Molecular Physiology of Kainate Receptors

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Lerma, Juan, Ana V. Paternain, Antonio Rodríguez-Moreno, and Juan C. López-García. Molecular Physiology of Kainate Receptors. Physiol Rev 81: 971–998, 2001.—A decade ago, our understanding of the molecular properties of kainate receptors and their involvement in synaptic physiology was essentially null. A plethora of recent studies has altered this situation profoundly such that kainate receptors are now regarded as key players in the modulation of transmitter release, as important mediators of the postsynaptic actions of glutamate, and as possible targets for the development of antiepileptic and analgesic drugs. In this review, we summarize our current knowledge of the properties of kainate receptors focusing on four key issues: 1) their structural and biophysical features, 2) the important progress in their pharmacological characterization, 3) their pre- and postsynaptic mechanisms of action, and 4) their involvement in a series of physiological and pathological processes. Finally, although significant progress has been made toward the elucidation of their importance for brain function, kainate receptors remain largely an enigma and, therefore, we propose some new roads that should be explored to obtain a deeper understanding of this young, but intriguing, class of proteins.

I. INTRODUCTION

Fast excitatory neurotransmission in the mammalian nervous system is mainly mediated by glutamate. Albeit the relevance of this amino acid for brain function has been repeatedly underscored, it is impossible to overestimate it. On the one hand, glutamate acts as the transmitter at most excitatory synapses, and it is involved in the induction of long-lasting changes in the efficacy of neurotransmission, changes thought to be cellular correlates of memory formation. On the other, glutamate has a crucial role during the ontogeny of the nervous system, participating in the outgrowth of processes, in the formation and elimination of synapses, and in the activity-dependent fine tuning of exquisitely precise patterns of connectivity in several brain areas. Finally, alterations of glutamatergic neurotransmission have been related to neuronal damage observed after episodes of ischemia and hypoglycemia, as well as to the etiology of a series of neurological conditions including epilepsy, Alzheimer’s disease, Huntington’s chorea, and amyotrophic lateral sclerosis. As a result of this multiplicity of functions, the number of studies devoted to the understanding of glutamate-mediated transmission has grown steadily over the years. Therefore, it is hardly surprising that ionotropic glutamate receptors, cationic channels responsible of converting the synaptic release of the amino acid into an immediate neuronal response, are among the most studied and best-understood molecules of the nervous system (77).

It is now well established that there exist three subtypes of ionotropic glutamate receptors, subtypes that have been named after their preferred ligands: α-amino-
3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-preferring, N-methyl-D-aspartate (NMDA)-preferring, and kainate-preferring receptors. The cloning of a large number of glutamate receptor proteins and the discovery of their structural relationships has confirmed the legitimacy of this pharmacological subdivision. At the same time, it has paved the way to most of our current understanding of the biophysical properties and the physiological role of each subtype in the mammalian brain.

Nowhere is this fact better exemplified than in the study of kainate receptors (27, 51, 90–93). Although the early classification schemes of glutamate receptors, based solely of pharmacological and radioligand binding assays (112, 197), did postulate the existence of a separate class of molecules selective for kainate, the evidence supporting this idea was ambiguous. For example, the fact that a given neuron could exhibit a rapidly desensitizing response upon the application of AMPA (or quisqualate, another agonist commonly used in the early studies) and a nondenstisitizing response to kainate was interpreted as an indication that each ligand was acting on a separate molecular entity. However, the cloning of GluR1, the first AMPA receptor subunit (68), led to the observation that homomeric channels made of this protein could be activated by AMPA and by kainate. Furthermore, the characteristics of the responses elicited by each ligand on GluR1 were very similar to those observed previously in nerve cells (15, 84, 87, 126). In this way, both agonists were shown to act on the same receptor protein, placing in serious doubt the mere existence of kainate receptors.

The subsequent cloning of additional glutamate receptor subunits led eventually to the unequivocal discovery of a group of true kainate receptors (10, 11, 40, 66, 185), channels with a strong preference for this agonist over AMPA, which displayed rapidly desensitizing responses, similar to those observed in preparations like the dorsal root ganglia (DRG) (71). Thus the cloning of a number of kainate receptor subunits was a real breakthrough in the study of these molecules and constitutes the foundation upon which the spectacular progress in their understanding has been built over the last decade.

