** 327–338.
38. Cossart, R., Tyzio, R., Dinocourt, C., Esclapez, M., Hirsch, J. C., Ben Ari, Y., and Bernard, C. (2001) Presynaptic kainate receptors that enhance the release of GABA on CA1 hippocampal interneurons. *Neuron* **29,** 497–508.
39. Lauri, S. E., Delany, C., Clarke, V.R J., Bortolotto, Z. A., Ornstein, P. L., Isaac, J. T. R., and Collingridge, G. L. (2001a) Synaptic activation of a presynaptic kainate receptor facilitates AMPA receptor-mediated synaptic transmission at hippocampal mossy fibre synapses. *Neuropharmacology* **41,** 907–915.
40. Clarke, V. R. J. and Collingridge, G. L. (2002) Characterisation of the effects of ATPA, a GLU_K5 receptor selective agonist, on excitatory synaptic transmission in area CA1 of rat hippocampal slices. *Neuropharmacology* **42,** 889–902.
41. Swanson, G. T., Green, T., and Heinemann, S. F. (1998) Kainate receptors exhibit differential sensitivities to (S)-5-iodowillardine. *Mol. Pharmacol.* **53,** 942–949.
42. Paternain, A. V., Rodriguez-Moreno, A., Villarroel, A., and Lerma, J. (1998) Activation and desensitization properties of native and recombinant kainate receptors. *Neuropharmacology* **37,** 1249–1259.
43. Wong, L. A. and Mayer, M. L. (1993) Differential modulation by cyclothiazide and concanavalin A of desensitization at native alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic. *Mol. Pharmacol.* **44,** 504–510.
44. Cui, C. and Mayer, M. L. (1999) Heteromeric kainate receptors formed by the coassembly of GluR5, GluR6, and GluR7. *J. Neurosci.* **19,** 8281–8291.
45. Wilding, T. J. and Huettner, J. E. (1996) Antagonist pharmacology of kainate- and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-preferring receptors. *Mol. Pharmacol.* **49,** 540–546.
46. Mulle, C., Sailer, A., Swanson, G. T., Brana, C., O'Gorman, S., Bettler, B., and Heinemann, S. F. (2000) Subunit composition of kainate receptors in hippocampal interneurons. *Neuron* **28,** 475–484.
47. Bleakman, R., Schoepp, D. D., Ballyk, B., Bufton, H., Sharpe, E. F., Thomas, K., et al. (1996) Pharmacological discrimination of GluR5 and GluR6 kainate receptor subtypes by (3S,4aR,6R,8aR)-6-[2-(1(2)H-tetrazole-5-yl)ethyl]decahyd roisdoquinoline-3 carboxylic-acid. *Mol. Pharmacol.* **49,** 581–585.
48. O'Neill, M. J., Bond, A., Ornstein, P. L., Ward, M. A., Hicks, C. A., Hoo, K., et al. (1998) Decahydroisoquinolines: novel competitive AMPA/kainate antagonists with neuroprotective effects in global cerebral ischaemia. *Neuropharmacology* **37,** 1211–1222.
49. Bortolotto, Z. A., Clarke, V. R., Delany, C. M., Parry, M. C., Smolders, I., Vignes, M., et al. (1999) Kainate receptors are involved in synaptic plasticity. *Nature* **402,** 297–301.

50. Smolders, I., Bortolotto, Z. A., Clarke, V. R. J., Warre, R., Khan, G. M., O'Neill, M. J., et al. (2002) Antagonists of GLU(K5)-containing kainate receptors prevent pilocarpine-induced limbic seizures. *Nat. Neurosci.* **5,** 796–804.
51. Mayer, M. L. and Vyklicky, L., Jr. (1989) Concanavalin A selectively reduces desensitization of mammalian neuronal quisqualate receptors. *Proc. Natl. Acad. Sci. USA* **86,** 1411–1415.
52. Thalhammer, A., Everts, I., and Hollmann, M. (2002) Inhibition by lectins of glutamate receptor desensitization is determined by the lectin's sugar specificity at kainate but not AMPA receptors. *Mol. Cell. Neurosci.* **21,** 521–33.
53. Yamada, K. A. and Rothman, S. M. (1992) Diazoxide blocks glutamate desensitization and prolongs excitatory postsynaptic currents in rat hippocampal neurons. *J. Physiol.* **458,** 409–423.
