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Commentary

Kainate receptors in hippocampal CA3 subregion: evidence for a role in regulating neurotransmitter release

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Abstract

The hippocampal CA3 subregion of the rat is characteristically enriched in kainate receptors. At the synaptic level, the subcellular localization of these receptors is still a matter of debate. The CA3 pyramidal cells are particularly sensitive to excitotoxicity induced by kainate, which is in agreement with the high levels of kainate receptors in the *stratum lucidum* of the hippocampal CA3 subregion. Immunocytochemical studies, using antibodies against kainate receptor subunits, clearly demonstrated the presence of postsynaptic kainate receptors. However, it was not possible at the time to identify the activity of postsynaptic kainate receptors as mediators of the synaptic transmission. There are also reports showing the labeling of unmyelinated axons and nerve terminals with antibodies against kainate receptor subunits. The evidence for the presence of presynaptic kainate receptors in the hippocampus is further substantiated by the demonstration that stimulation of kainate receptors in synaptosomes isolated from the rat hippocampal CA3 subregion increases the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) coupled to the release of glutamate. These results support the model proposed by Coyle (1983), in which the excitotoxicity induced by kainate involves the activation of presynaptic kainate receptors, causing the release of glutamate. According to this model, the neurotoxic effect of kainate in the rat hippocampal CA3 subregion involves a direct effect on presynaptic kainate receptors and an indirect effect on postsynaptic glutamate receptors due to the enhanced release of glutamate.

L-Glutamate is the major excitatory neurotransmitter in the brain. The effects of glutamate are mediated through the interaction with glutamate receptors located presynaptically, postsynaptically, or on glial cells. In nerve terminals, glutamate receptors may induce the release of neurotransmitters, or modulate the response of the synapse to the next stimulus (Ruzicka and Jhamandas, 1993; Verhage *et al.*, 1994).

Glutamate receptors may be classified as metabotropic, which are coupled to G-protein second messenger pathway systems, or may be ionotropic allowing the direct influx of cations (Ruzicka and Jhamandas, 1993; Schoepp and Conn, 1993; Pin and Bockaert, 1995). Ionotropic glutamate receptors form a complex family of glutamate receptors with both NMDA and non-NMDA receptors. These two groups have different glutamate receptor subunit compositions and also distinct pharmacological characteristics (Hollmann and Heinemann, 1994). In contrast to non-NMDA receptors, the NMDA receptors are efficiently activated by NMDA, require the presence of glycine to be fully active, are blocked by physiological concentrations of Mg^{2+} and are insensitive to either AMPA or kainate (Hollmann and Heinemann, 1994).

Non-NMDA glutamate ionotropic receptors are composed by homomeric or heteromeric associations of either AMPA receptor subunits, $GluR_{A\text{-}D}$, or kainate receptor subunits, $GluR_{5\text{-}7}$ and $KA_{1\text{-}2}$ (Hollmann and Heinemann, 1994; Bettler and Mulle, 1995). The different non-NMDA ionotropic receptors studied to date have a permeability to Ca^{2+} lower than that of NMDA receptors (Bettler and Mulle, 1995; Mori and Mishina, 1995). However, several factors may contribute to the existence of a diversity of non-NMDA glutamate receptors with different Ca^{2+} permeability, such as subunit composition (Hollmann *et al.*, 1991; Verdoorn *et al.*, 1991; Burnashev *et al.*, 1992), mechanisms of alternative splicing (Sommer *et al.*, 1990; Sommer and Seeburg, 1992), and editing of mRNA

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(Bettler and Mulle, 1995; Seeburg, 1993; Köhler *et al.*, 1993).