In this review, we summarize our current knowledge on kainate receptors by first discussing their molecular, biophysical, and pharmacological properties, arenas where the insights derived from the analysis of AMPA and NMDA receptors have been a very valuable asset. We then highlight the possible roles of these receptor molecules during synaptic transmission and illustrate their relevance for brain function with some recent examples where the involvement of kainate receptors at the systems level has been demonstrated. Finally, we speculate on the possibility that this receptor subtype may participate in certain pathological conditions, mainly epilepsy, pointing to the necessity of developing more selective pharmacological agents to test the potential of kainate receptors as targets of antiepileptic drugs.

II. MOLECULAR BIOLOGY OF KAINATE RECEPTORS

A. Identification and Distribution of Kainate Receptor Subunits

Low-stringency, homology-based cloning studies performed during the early 1990s (10, 11, 40, 66, 114, 148, 185) led to the discovery of five kainate receptor subunits termed GluR5, GluR6, GluR7, KA1, and KA2, proteins with molecular masses of ~100 kDa. The degree of similarity among the primary structure of these proteins and other ionotropic glutamate receptor subtypes was shown to be low but significant: ~40% homologous to AMPA receptors and ~20% to NMDA receptors. Based on their amino acid sequence, kainate receptor subunits have been further subdivided into two classes. GluR5, GluR6, and GluR7 share a 75–80% homology, whereas KA1 and KA2 are 68% similar. In contrast, the similarity between the two subclasses is just 45%. A remarkable observation related to the existence of these two subgroups is that, in addition to their marked differences in primary structure, the same division can be traced based on two of their functional characteristics. First, the heterologous expression of GluR5–7 leads to the formation of homomeric channels that can be gated by kainate (40, 152, 160). KA1 and KA2, in contrast, do not form channels on their own but only when coexpressed with the other subunits (66, 185). The resulting receptors possess properties different from the homomers, revealing the ability of KA1 and KA2 to coassemble into functional heteromeric channels (Figs. 1 and 2). Second, binding assays on recombinant receptors have shown that KA1 and KA2 have a significantly higher affinity for kainate than GluR5–7. This observation has led to the suggestion that the two classes correspond to the high- and low-affinity kainate binding sites originally described by the early autoradiographic studies (112, 113). Although definitive proof is still missing, in situ hybridization studies have lent some support to this idea by showing that the distribution of the low- and high-affinity radioligand binding sites roughly predicts the observed localization of each subunit.

In addition to the identification of these bona fide mammalian kainate receptors, several additional homologous proteins capable of binding kainate have been cloned from other species including chick (58), frog (73, 179, 184), and goldfish (192). These kainate binding proteins cannot form functional channels alone or when coexpressed with other subunits (65). It is likely that this phenotype is the result of their inability of translating ligand binding into channel opening. Evidence in favor of
this idea has been obtained by the generation of functional chimeric receptors consisting of the GluR6 ligand binding domain and the ion channel domain of the different kainate binding proteins (177).

The analysis by in situ hybridization of the distribution of kainate receptors has revealed that they are widely expressed throughout the nervous system. However, the expression patterns of the different subunits are very heterogeneous (4, 191). Thus the GluR5 transcript is mainly present in DRG neurons, in the subiculum, in the septal nuclei, in the piriform and the cingulate cortices, and in Purkinje cells of the cerebellum (Fig. 3). GluR6 is most abundant in the cerebellar granule cells, in the dentate gyrus and the CA3 region of the hippocampus (Fig. 3), and in the striatum. The GluR7 mRNA is expressed at low levels throughout the brain but particularly in the deep layers of the cerebral cortex, in the striatum, and in the inhibitory neurons of the molecular layer of the cerebellum. KA1 is almost exclusively restricted to the CA3 region, although it is also expressed at lower levels in the dentate gyrus, in the amygdala, and in the entorhinal cortex. Finally, the KA2 message can be found in essentially every part of the nervous system. Although the different kainate receptor subunits are already present in the embryo, most of these patterns of expression emerge during the early postnatal period (4). Thus the amount of GluR5 mRNA peaks between postnatal day (P) 0 and P5 and then begins to decline toward its adult level by P12. Similarly, the GluR6 and KA1 expression patterns observed within the hippocampal formation begin to differ at around P0 (GluR6) and P12 (KA1).