54. Palmer, A. J. and Lodge, D. (1993) Cyclothiazide reverses AMPA receptor antagonism of the 2,3-benzodiazepine, GYKI 53655. *Eur. J. Pharmacol.* **244,** 193–194.
55. Patneau, D. K., Vyklicky, L., Jr., and Mayer, M. L. (1993) Hippocampal neurons exhibit cyclothiazide-sensitive rapidly desensitizing responses to kainate. *J. Neurosci.* **13,** 3496–3509.
56. Pook, P., Brugger, F., Hawkins, N. S., Clark, K. C., Watkins, J. C., and Evans, R. H. (1993) A comparison of the actions of agonists and antagonists at non-NMDA receptors of C fibres and motoneurones of the immature rat spinal cord in vitro. *Br. J. Pharmacol.* **108,** 179–184.
57. Wilding, T. J. and Huettner, J. E. (1995) Differential antagonism of alpha-amino-3-hydroxy-5-methyl-4- isoxazolepropionic acid-preferring and kainate-preferring receptors by 2,3-benzodiazepines. *Mol. Pharmacol.* **47,** 582–587.
58. Tarnawa, I., Farkas, S., Berzsenyi, P., Pataki, A., and Andrasi, F. (1989) Electrophysiological studies with a 2,3-benzodiazepine muscle relaxant: GYKI 52466. *Eur. J. Pharmacol.* **167,** 193–199.
59. Donevan, S. D. and Rogawski, M. A. (1993) GYKI 52466, a 2,3-benzodiazepine, is a highly selective, noncompetitive antagonist of AMPA/kainate receptor responses. *Neuron* **10**(1), 51–59.
60. Zorumski, C. F., Yamada, K. A., Price, M. T., and Olney, J. W. (1993) A benzodiazepine recognition site associated with the non-NMDA glutamate receptor. *Neuron* **10,** 61–67.
61. Bleakman, D., Ballyk, B. A., Schoepp, D. D., Palmer, A. J., Bath, C. P., Sharpe, E. F., et al. (1996) Activity of 2,3-benzodiazepines at native rat and recombinant human glutamate receptors in vitro: stereospecificity and selectivity profiles. *Neuropharmacology* **35,** 1689–1702.
62. Wilding, T. J. and Huettner, J. E. (1997) Activation and desensitization of hippocampal kainate receptors. *J. Neurosci.* **17,** 2713–2721.
63. Frerking, M., Malenka, R. C., and Nicoll, R. A. (1998) Synaptic activation of kainate receptors on hippocampal interneurons. *Nat. Neurosci.* **1,** 479–486.
64. Kidd, F. L. and Isaac, J. T. R. (1999) Developmental and activity-dependent regulation of kainate receptors at thalamocortical synapses. *Nature* **400,** 569–573.
65. Bureau, I., Dieudonne, S., Coussen, F., and Mulle, C. (2000) Kainate receptor-mediated synaptic currents in cerebellar Golgi cells are not shaped by diffusion of glutamate. *Proc. Natl. Acad. Sci. USA* **97,** 6838–6843.
66. Li, H. and Rogawski, M. A. (1998) GluR5 kainate receptor mediated synaptic transmission in rat basolateral amygdala in vitro. *Neuropharmacology* **37,** 1279–1286.
67. DeVries, S. H. and Schwartz, E. A. (1999) Kainate receptors mediate synaptic transmission between cones and 'Off' bipolar cells in a mammalian retina. *Nature* **397,** 157–160.
68. Kidd, F. L. and Isaac, J. T. (2001) Kinetics and activation of postsynaptic kainate receptors at thalamocortical synapses: role of glutamate clearance. *J. Neurophysiol.* **86,** 1139–1148.
69. Cossart, R., Epsztein, J., Tyzio, R., Becq, H., Hirsch, J., Ben Ari, Y., and Crepel, V. (2002) Quantal release of glutamate generates pure kainate and mixed AMPA/kainate EPSCs in hippocampal neurons. *Neuron* **35,** 147–159.

70. Raymond, L. A., Blackstone, C. D., and Huganir, R. L. (1993) Phosphorylation and modulation of recombinant GluR6 glutamate receptors by cAMP-dependent protein kinase. *Nature* **361,** 337–341.