Both AMPA and kainate receptors are non-NMDA ionotropic glutamate receptors efficiently activated by either AMPA or kainate. However, the order of agonist potency for these two groups of receptors is quite different. The usual agonist potency order for the activation of the AMPA receptors is: quisqualate > AMPA > glutamate > kainate (Keinänen *et al.*, 1990; Hollmann and Heinemann, 1994; Bettler and Mulle, 1995), but the relative potency of each agonist depends on the subunit composition of the receptors (Hollmann and Heinemann, 1994). Kainate receptors are activated by lower concentrations of kainate as compared to AMPA. Also, kainate receptors are formed by two different groups, which are low-affinity kainate receptors ($GluR_{5-7}$) and high-affinity kainate receptors (KA_{1-2}). The agonist potency order is domoate > kainate > glutamate > AMPA, for the low affinity kainate receptors, or kainate > domoate > glutamate > > AMPA, for high-affinity kainate receptors (Hollmann and Heinemann, 1994; Bettler and Mulle, 1995).

The quinoxaline derivative, CNQX, is the most well known competitive antagonist of non-NMDA glutamate receptors (Honoré *et al.*, 1988; Egebjerg *et al.*, 1991). Another quinoxaline compound, NBQX, is a stronger inhibitor of AMPA receptors as compared to kainate receptors (Sheardown *et al.*, 1990). By comparing the inhibitory effects of the two compounds, it is possible to distinguish between the activity of kainate receptors and AMPA receptors (Sheardown *et al.*, 1990). Recently, a new compound, 5-nitro-6,7,8,9, tetrahydrobenzo[g]indole-2,3-dione-3-oxime (NS-102), was used as a specific competitive antagonist of low affinity kainate receptors (Johansen *et al.*, 1993; Verdoorn *et al.*, 1994). Also, a new non-competitive and specific inhibitor of AMPA receptors, GYKI 53655, is a useful tool to distinguish between the activity of AMPA and kainate receptors, since kainate receptors are insensitive to this compound (Partenain *et al.*, 1995).

1. Kainate receptors

The physiology, subunit constitution and subcellular localization of native kainate receptors in the CA3 subregion of the hippocampus persist largely unknown, although, in recent years, several groups of investigators contributed to partially clarifying this important problem (Lerma *et al.*, 1997; Huettner, 1997; Petralia, 1997).

1.1. *[^{3}H]kainate binding sites*

The labeling of brain slices with [^{3}H]kainate showed the existence of two different binding sites; low-affinity binding sites ($Kd \sim 60$ nM) and high-affinity binding sites ($Kd \sim 10$ nM) (Foster *et al.*, 1981; Unnerstall and Wamsley, 1983; Sommer and Seeburg, 1992; Garcia-Ladona and Gombos, 1993). Both in mice and in rat hippocampal CA3 subregion, there exist a large number of kainate binding sites in the *stratum lucidum*, corresponding to the mossy fiber nerve terminals region (Foster *et al.*, 1981; Unnerstall and Wamsley, 1983; Represa *et al.*, 1987; Miller *et al.*, 1990; Garcia-Ladona and Gombos, 1993; Frotscher *et al.*, 1994). The presence of high-affinity kainate binding sites in the *stratum lucidum* of the rat CA3 subregion is an indication of a synaptic localization of high-affinity kainate receptors. Accordingly, the ontogeny of [^{3}H]kainate binding sites occurs within the period of maturation of synapses formed between the mossy fiber projections and the CA3 pyramidal cells (Miller *et al.*, 1990; Dessi *et al.*, 1991). It was shown that these binding sites are particularly concentrated in synaptic plasma membranes, specially at the synaptic junctions (Foster *et al.*, 1981), in contrast with the subcellular localization of [^{3}H]AMPA binding sites, since the latter are specially abundant in the microsomal fraction (Henley, 1995).

