Neuron, Vol. 32, 561–564, November 20, 2001, Copyright ©2001 by Cell Press

Presynaptic Kainate Receptors in the Hippocampus: Slowly Emerging from Obscurity

Minireview

Dimitri M. Kullmann[1]
Institute of Neurology
University College London
Queen Square
London WC1N 3BG
United Kingdom

Summary

Kainate receptor agonists depress transmitter release at several synapses in the hippocampus. Distinct mechanisms appear to underlie this phenomenon at different synapses. Recently, it has emerged that presynaptic kainate receptors can also potentiate the release of both GABA and glutamate and that axonal kainate receptors can trigger ectopic action potentials in interneurons. Because synaptically released glutamate mimics many of the actions of exogenous agonists, presynaptic kainate receptors potentially play an extensive role in hippocampal signaling.

Among the ionotropic glutamate receptors, the kainate subtype has, for a long time, been the poor relative of its illustrious cousins, the AMPA and NMDA receptors. Part of the reason for this obscurity is that selective AMPA receptor antagonists (in particular the 2,3 benzodiazepines) have only recently become available. Attention has increasingly shifted to the presynaptic side of the synaptic cleft with the realization that kainate receptors can affect the release of both GABA and glutamate. This minireview addresses the current state of knowledge of these presynaptic actions of kainate receptors in the hippocampus and focuses on three questions: (1) what is the consequence of kainate receptor activation for neurotransmitter release, (2) what are the subcellular mechanisms that underlie these effects, and (3) what is the evidence that endogenous glutamate, released from excitatory terminals, can mimic the effects of exogenous kainate?

Kainate Receptor-Mediated Modulation of GABAergic Transmission

Much attention has been devoted to the finding that exogenous kainate receptor agonists depress monosynaptic IPSPs and IPSCs recorded in hippocampal pyramidal neurons (reviewed by Lerma et al., 2001). This phenomenon has recently been overshadowed by the observation that submicromolar kainate causes interneurons to fire spontaneously, either by acting on synaptic kainate receptors (Ben-Ari and Cossart, 2000; Frerking and Nicoll, 2000) or by triggering ectopic action potentials in axons (Semyanov and Kullmann, 2001). The spontaneous firing of interneurons could potentially explain the kainate-evoked depression of evoked IPSCs without having to invoke a direct action at presynaptic receptors. Extracellular GABA accumulation both desensitizes postsynaptic $GABA_A$ receptors and activates presynaptic $GABA_B$ receptors.

One observation, nevertheless, argues that kainate receptors may have a direct depressant effect on GABA release. Kainate has been reported to reduce the frequency of action potential independent miniature IPSCs (mIPSCs) (Lerma et al., 2001). However, this effect of kainate has not been observed universally, in spite of apparently similar experimental conditions (Ben-Ari and Cossart, 2000; Frerking and Nicoll, 2000). The reasons for this discrepancy remain unclear. It is, in fact, surprising that kainate receptors, which gate a cation channel, should decrease transmitter release, since direct depolarization of presynaptic terminals generally has the opposite effect. Rodriguez-Moreno and Lerma (1998) have argued that kainate receptors depress GABA release not via depolarization of presynaptic terminals, but by triggering a metabolic cascade involving both G proteins and protein kinase C. This controversial suggestion has received some support from the examination of the effect that kainate receptor agonists have on GABA release from synaptosomes (Cunha et al., 2000). A metabotropic action of kainate receptors (albeit one that does not involve protein kinases) has also been reported at glutamatergic synapses on CA1 pyramidal neurons (Frerking et al., 2001, see below).