It should be noted that the information gained by the in situ hybridization analysis, albeit correct in general, remains incomplete in the details. On the one hand, we still need to know what particular subunits are expressed by specific cell types within a given brain structure. For instance, a recent analysis of the coexpression of GluR5 or GluR6 with glutamic acid decarboxylase (GAD), the synthesizing enzyme of GABA, revealed that many hippocampal interneurons contained both GAD and one of the kainate receptor subunits (122) (Fig. 3). The percentage of interneurons that expressed GluR5 and GAD plus those expressing GluR6 and GAD was larger than 100, suggesting that a significant fraction of individual cells expressed the two kainate receptor subunits. This finding led to the subsequent demonstration that members of the GluR5–7 subtype can coassemble to form heteromers (32, 122) (Fig. 4). On the other hand, the lack of subunit-specific kainate receptor antibodies has limited our understanding of their localization and compartmentalization within the nerve cell. The use of anti-GluR5/6/7 or anti-GluR6/7 antibodies has mapped kainate receptors to dendrites and postsynaptic membranes (56, 72, 132, 156). However, the advent of better antibodies against these proteins should help us to establish unambiguously where in the neuron each of them is localized and to determine what specific subunits mediate the different physiological effects of kainate receptor activation. In particular, the development of better antibodies against kainate receptors should help us to identify and dissociate between the effectors of its presynaptic versus its postsynaptic actions, as discussed in section IV.

B. Structural Diversity

Kainate receptors are believed to share the same transmembrane topology and stoichiometry as AMPA and NMDA receptors (7, 67, 88, 143). Thus they are thought to be tetramers in which each monomer carries its own
ligand binding site and contributes with a specific amino acid stretch to form the channel lumen, the so-called M2 segment, composed of hydrophobic residues that dip into the membrane forming a hairpin-like structure (Fig. 1A).

In addition to this hydrophobic sequence, each subunit has three transmembrane segments (M1, M3, and M4) arranged such that the NH2-terminal domain of each protein is extracellular and the COOH-terminal region lies intracellularly (139, 167, 192). By analogy to the structural data available for AMPA receptors (3), the glutamate binding site of kainate receptors is supposed to consist of the homologous residues distributed throughout both the loop between M3 and M4 and the NH2-terminal domain (163). However, the actual structure of the binding pocket in kainate receptors is bound to be significantly different from the AMPA receptor. These likely differences should help to explain, among other things, why AMPA receptors can bind kainate with high affinity, whereas kainate receptors bind AMPA very poorly or not at all (Fig. 1B). In this regard, it is noteworthy that a specific residue of the extracellular loop between M3 and M4 has been identified as relevant for the moderate sensitivity of GluR5 to AMPA (165). When this amino acid is transplanted to the equivalent position in GluR6 (N721), the chimeric receptor can respond to the application of this agonist.

The identification of a number of isoforms derived from alternative splicing has revealed that the structural repertoire of the kainate receptor subtype is not limited to the five proteins mentioned earlier. The rat GluR5, for instance, can be found as two different variants (10): GluR5–1, an isoform that contains 15 extra amino acids in the NH2-terminal region, and GluR5–2. GluR5–2 has been found to have three splice variants that differ in the sequence of their COOH-terminal domains. GluR5–2a is 49 amino acids shorter than the GluR5–2b because of the introduction of a premature stop codon. GluR5–2c contains an extra in-frame exon that makes it 29 amino acids longer than the GluR5–2b isoform. The COOH-terminal domain for GluR5–1 is as in GluR5–2b. B: typical responses induced by different agonists at homomeric GluR5–2a receptors. Kainate induces faster and deeper desensitization at heteromeric GluR5/KA2 receptors (unpublished data). [From Sommer et al. (160). Reprinted by permission of Oxford University Press. C: GluR7 undergoes alternative splicing at the COOH-terminal domain. The insertion of a 13-amino acid residue out of the reading frame of the transcript leads to the generation of a protein 9 amino acids shorter (GluR7b). D: both isoforms can form homomeric channels sensitive to glutamate and kainate but insensitive to domoate and AMPA. Heteromeric GluR7/KA1, in contrast, responds to the application of AMPA. [Data from Schiffer et al. (152). Reprinted from Neuron with permission of Elsevier Science.]