1.2. *Distribution of mRNA for kainate receptor subunits in the hippocampus*

The distribution of mRNA for Glu R_6, KA_1 and KA_2 subunits is in agreement with the high levels of high-affinity kainate binding sites, specially in the CA3 subregion of the hippocampus. High levels of KA_2 subunits are expressed in all hippocampal subregions (Herb *et al.*, 1992; Wisden and Seeburg, 1993b); Bahn *et al.*, 1994). The presence of mRNA for KA_1 subunits is characteristic of CA3 pyramidal cells, since this kainate receptor subunit is specially abundant in the CA3 subregion, with lower levels in the dentate gyrus (Werner *et al.*, 1991; Herb *et al.*, 1992; Wisden and Seeburg, 1993b; Bahn *et al.*, 1994). Low-affinity kainate receptor subunits are also expressed in the hippocampus. For $GluR_6$, the mRNA is abundant in the dentate gyrus and also in the CA3 subregion (Egebjerg *et al.*, 1991; Wisden and Seeburg, 1993b; Bahn *et al.*, 1994). $GluR_7$ is not expressed in high levels in the hippocampus, but dentate gyrus granule cells show moderate levels of mRNA for $GluR_7$ (Bettler *et al.*, 1992; Lomeli *et al.*, 1992; Wisden and Seeburg, 1993b; Bahn *et al.*, 1994). $GluR_5$ is expressed in low levels in CA1 subregion and is almost absent in other subregions of the hippocampus (Bettler *et al.*, 1990; Wisden and Seeburg, 1993b; Bahn *et al.*, 1994). However, the scattered pattern of the in situ hybridization labeling for the $GluR_5$ mRNA indicates that some interneurons may express high levels of mRNA for $GluR_5$ (Bahn *et al.*, 1994).

The distribution of mRNA for the various kainate receptor subunits suggests that CA3 pyramidal cells may express kainate receptors having $GluR_6$, KA_1 or KA_2 subunits, and that dentate gyrus granule cells may express

receptors with $GluR_6$, $GluR_7$, KA_1 or KA_2 subunits (Bahn and Wisden, 1997). The latter constitution for kainate receptors may be expected for the subunit arrangement of the putative mossy fiber nerve terminal kainate receptors, since the mRNA for these receptors may be expected to reside on dentate gyrus granule cells.

1.3. *Immunocytochemistry*

The use of antibodies recognizing $GluR_{6/7}$ (Petralia *et al.*, 1994), $GluR_{5/6/7}$ (Huntley *et al.*, 1993; Siegel *et al.*, 1995), or KA_2 subunits (Petralia *et al.*, 1994; Roche and Huganir, 1995) revealed a similar distribution of glutamate receptor subunits and of [^{3}H]kainate binding sites. In the rat and monkey hippocampus, the immunoreactivity with these antibodies occurs mainly in the hilus and in the CA3 subregion (Petralia *et al.*, 1994; Siegel *et al.*, 1995). At the synaptic level, the localization of kainate receptor subunits appears to be principally at the postsynaptic membranes (Petralia *et al.*, 1994; Huntley *et al.*, 1993; Siegel *et al.*, 1995). However, presynaptic KA_2 subunits were also identified in nerve terminals of the rat cerebral and cerebellar cortex (Petralia *et al.*, 1994). Also, antibodies against $GluR_{6/7}$ (Petralia *et al.*, 1994) react strongly with unmyelinated axons, which possibly are mossy fibers. Mossy fibers in the CA3 subregion of the monkey hippocampus are also stained with antibodies against $GluR_{5/6/7}$ (Siegel *et al.*, 1995). Some myelinated axons of the CA3 subregion of the rat hippocampus and also some unmyelinated axons of the cerebral and cerebellar cortex of the rat are recognized by an antibody against KA_2 subunit (Petralia *et al.*, 1994).