The action of kainate on inhibitory transmission recently took an unexpected turn with the report that kainate in submicromolar concentrations can actually enhance GABAergic transmission. Cossart et al. (2001) reported this effect when recording either evoked IPSCs or mIPSCs in interneurons, leading to the conclusion that kainate directly potentiates GABA release at synapses among interneurons. They, however, saw no effect of kainate on IPSCs in pyramidal neurons, suggesting that the identity of the postsynaptic neuron might determine the distribution and/or function of presynaptic kainate receptors. Mulle et al. (2000) also reported a kainate-evoked increase in mIPSC frequency in interneurons, although with a relatively high concentration (10 μM). A recent report by Semyanov and Kullmann (2001), however, calls for caution in interpreting an enhancement of evoked IPSCs, while at the same time arguing that depolarization of the axons of interneurons is potentially a major mechanism underlying the complex actions of kainate. Submicromolar kainate reduced the threshold for electrically evoking antidromic action potentials in the axons of interneurons and even triggered the appearance of spontaneous ectopic action potentials. This finding implies that the kainate-evoked increase in evoked IPSC amplitude reported by Cossart et al. (2001) could be at least partially due to the recruitment of more axons by the stimulating electrode.

The observation that kainate enhanced the frequency of mIPSCs (Cossart et al., 2001; Mulle et al., 2000) nevertheless argues for a direct effect on the terminals of interneurons. However, this finding too has not been universally reproduced, although differences between species may hold the key (Semyanov and Kullmann, 2001). A possible explanation is that axonal kainate receptors are positioned at variable distances from the

[1] Correspondence: d.kullmann@ion.ucl.ac.uk

GABA release sites. In the presence of tetrodotoxin, the axonal depolarization (and/or Ca^{2+} influx) triggered by kainate receptor activation may not always be sufficient to spread to the GABA release sites to affect spontaneous exocytosis.

Paired recordings of both presynaptic and postsynaptic neurons have an advantage in the fact that changes in axonal excitability should not affect the initiation of orthodromic action potentials (although intermittent failure of the invasion of axonal terminals could confuse the issue). To date, this approach has only been applied to study interneuron-pyramidal neuron transmission. Jiang et al. (2001) reported a complex dose-dependent effect of kainate on unitary IPSCs. At a concentration of 5 μM, kainate depressed GABAergic transmission, although only at connections where presynaptic action potentials had an initially high probability of evoking an IPSC. This result is consistent with early studies on IPSPs/IPSCs evoked by extracellular stimulation (Lerma et al., 2001), although it does not distinguish between a direct effect on presynaptic release sites and an indirect effect involving spontaneous firing of interneurons and extracellular GABA accumulation. However, when a submicromolar concentration was applied (0.3 μM), kainate enhanced GABAergic transmission. Again, this was only seen at a subset of connections, although in this case those where presynaptic action potentials had an initially low probability of evoking an IPSC.

The mechanisms by which kainate enhances transmission remain to be elucidated. Depolarization of presynaptic terminals might enhance transmitter release by promoting inactivation of K^+ channels. Alternatively, because kainate receptors themselves can be Ca^{2+} permeable, a Ca^{2+} influx might interact with the Ca^{2+} transient triggered by an action potential invasion. A further possibility is that kainate receptor-mediated depolarization enhances action potential invasion of axonal branches (although see Jiang et al., 2001).

An alternative method to probe the role of kainate receptors in modulating GABA release is to delete individual receptor subunits selectively (Mulle et al., 2000). Knocking out GluR5 and GluR6 receptors together profoundly reduced the depolarization of interneurons by kainate or domoate and also reduced the depression of IPSCs evoked in pyramidal neurons. Because deletion of one subunit alone failed to abolish these effects, Mulle et al. (2000) proposed that heteromeric receptors containing GluR5 and GluR6 mediate both phenomena (see also Lerma et al., 2001). These results are consistent with the hypothesis that the depression of evoked inhibition is actually caused by the intense spontaneous firing among interneurons, leading to extracellular GABA accumulation (Frerking and Nicoll, 2000), though they do not rule out an additional direct effect on interneuron terminals. As for the enhancement of GABA release, only preliminary results have been reported: a relatively high concentration of kainate increased the frequency of mIPSCs recorded in interneurons in wild-type and GluR5 knockout mice, but not in GluR6 knockouts (Mulle et al., 2000). It will be important to repeat this study with submicromolar kainate in an effort to relate this result to the phenomena studied by Cossart et al. (2001), Semyanov and Kullmann (2001), and Jiang et al. (2001).