FIG. 2. Structure and function of GluR5 and GluR7 receptors. A: the rat GluR5 can be found as two different variants: GluR5–1, which contains 15 extra amino acids in the NH2-terminal region, and GluR5–2. GluR5–2 has been found to have three splice variants that differ in the sequence of their COOH-terminal domains. GluR5–2a is 49 amino acids shorter than the GluR5–2b because of the introduction of a premature stop codon. GluR5–2c contains an extra in-frame exon that makes it 29 amino acids longer than the GluR5–2b isoform. The COOH-terminal domain for GluR5–1 is as in GluR5–2b. B: typical responses induced by different agonists at homomeric GluR5–2a receptors. Kainate induces faster and deeper desensitization at heteromeric GluR5/KA2 receptors (unpublished data). [From Sommer et al. (160). Reprinted by permission of Oxford University Press. C: GluR7 undergoes alternative splicing at the COOH-terminal domain. The insertion of a 13-amino acid residue out of the reading frame of the transcript leads to the generation of a protein 9 amino acids shorter (GluR7b). D: both isoforms can form homomeric channels sensitive to glutamate and kainate but insensitive to domoate and AMPA. Heteromeric GluR7/KA1, in contrast, responds to the application of AMPA. [Data from Schiffer et al. (152). Reprinted from Neuron with permission of Elsevier Science.]

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The role of the different kainate receptor splice variants is unknown. The 15 additional extracellular amino acids present in GluR5–1 do not map directly to the putative glutamate binding pocket of the receptor, but the possible influence of these residues on ligand binding has not been tested directly. Similarly, the possibility that this sequence may be involved in some type of intersubunit interaction remains unexplored. The rest of the known splice variants bear modifications in the COOH-terminal region. It is well established that the COOH-terminal domains of the different ionotropic glutamate receptor subtypes are important for their interaction with an increasing number of proteins involved in the organization and maintenance of synaptic structure. In the case of kainate receptors, this interaction is best exemplified by their ability to bind to members of the SAP90/PSD-95 class of proteins (54). Thus it is possible that the differences among the splice variants confer them with the selectivity necessary to interact with specific intracellular partners, providing the synapse with a system for the control of the activity of kainate receptors. This idea has not been explored in detail yet.

Another source of structural variability for kainate receptors is mRNA editing. As is the case for the GluR2 AMPA receptor (161), GluR5 and GluR6 (but not GluR7, KA1, and KA2) are susceptible of undergoing this kind of posttranscriptional modification at a specific position of their M2 segment, the so-called glutamine/arginine (Q/R) site. It has been shown that the presence of a R residue at this position in GluR2 is enough to change the rectification properties of AMPA receptors and, more importantly, to abolish their calcium permeability, a dominant trait observed in homomeric as well as in heteromeric AMPA channels (155, 161). In the case of homomeric kainate receptors assembled from GluR6(R), the Q-to-R substitution also decreases the permeability to calcium (20, 41) whilst increasing their chloride permeability (19). At the same time, the presence of a R at this site transforms the rectification properties of these receptors from inwardly rectifying to linear or slightly outwardly rectifying and