Several factors may account for the labeling of axons with kainate receptor antibodies, and may include nonspecific staining, nonfunctional transport of receptor subunits along the axon, nonsynaptic localization of kainate receptors, or presynaptic kainate receptors (Petralia, 1997). The presence of functional presynaptic kainate receptors in the CA3 subregion of the rat hippocampus is supported by several biochemical and physiological observations (Represa *et al.*, 1987; Ben-Ari and Gho, 1988) and by our demonstration, for the first time, that kainate receptor stimulation increases the $[Ca^{2+}]_i$ in hippocampal synaptosomes (Malva *et al.*, 1995). However, there are no clear immunocytochemical demonstrations of these presynaptic receptors, which may be due, in part, to conformational changes, to association of the receptors with other molecules, or to the presence of undetectable low levels of kainate receptor subunits (Petralia, 1997).

1.4. *Electrophysiology*

One of the most important problems in investigating kainate receptors is the lack of electrophysiological activity of kainate receptors in hippocampal slices, using patch-clamp techniques (Jonas and Sakmann, 1992; McBain and Dingledine, 1993). Several factors may account for this difficulty, including the localization of kainate receptors in distal dendrites or in nerve terminals, the localization of kainate receptors in glial cells, and also the AMPA receptors-mediated non-desensitizing currents elicited by kainate (Wisden and Seeburg, 1993a; Bettler and Mulle, 1995) which may mimic the effect of kainate on kainate receptors.

The use of the novel AMPA receptor antagonists, GYKI 52466 and GYKI 53655, allows identification of the rapidly desensitizing currents elicited by kainate, attributed to the activity of kainate receptors (Partenain *et al.*, 1995) in hippocampal neuronal microcultures. However, kainate receptors do not mediate synaptic transmission in these hippocampal cultures (Lerma *et al.*, 1997), although there are strong immunocytochemical evidences for the presence of postsynaptic kainate receptors in these cells (Lerma *et al.*, 1997). These observations are consistent with the lack of detection of kainate receptor-induced currents in apical dendrites of the CA3 and CA1 pyramidal cells in slices of the hippocampus (Spruston *et al.*, 1995).

1.5. *Excitotoxicity*

The high levels of kainate receptors in the CA3 subregion of the rat hippocampus (Hollmann and Heinemann, 1994) is in accordance with observations that the CA3 pyramidal cells are particularly sensitive to kainate receptor agonists-induced excitotoxicity (Nadler *et al.*, 1978; Coyle, 1983; Represa *et al.*, 1987). The best evidence for presynaptic kainate receptors was obtained through lesion studies. It was observed that [^{3}H]kainate binding sites in the stratum lucidum of the rat hippocampus are more sensitive to colchicine lesion of granule cells than to kainate induced CA3 pyramidal cells death (Represa *et al.*, 1987). Also, neonatal γ-ray irradiation reduce the number of granule cells and prevent the epileptic action of kainate (Gaiarsa *et al.*, 1994).

The kindling model of experimental epileptogenesis involves the generation of spontaneous seizures together with reorganization of mossy fiber synapses (McNamara, 1988; Sutula *et al.*, 1988; Ben-Ari and Represa, 1990; Cavazos *et al.*, 1991). It is interesting that together with synaptic reorganization of mossy fiber projections there appeared new [^{3}H]kainate binding sites in the infrapyramidal cell layer of the CA3 subregion (Ben-Ari and Represa, 1990). One may speculate that the new [^{3}H]kainate binding sites appearing in the infrapyramidal cell layer are presynaptic kainate receptors in mossy fiber terminals. However, it is also possible that this [^{3}H]kainate binding sites are postsynaptic kainate receptors which appear after the formation of these new abnormal synapses.

1.6. *Presynaptic kainate receptors and regulation of neurotransmitter release*

The model proposed by Joseph T. Coyle (1983) to explain the specially high sensitivity of CA3 pyramidal cells to kainate-induced toxicity, postulates the involvement of presynaptic kainate receptors in mediating the release of glutamate in this brain region. However, until very recently, it had not been possible to clearly show physiological activity of presynaptic kainate receptors in CA3 nerve terminals.