What is the (mal)adaptive significance of presynaptic kainate receptors? Axo-axonic glutamatergic synapses are not known to occur in the hippocampus. Therefore, if endogenous glutamate is able to reach presynaptic kainate receptors, it is likely to diffuse from neighboring excitatory synapses. Evidence exists to support both decreases and increases in GABAergic transmission to pyramidal neurons in response to endogenous glutamate release. Brief trains of stimuli to Schaffer collaterals have been shown to depress GABAergic IPSCs evoked within 100 ms, through a kainate receptor-mediated mechanism (Min et al., 1999). Jiang et al. (2001), on the other hand, reported that brief trains of stimuli delivered to stratum radiatum were followed by a prolonged increase in unitary IPSC amplitude and synaptic success rate, which was sensitive to kainate receptor blockers. This enhancement lasted several minutes, even though glutamate uptake is generally thought to clear the neurotransmitter from the extracellular space within a few milliseconds. If a low concentration of glutamate can persist in the extracellular space for this length of time, the kainate receptors underlying the enhancement may have a very high sensitivity to glutamate indeed.

GABAergic inhibition among interneurons can also be enhanced by glutamate spillover from neighboring excitatory synapses acting on kainate receptors (Cossart et al., 2001). However, because the IPSCs were evoked by extracellular stimulation, this observation does not distinguish between an enhancement of release probability and increased excitability of presynaptic axons. Indeed, Semyanov and Kullmann (2001) showed that brief trains of stimuli to glutamatergic axons increased the success rate for evoking antidromic action potentials in the axons of interneurons.

Although many inconsistencies remain, a tentative synthesis of the above results is that low concentrations of kainate receptor agonists (whether endogenous or exogenous) potentiate GABA release, while higher concentrations depress it (Figure 1). The enhancement can be attributed to presynaptic and/or axonal depolarization. If the main target is pyramidal neurons, the phenomenon offers an obvious homeostatic mechanism to regulate circuit excitability—a build up of extracellular glutamate will enhance inhibition of principal neurons. As for the role of depression of inhibition by higher concentrations of agonist, it is at least partly explained by the spontaneous firing of interneurons depolarized via axonal and somatodendritic receptors. A direct metabotropic action via presynaptic kainate receptors may also contribute. Its role is difficult to predict; the heterosynaptic depression of GABAergic transmission to pyramidal neurons could disinhibit the circuitry and trigger the initiation of seizures. However, because this is accompanied by an increase in interneuron firing, the net effect might still be to dampen hippocampal activity.

Kainate Receptor-Mediated Modulation of Glutamatergic Transmission

The role of presynaptic kainate receptors in modulating glutamate release has undergone a similar shift, with initial reports of depression eclipsed by the discovery that, at least at mossy fiber synapses, kainate receptors can actually enhance glutamate release.

Early indications that kainate depresses excitatory signaling came from examining glutamate efflux from hippocampal synaptosomes and evoked NMDA receptor-mediated EPSCs in the CA1 region (Chittajallu et al.,

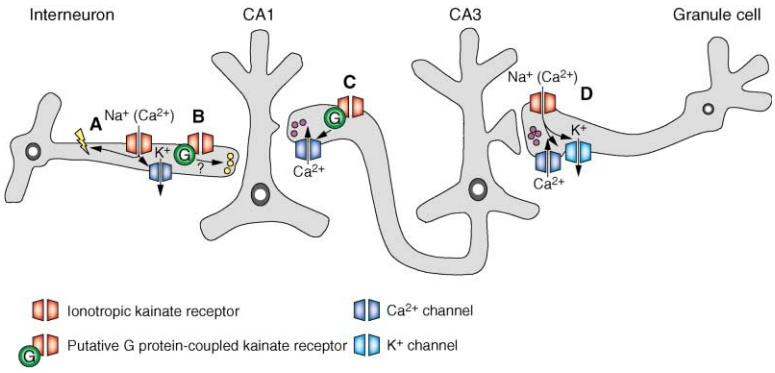

Figure 1. Presynaptic and Axonal Kainate Receptors Have Varied Roles at Different Hippocampal Synapses

(A) Presynaptic receptors in interneurons depolarize axons, trigger ectopic action potentials, and enhance GABA release (possibly by inactivating K^+ channels and/or by directly mediating Ca^{2+} influx). (B) Receptors at interneuron-pyramidal neuron synapses have also been reported to depress GABA release via a metabotropic action.