FIG. 3. Expression pattern of GluR5 and GluR6 mRNA. In the cerebellum (A), GluR5 is expressed in the Purkinje cell layer (P.C.L.) and in Golgi cells of the granule cell layer (G.C.L.), whereas GluR6 is abundantly expressed by the granule cells (B). The asterisks indicate unlabeled Purkinje cell bodies. Insets show a magnification of the Purkinje cell layer. In the hippocampus, GluR5 is mainly expressed by interneurons (C), and GluR6 is predominantly expressed in the principal cells (D). Double in situ hybridization with glutamic acid decarboxylase (GAD) and either GluR5 (E) or GluR6 (F) probes is shown. The expression of GAD is labeled red (white arrowheads). The expression of GluR5 or GluR6 is labeled blue (black arrowheads). Coexpression appears as a brown precipitate (black arrows). E and F correspond to a close view of the CA1 and CA3 regions, respectively. S.O., stratum oriens; S.P., stratum pyramidale; S.R., stratum radiatum; S.L., stratum lucidum. [C–F from Paternain et al. (122).]
reduces the unitary conductance of the channels (41, 86, 164). It should be noted, however, that the edition at the Q/R site is not the only change capable of altering the calcium permeability of GluR6 homomeric channels. Two additional positions prone to mRNA editing have been identified in the M1 segment of this subunit: the I/V site, where a valine can substitute for an isoleucine, and the Y/C site, where a tyrosine can be replaced by a cysteine (86). Editing at these positions modulates the effect of the Q/R site in calcium flow such that the fully edited subunit exhibits null passage of this cation. The mechanism of interaction among these three sites remains to be elucidated.

Heteromeric GluR5(Q)/GluR5(R) or GluR6(Q)/GluR6(R) receptors retain the rectification properties and the null permeability to calcium of the fully edited homomers (86, 160), indicating that the characteristics conferred by the presence of a R at M2 are dominant. In fact, this dominant character has recently been used to demonstrate that heteromeric receptors containing solely GluR5–7 subunits can exist. For instance, the coexpression of GluR6(Q) with GluR5(R) leads to the appearance of receptors with the characteristics of GluR6 channels but, at the same time, their current-voltage relationship is outward rectifying (32, 122). It must be noted, however, that not all of the functional changes introduced by mRNA editing are dominant as, for instance, GluR6(Q)/GluR6(R) channels remain chloride impermeable (19). Similarly, the reduction of the unitary conductance observed in the fully edited homomers is partly reverted in heteromeric assemblies. Thus the coexpression of KA2, a subunit susceptible to mRNA editing, with GluR5(R) or GluR6(R) generates receptors with unitary conductance larger than for the corresponding homomeric channels (70, 164). As mentioned above, M2 is thought to form the pore of the channel. Therefore, it has been proposed that, in principle, the presence of a positively charged R within this segment is sufficient to explain the functional behavior of the edited receptor. First, the existence of a ring of positive charges at the pore reduces unitary conductance because of an increase in the energy barrier for the flow of positive charges, an increase with a more significant effect on divalent cations such as calcium. At the same time, the electrostatic cloud of positive charges could favor the passage of anions like chloride, although, in order for this to occur, it seems necessary to maximize the reduction of the energy barrier by editing all of the Q/R sites. Finally, the presence of an R residue at the pore prevents the entry of polyamines, molecules responsible for the inward rectification observed in kainate as well as in other receptors. This fact leads to the linear or to the outwardly rectifying current-voltage relationships observed in the edited channels (16, 19, 79). However, this model fails to account for the calcium permeability of GluR6 and its dependence on the editing at the M1 segment, as mentioned above.