71. Wang, L. Y., Taverna, F. A., Huang, X. P., MacDonald, J. F., and Hampson, D. R. (1993) Phosphorylation and modulation of a kainate receptor (GluR6) by cAMP-dependent protein kinase. *Science* **259,** 1173–1175.
72. Traynelis, S. F. and Wahl, P. (1997) Control of rat GluR6 glutamate receptor open probability by protein kinase A and calcineurin. *J. Physiol.* **503,** 513–531.
73. Garcia, E. P., Mehta, S., Blair, L. A., Wells, D. G., Shang, J., Fukushima, T., et al. (1998) SAP90 binds and clusters kainate receptors causing incomplete desensitization. *Neuron* **21,** 727–739.
74. Hirbec, H., Francis, J. C., Lauri, S. E., et al. (2003) Rapid and differential regulation of AMPA and Kainate receptors at hippocampal mossy fibre synapses by PICK1 and GRIP. *Neuron* **37,** 625–638.
75. Vignes, M., Bleakman, D., Lodge, D., and Collingridge, G. L. (1997) The synaptic activation of the GluR5 subtype of kainate receptor in area CA3 of the rat hippocampus. *Neuropharmacology* **36,** 1477–1481.
76. Mulle, C., Sailer, A., Perez-Otano, I., Dickinson-Anson, H., Castillo, P. E., Bureau, I., et al. (1998) Altered synaptic physiology and reduced susceptibility to kainate-induced seizures in GluR6-deficient mice. *Nature* **392,** 601–605.
77. Lauri, S. E., Bortolotto, Z. A., Bleakman, D., Ornstein, P. L., Lodge, D., Isaac, J. T., and Collingridge, G. L. (2001b) A critical role of a facilitatory presynaptic kainate receptor in mossy fiber LTP. *Neuron* **32,** 697–709.
78. Kerchner, G. A., Wilding, T. J., Huettner, J. E., and Zhuo, M. (2002) Kainate receptor subunits underlying presynaptic regulation of neurotransmitter release in the dorsal horn. *J. Neurosci.* **22,** 8010–8017.
79. Huettner, J. E. (2001) Kainate receptors: knocking out plasticity. *Trends Neurosci.* **24,** 365–366.
80. McGehee, D. S. and Role, L. W. (1996) Presynaptic ionotropic receptors. *Curr. Opin. Neurobiol.* **6,** 342–349.
81. Coyle, J. T. (1983) Neurotoxic action of kainic acid. *J. Neurochem.* **41,** 1–11.
82. Kehl, S. J., McLennan, H., and Collingridge, G. L. (1984) Effects of folic and kainac acids on synaptic responses of hippocampal neurones. *Neuroscience* **11(1),** 111–124.
83. Fisher, R. S., and Alger, B. E. (1984) Electrophysiological mechanisms of kainic acid-induced epileptiform activity in the rat hippocampal slice. *J. Neurosci.* **4(5),** 1312–1323
84. Kullmann, D. M. (2001) Presynaptic kainate receptors in the hippocampus: slowly emerging from obscurity. *Neuron* **32,** 561–564.
85. Contractor, A., Swanson, G., and Heinemann, S. F. (2001) Kainate receptors are involved in short- and long-term plasticity at mossy fiber synapses in the hippocampus. *Neuron* **29,** 209–216.
86. Jiang, L., Xu J., Nedergaard, M., and Kang, J. (2001) A kainate receptor increases the efficacy of GABAergic synapses. *Neuron* **30,** 503–513.
87. Kidd, F. L., Coumis, U., Collingridge, G. L., Crabtree, J. W., and Isaac, J. T. (2002) A presynaptic kainate receptor is involved in regulating the dynamic properties of thalamocortical synapses during development. *Neuron* **34,** 635–646.
88. Chittajallu, R., Vignes, M., Dev, K. K., Barnes, J. M., Collingridge, G. L., and Henley, J. M. (1996) Regulation of glutamate release by presynaptic kainate receptors in the hippocampus. *Nature* **379,** 78–81.