(C) Presynaptic receptors at Schaffer collateral-CA1 pyramidal neuron synapses depress glutamate release, apparently through a metabotropic mechanism that acts upon Ca^{2+} channels.

(D) At mossy fiber synapses on CA3 pyramidal neurons, kainate receptor agonists have a biphasic effect. At low concentrations they enhance glutamate release, possibly by inactivating K^+ channels, and at higher concentrations they depress glutamate release, possibly by inactivating Ca^{2+} channels. Evoking glutamate spillover from neighboring synapses has mimicked most of the above phenomena.

1996). GABA receptor antagonists failed to prevent the effect of kainate, implying a direct presynaptic action. Interestingly, submicromolar kainate (0.3 μM) actually increased EPSCs, an early hint that kainate receptors might have a complex effect on transmission. Kamiya and Ozawa (2000) reported that the depression of glutamatergic transmission by kainate is accompanied by a decrease in Ca^{2+} influx into presynaptic terminals, but no change in fiber volley. Although this implies a decrease in Ca^{2+} influx downstream of action potential invasion, confounding effects of interneuron firing and presynaptic $GABA_B$ receptor activation were not ruled out in this study.

Surprisingly, Frerking et al. (2001) reported that *N* ethylmaleimide and pertussis toxin prevent the action of kainate on CA1 synapses, implying a role for G proteins, in particular G_i/G_o. The phenomenon persisted in the presence of a variety of blockers of seven transmembrane domain G protein-coupled receptors and was also unaffected by manipulations to prevent interneuron firing. In contrast to the metabotropic cascade implicated in the depression of GABA release, protein kinase inhibitors were without effect. A parsimonious explanation is that G protein subunits dissociate upon activation of presynaptic kainate receptors followed by a direct interaction with Ca^{2+} channels (Figure 1). However, the results do not conclusively rule out an alternative model where G proteins have only a permissive role. In this instance, cation flux through presynaptic kainate receptors could conceivably decrease transmitter release through another mechanism that is under the tonic control of G proteins.

Although the molecular identity of the kainate receptors mediating the depression of transmission in CA1 is not known, these synapses share many features with associational-commisural synapses on CA3 pyramidal neurons. Kainate application has been shown to depress transmission here too, and this effect is abolished in GluR6 (but not GluR5) knockouts (Contractor et al., 2000). Another excitatory input to the distal dendrites of CA3 pyramidal neurons, the perforant path, was actually potentiated by 3 μM kainate. Knocking out either GluR5 or GluR6 prevented this effect. It remains to be determined whether this phenomenon reflects a GluR5/6-mediated increase in presynaptic axon excitability or a direct effect on transmitter release.

Kainate receptors are especially abundant in stratum lucidum, the termination zone of mossy fibers. Lesioning granule cells profoundly reduces kainate binding in stratum lucidum (Ben-Ari and Cossart, 2000), implying a presynaptic location on mossy fibers themselves. Both kainate and ATPA, an agonist with some preference for GluR5 containing receptors, depress mossy fiber EPSCs recorded in CA3 pyramidal neurons (Kamiya and Ozawa, 2000; Vignes et al., 1998). Action potential-dependent Ca^{2+} influx into mossy fiber terminals is also decreased (Kamiya and Ozawa, 2000). However, because $GABA_B$ receptors were not blocked in these studies, at least part of the depression could potentially be reinterpreted as an indirect action mediated by interneuron firing. Nevertheless, when these receptors were blocked, kainate was still effective (Schmitz et al., 2000), and knockout experiments implicated GluR6 rather than GluR5 (Contractor et al., 2000). This finding is consistent with the finding that GluR6 is abundant in mossy fibers and granule cells (reviewed by Schmitz et al., 2001a).