Presynaptic kainate receptors in rat mossy fiber synaptosomes may be involved in the domoate or kainate-induced increase in glutamate release elicited by KCl depolarization (Gannon and Terrian, 1991; Terrian *et al.*, 1991). However, due to the high concentration of agonists used, activation of AMPA receptors may also occur. In apparent contradiction, it was also shown that stimulation of presynaptic kainate receptors reduces the release of [^{3}H]glutamate induced by 4-aminopyridine or KCl depolarization (Chittajallu *et al.*, 1996), and these authors also observed a decrease in synaptic transmission in CA1 Schaffer collateral-commissural synapses (Chittajallu *et al.*, 1996). However, interestingly, the authors observed that in some synapses this inhibitory effect is preceded by a transient increase in synaptic transmission, which the authors attributed to an initial and transient release of glutamate (Chittajallu *et al.*, 1996).

The inhibitory synaptic transmission in the hippocampus may be depressed following activation of presynaptic kainate receptors (Clarke *et al.*, 1996), which may be attributed to the inhibition of GABA release, in accordance to the observation made in rat hippocampal synaptosomes (Cunha *et al.*, 1997). Inhibition of GABA release by presynaptic kainate receptors may contribute to the increase in neuronal excitability induced by kainate receptor agonists, eventually causing neuronal toxicity. The presence of presynaptic kainate receptor subunits in non-glutamatergic synapses was also shown in the cerebellar and cerebral cortex of the rat, using an antibody against KA_2 kainate receptor subunit (Petralia *et al.*, 1994).

Recently, we identified the activity of glutamate receptors, in synaptosomes isolated from the CA3 subregion of rat hippocampus, which modulate the $[Ca^{2+}]_i$ (Malva *et al.*, 1995). The stimulation of these receptors increased the $[Ca^{2+}]_i$ with the following agonist potency order: domoate (EC_{50}, 0.16 μM) > kainate (EC_{50}, 0.86 μM) > AMPA (EC_{50}, 43.04 μM) (Malva *et al.*, 1996). This agonist potency order, the EC_{50} values, and the sensitivity to the inhibition by CNQX (Malva *et al.*, 1995) are clearly compatible with the activity expected for a kainate receptor (Hollmann and Heinemann, 1994). We also found that the stimulation of kainate receptors induced the exocytotic release of glutamate (Malva *et al.*, 1996), specially in synaptosomes from the hippocampal CA3 subregion. The influx of Ca^{2+} which is coupled to the exocytotic release of neurotransmitters occurs in part through class A and class B voltage sensitive calcium channels (Carvalho *et al.*, 1995).

2. Conclusion

The existing evidence indicates that the rat CA3 hippocampal subregion is specially enriched in kainate receptors. Also, the presence of kainate receptors in this brain subregion is compatible with the specially high sensitivity of CA3 pyramidal neurons to excitotoxicity induced by kainate receptor agonists, like kainate or domoate. For several years the subcellular localization of these kainate receptors was a matter of debate, and even now this problem remains essentially unsolved. However, a large consensus exists that CA3 kainate receptors in the rat hippocampus are synaptic receptors, and also strong immunocytochemical evidences suggest that some receptors may be localized in the postsynaptic membranes. However, physiological studies do not strongly support this hypothesis. Some evidence exists, based on immunocytochemical studies, for the localization of kainate receptors in nerve terminals, including mossy fiber terminals. However, the stronger evidence for the presence of presynaptic kainate receptors was obtained through lesion studies, and by the identification of physiological active kainate receptors in modulating the $[Ca^{2+}]_i$ coupled to the release of glutamate in synaptosomes, in accordance to the high sensitivity of CA3 to excitotoxicity.

Note added in proof

After the acceptance of the present paper two different groups reported the identification of the activity of postsynaptic kainate receptors in CA3 pyramidal cells (*Nature*, *388*, 179–182; *Nature*, *388*, 182–186).

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