**FIG. 4.** Pharmacological and functional properties of some heteromeric kainate receptors. **A:** heteromeric GluR6/KA2 kainate receptors desensitize in response to glutamate but not to (R5)-α-amino-3(3-hydroxy-5-tert-butylisoxazol-4-yl)propanoic acid (ATPA), a drug previously thought to be a GluR5-specific agonist. In contrast, GluR5/KA2 heteromers fully desensitize in the presence of ATPA, whereas GluR5/ GluR6 heteromers exhibit only a partial desensitization at high concentrations of this compound. **B:** dose-response curves of ATPA for GluR5/KA2 and for GluR6/KA2 heteromers. The EC₅₀ values of the agonist for the two assemblies are 6.3 and 84 μM, respectively. **C:** dose-response curves of ATPA for GluR5/GluR6 heteromers. Preincubation with concanavalin A (ConA), a lectin that eliminates receptor desensitization, reduces the EC₅₀ from 21 to 1.1 μM. [Modified from Paternain et al. (122).]
Two additional facts about mRNA editing of kainate receptors deserve attention. First, the process is developmentally regulated, and it has been shown that a significant proportion of edited receptors exists both in embryonic and adult brains. The majority of GluR6 editing occurs earlier than for GluR5, and it is more thoroughly completed in the mature nervous system: up to 95% of GluR6 transcripts are edited, compared with 50-60% observed for the GluR5 subunit (9, 119–121, 153). Second, there seems to exist a site- and cell-specific regulation of kainate receptor mRNA editing. Studies of single hippocampal neurons in culture have shown that a given cell can coexpress several edited variants and, furthermore, that the degree of editing varies significantly across the different sites within the same neuron (144). Thus some cells have been found to possess, for instance, mRNA fully edited at the I/V site but totally unedited at the Q/R position. At the same time, only half of these transcripts appear edited at the Y/C site. Similar findings have been obtained in developing cerebellar granule cells (5) and in adult hippocampal neurons (102). The mechanisms responsible for the generation of this heterogeneity remain a matter of speculation and, similarly, its functional implications are not understood. For instance, mice with mutations at the Q/R editing site in GluR5 have been found to exhibit a reduction of kainate receptor-mediated currents in their sensory neurons (147), but the responses of these animals to painful stimuli are not altered. Furthermore, they do not show developmental abnormalities or deficits in the performance of several behavioral tasks. These observations stand in sharp contrast to the finding that knocking out the editing enzyme of the AMPA receptor subunit GluR2 leads to the generation of epileptic animals (45).

### III. FUNCTIONAL PROPERTIES OF KAINATE RECEPTORS

#### A. Biophysical Properties

The kinetic properties of the kainate receptor subtype have been studied mostly in heterologous expression systems. Under these conditions, stationary noise analysis has revealed that the single-channel conductance of homomeric GluR5(Q) or GluR6(Q) receptors is in the picrosiemens range with values of 2.9 and 5.4, respectively (164). A detailed study of the resolvable channel openings has revealed the existence of three subconductance levels that are approximately twice or three times the size of the smallest current (164). It is interesting to consider these data in relationship to the tetrameric model proposed for AMPA receptors, where current through the channel is a function of the number of agonist molecules bound to the receptor (143). Do the subconductance levels observed in kainate receptors correspond to the opening of channels bearing a different number of ligand molecules, or is it the expression of a fundamentally distinct process? In any case, the existence of subconductance states may help to explain why studies performed under nonstationary conditions have obtained larger conductance values for GluR6 receptors. The larger conductance may reflect a preponderance of channels working at the higher subconductance levels (172).

As mentioned above, Q/R editing reduces the single-channel conductance of GluR5 and GluR6 receptors by more than one order of magnitude, an effect that is partly reversed by the coexpression of the KA2 subunit (70, 164). Thus the conductance of homomeric GluR5(R) channels is less than 200 fS, but it increases to 950 fS upon assembly with KA2. Similarly, GluR6(R) homomers have a single-channel conductance between 230 and 260 fS, whereas the value for GluR6(R)/KA2 heteromers lies between 570 and 700 fS. The effect of KA2 on the conductance of homomeric GluR5(Q) or GluR6(Q) receptors is more subtle: an increase to 4.5 and 7.1 pS, respectively, which is accompanied, in the case of GluR5(Q), by a decrease in burst length (164). A paradoxical effect of the addition of KA2 subunits, the molecules with the highest affinity for kainate, to assemblies of GluR5 or GluR6 is that the heteromers exhibit a reduced affinity for the ligand (70, 164). This fact illustrates how little we understand about subunit interactions in kainate receptors and underscores other related questions that await an answer: how many KA1 or KA2 subunits can be incorporated into a receptor before it loses its functionality? Is it possible to find a subunit combination that has the highest affinity and the largest unitary conductance or does a compromise always exist between the two properties?

Some studies have tried to determine the single-channel conductance of kainate receptors expressed in neurons. In DRG cells, for instance, a value of 2–4 pS was determined (71) and three subconductance levels similar to those found for GluR5(Q) of GluR5(Q)/KA2 were observed. These findings agree with the fact that DRG neurons express GluR5 abundantly. In addition, the conductance of kainate receptors expressed in cerebellar granule neurons has been determined to be ~1 pS under conditions that reduce their desensitization (129) and ~4 pS in immature, proliferating cells (159).