In striking contrast to the effect in CA1, the action of kainate is accompanied by an enhancement of the presynaptic fiber volley (Kamiya and Ozawa, 2000), and this persists in the presence of GABA receptor antagonists (Schmitz et al., 2000). Mossy fibers are also rendered more excitable, as witnessed by a decrease in the threshold for evoking antidromic action potentials recorded in granule cells. Schmitz et al. (2000) further showed that brief trains of stimuli delivered to other glutamatergic axons mimic the effect of exogenous agonist application on the mossy fiber volley and threshold. This finding implies that glutamate can both diffuse a relatively long distance to the mossy fibers and affect them downstream of the initiation of action potentials by the electrical stimulus.

Schmitz et al. (2001a, 2001b) reported that concentrations of kainate as low as 50 nM increase the mossy fiber volley. This prompts a reexamination of the effects on synaptic transmission. Although 0.5 μM kainate depressed NMDA receptor-mediated EPSCs recorded in CA3 pyramidal neurons, lower concentrations (20–50 nM) actually potentiated them (Schmitz et al., 2001b). The physiological counterpart of this biphasic effect was shown by reducing the number of conditioning pulses delivered to stratum radiatum: heterosynaptic depression was converted into heterosynaptic facilitation.

These results imply that presynaptic kainate receptors have a complex role in filtering information at these strategically important hippocampal synapses (Figure 1). The underlying biophysical mechanisms remain to be resolved but may include the inactivation of K^+ channels with modest depolarization and the inactivation of Ca^{2+} channels with more profound depolarization.

Kainate receptors also contribute to the pronounced facilitation seen at mossy fiber synapses when the presynaptic axons are repeatedly stimulated at intermediate frequencies (0.33–100 Hz) (Schmitz et al., 2001b). Contractor et al. (2000) showed that deleting GluR6 reduced frequency-dependent facilitation at mossy fibers, although deleting GluR5 had no effect. A possible interpretation of this finding is that GluR6-containing kainate receptors act as facilitatory autoreceptors, amplifying glutamate release from the presynaptic terminal in response to extracellular glutamate accumulation. It remains to be determined whether this phenomenon can be evoked at a single mossy fiber or whether it depends on glutamate spillover among multiple discharging mossy fibers. Contractor et al. (2000) further reported that deleting GluR6 (but not GluR5) profoundly reduced the magnitude of mossy fiber LTP elicited by tetanic stimulation. This suggests that kainate receptor-mediated presynaptic depolarization may help to trigger the LTP induction cascade. However, given the bimodal effect of kainate on transmitter release (Schmitz et al., 2001b), one might expect the opposite: intense kainate receptor-mediated depolarization could inactivate presynaptic Ca^{2+} channels.

Evidence for the involvement of kainate receptors in mossy fiber LTP actually predates these reports: Bortolotto et al. (1999) showed that mossy fiber LTP was abolished when kainate receptors were blocked by a novel compound, LY382884, which spares NMDA and AMPA receptors. Very recently, Lauri et al. (2001) reported that the same antagonist also attenuates frequency-dependent facilitation of mossy fiber EPSCs. LTP at mossy fiber synapses has previously been reported to reduce short-term facilitation when brief trains of stimuli are repeatedly applied to presynaptic axons. Interestingly, the effect of LY382884 on facilitation is also profoundly reduced after induction of LTP. On the basis of this occlusion, Lauri et al. (2001) proposed that mossy fiber LTP and kainate receptor activation converge on a common expression mechanism. This mechanism could be none other than depolarization itself or a downstream consequence of depolarization (such as K^+ channel inactivation) because LTP also occluded the effect of elevating the extracellular K^+ concentration on transmission.

A puzzle, however, surrounds the results obtained by applying LY382884. This compound has been reported to be selective for GluR5-containing receptors and is inactive at GluR6 receptors (Bortolotto et al., 1999). It is thus difficult to reconcile the profound effects of LY382884 with the failure of GluR5 deletion to affect either frequency facilitation or LTP at mossy fibers (Contractor et al., 2000; Huettner, 2001). Further complicating matters, mossy fiber LTP has even been induced in the temporary presence of broad spectrum ionotropic glutamate receptor blockers (Schmitz et al., 2001a), ruling out an absolute requirement for presynaptic kainate receptor activation in LTP induction.