89. Kamiya, H. and Ozawa, S. (1998) Kainate receptor-mediated inhibition of presynaptic Ca2+ influx and EPSP in area CA1 of the rat hippocampus. *J. Physiol.* **509 (Pt 3),** 833–845.
90. Frerking, M., Schmitz, D., Zhou, Q., Johansen, J., and Nicoll, R. A. (2001) Kainate receptors depress excitatory synaptic transmission at CA3-CA1 synapses in the hippocampus via a direct presynaptic action. *J. Neurosci.* **21,** 2958–2966.

91. Contractor, A., Swanson, G. T., Sailer, A., O'Gorman, S., and Heinemann, S. F. (2000) Identification of the kainate receptor subunits underlying modulation of excitatory synaptic transmission in the CA3 region of the hippocampus. *J. Neurosci.* **20,** 8269–8278.
92. Kamiya, H. and Ozawa, S. (2000) Kainate receptor-mediated presynaptic inhibition at the mouse hippocampal mossy fibre synapse. *J. Physiol.* **523,** 653–665.
93. Rodríguez-Moreno, A., Herreras, O., and Lerma, J. (1997) Kainate receptors presynaptically downregulate GABAergic inhibition in the rat hippocampus. *Neuron* **19,** 893–901
94. Rodríguez-Moreno, A. and Lerma, J. (1998) Kainate receptor modulation of GABA release involves a metabotropic function. *Neuron* **20,** 1211–1218.
95. Cunha, R. A., Malva, J. O., and Ribeiro, J. A. (1999) Kainate receptors coupled to G(i)/G(o) proteins in the rat hippocampus. *Mol. Pharmacol.* **56,** 429–433.
96. Vignes, M. (2001) Regulation of spontaneous inhibitory synaptic transmission by endogenous glutamate via non-NMDA receptors in cultured rat hippocampal neurons. *Neuropharmacology* **40,** 737–748.
97. Frerking, M., Petersen, C. C., and Nicoll, R. A. (1999) Mechanisms underlying kainate receptor-mediated disinhibition in the hippocampus. *Proc. Natl. Acad. Sci. USA* **96,** 12917–12922.
98. Bureau, I., Bischoff, S., Heinemann, S. F., and Mulle, C. (1999) Kainate receptor-mediated responses in the CA1 field of wild-type and GluR6-deficient mice. *J. Neurosci.* **19,** 653–663
99. Kerchner, G. A., Wang, G. D., Qiu, C. S., Huettner, J. E., and Zhuo, M. (2001) Direct presynaptic regulation of GABA/glycine release by kainate receptors in the dorsal horn: an ionotropic mechanism. *Neuron* **32,** 477–488.
100. Schmitz, D., Mellor, J., and Nicoll, R. A. (2001) Presynaptic kainate receptor mediation of frequency facilitation at hippocampal mossy fiber synapses. *Science* **291,** 1972–1976.
101. Kamiya, H., Ozawa, S., and Manabe, T. (2002) Kainate receptor-dependent short-term plasticity of presynaptic Ca2 influx at the hippocampal mossy fiber synapses. *J. Neurosci.* **22(21),** 9237–9243.
102. Kamiya, H. (2002) Kainate receptor-dependent presynaptic modulation and plasticity. *Neurosci. Res.* **42,** 1–6.
103. Semyanov, A. and Kullmann, D. M. (2001) Kainate receptor-dependent axonal depolarization and action potential initiation in interneurons. *Nat. Neurosci.* **4,** 718–723.
104. Lauri, S. E., Bortolotto, Z. A., Bleakman D., et al. (2003) A role for Ca^{2+} stores in kainate-dependent synaptic facilitation and LTP at mossy fibre synapses in the hippocampus. *Neuron* **39,** 327–341.
105. Nicoll, R. A. and Malenka, R. C. (1995) Contrasting properties of two forms of long-term potentiation in the hippocampus. *Nature* **377,** 115–118.
106. Mellor, J., Nicoll, R. A., and Schmitz, D. (2002) Mediation of hippocampal mossy fiber long-term potentiation by presynaptic Ih channels. *Science* **295,** 143–147.
107. Li, H., Chen, A., Xing, G., Wei, M. L., and Rogawski, M. A. (2001) Kainate receptor-mediated heterosynaptic facilitation in the amygdala. *Nat. Neurosci.* **4,** 612–620.