Clearly, there is a long way to go before the uncertainties surrounding the role of kainate receptors in mossy fiber LTP are resolved. In particular, it will be important to document more fully the subunit composition of native presynaptic and postsynaptic kainate receptor, in order to explain the apparent selectivity profile of LY382884. Nevertheless, it is safe to conclude that these presynaptic receptors have a radically different role from kainate receptors at Schaffer collateral synapses on CA1 pyramidal neurons where they depress glutamate release, possibly through a metabotropic cascade.

Conclusion

Our understanding of the roles of presynaptic kainate receptors has taken two roughly parallel but tortuous routes through the last few years. Evidence exists for both enhancement and depression of release of both GABA and glutamate. Although there are important differences in location, mechanism, and subunit composition, both metabotropic and ionotropic cascades have been implicated. Kainate receptors will no doubt continue to surprise us, now that they have come into the limelight.

Selected Reading

Ben-Ari, Y., and Cossart, R. (2000). Trends Neurosci. *23*, 580–587.

Bortolotto, Z.A., Clarke, V.R., Delany, C.M., Parry, M.C., Smolders, I., Vignes, M., Ho, K.H., Miu, P., Brinton, B.T., Fantaske, R., et al. (1999). Nature *402*, 297–301.

Chittajallu, R., Vignes, M., Dev, K.K., Barnes, J.M., Collingridge, G.L., and Henley, J.M. (1996). Nature *379*, 78–81.

Contractor, A., Swanson, G.T., Sailer, A., O'Gorman, S., and Heinemann, S.F. (2000). J. Neurosci. *20*, 8269–8278.

Cossart, R., Tyzio, R., Dinocourt, C., Esclapez, M., Hirsch, J.C., Ben-Ari, Y., and Bernard, C. (2001). Neuron *29*, 497–508.

Cunha, R.A., Malva, J.O., and Ribeiro, J.A. (2000). FEBS Lett. *469*, 159–162.

Frerking, M., and Nicoll, R.A. (2000). Curr. Opin. Neurobiol. *10*, 342–351.

Frerking, M., Schmitz, D., Zhou, Q., Johansen, J., and Nicoll, R.A. (2001). J. Neurosci. *21*, 2958–2966.

Huettner, J.E. (2001). Trends Neurosci. *24*, 365–366.

Jiang, L., Xu, J., Nedergaard, M., and Kang, J. (2001). Neuron *30*, 503–513.

Kamiya, H., and Ozawa, S. (2000). J. Physiol. *523*, 653–665.

Lauri, S.E., Bortolotto, Z.A., Bleakman, D., Ornstein, P.L., Lodge, D., Isaac, J.T.R., and Collingridge, G.L. (2001). Neuron *32*, this issue, 697–709.

Lerma, J., Paternain, A.V., Rodriguez-Moreno, A., and Lopez-Garcia, J.C. (2001). Physiol. Rev. *81*, 971–998.

Min, M.Y., Melyan, Z., and Kullmann, D.M. (1999). Proc. Natl. Acad. Sci. USA *96*, 9932–9937.

Mulle, C., Sailer, A., Swanson, G.T., Brana, C., O'Gorman, S., Bettler, B., and Heinemann, S.F. (2000). Neuron *28*, 475–484.

Rodriguez-Moreno, A., and Lerma, J. (1998). Neuron *20*, 1211–1218.

Schmitz, D., Schmitz, D., Frerking, M., and Nicoll, R.A. (2000). Neuron *27*, 327–338.

Schmitz, D., Mellor, J., Frerking, M., and Nicoll, R.A. (2001a). Proc. Natl. Acad. Sci. USA *98*, 11003–11008.

Schmitz, D., Mellor, J., and Nicoll, R.A. (2001b). Science *291*, 1972–1976.

Semyanov, A., and Kullmann, D.M. (2001). Nat. Neurosci. *4*, 718–723.

Vignes, M., Clarke, V.R., Parry, M.J., Bleakman, D., Lodge, D., Ornstein, P.L., and Collingridge, G.L. (1998). Neuropharmacology *37*, 1269–1277.