#### B. Desensitization of Kainate Receptors

Rapid desensitization is one of the most characteristic features of kainate receptors (Fig. 5A). The time course of current decay upon the constant presence of agonist follows a single exponential curve (94, 124), although double exponential decays have also been described (94, 144, 189). However, in addition to the fact
that the speed of desensitization is dependent on the receptor subtype and on the cell type being analyzed, its actual value has been difficult to determine for different reasons (92). On the one hand, if the desensitization time constant is obtained purely from the measurement of current decay upon agonist perfusion, then a slow binding process could constitute a source of errors. On the other hand, the speed of current relaxation upon ligand application approaches the level of resolution of fast perfusion systems, raising the possibility that solution changes could also modify the measured desensitization rate. One way to circumvent these problems has been to measure the time constant of current relaxation at very high concentrations of agonist. Under these conditions, the binding rate is no longer a limiting factor and, as ligand concentration increases, the rate of current decay approaches an asymptotic value: the value of the real desensitization time constant. With the use of this strategy, it has been determined (124) that GluR6 homomers and native hippocampal kainate receptors have desensitization time constants of 11–13 ms, a value similar to what has been found for recombinant channels in excised patches (63, 172) or lifted cells (164, 165). Whether the small difference that persists between the two measurements is due to an effect of the excision of the patch on the channel properties, as has been described for the NMDA receptor (151), remains to be determined.

The recovery from the desensitized state proceeds slowly for kainate receptors, and it is dependent on the nature of the agonist (Fig. 5B). For instance, when glutamate is applied, two pulses evoke responses of similar sizes if they are separated by 15 s. In contrast, 1 min has to elapse when the desensitizing stimulus is a pulse of kainate (124). Another factor capable of affecting recovery from desensitization is the subunit composition (166). Thus GluR5 homomeric receptors recover in minutes, whereas the time constant of recovery for GluR5/KA2 heteromers is just 12 s. As mentioned earlier, the incorporation of KA2 subunits to kainate receptors reduces the affinity for the ligand. Thus it is possible that the faster recovery is due to a faster rate of agonist dissociation. However, it is unlikely that the exit from the desensitized state is a simple function of ligand unbinding because domoate, an agonist that desensitizes the receptors only partially, dissociates from the binding site with a faster time constant than the recovery time of the channel (94, 165). In any case, the profound differences in time scale between desensitization and recovery imply that the equilibrium between the two states is strongly displaced toward the former. In other words, the receptors spend most of their time in the desensitized state.

What are the implications of this propensity of kainate receptors to dwell on an inactive state? To visualize them more clearly, it is helpful to first look at the kinetic properties of their activation (Fig. 6). In cultured hippocampal neurons, glutamate has an EC${}_{50}$ of 330 μM; 3 μM of this agonist does not induce a significant response, and 3 mM is a saturating concentration (124). In contrast, desensitization occurs at ligand concentrations two orders of magnitude lower, which elicits no activation of the channel (EC${}_{50}$ = 2.8 μM). A similar situation has been observed in recombinant GluR6 homomers or when kainate is used as the agonist. In this case, the EC${}_{50}$ for activation is 22 μM, whereas it is just 0.31 μM for inactivation (124). These observations imply that kainate receptors are very sensitive to resting levels of glutamate and,
more importantly, that a large fraction of channels are already inactive before they can be activated, two factors that must have a profound influence in their physiological role. In addition, it is important to note that the kainate receptor activation and inactivation curves overlap to a significant extent (Fig. 6). This fact indicates that there are concentrations of the agonist high enough to open the channels and, at the same time, low enough to produce incomplete desensitization. The theoretical current predicted to exist under steady-state conditions of receptor activation is represented (dotted bell-shaped curve), and it reveals a steady activation of kainate receptors at 100 μM glutamate. [From Paternain et al. (124).]

C. Pharmacological Profile

A major hindrance in our way to understand kainate receptors has been the lack of specific agonists (Tables 1 and 2) and antagonists (Table 3). Although a clear pharmacological boundary has been traced between NMDA and the other ionotropic glutamate receptor classes, the separation between AMPA and kainate receptors has only been vaguely sketched and, for the longest time, the two types have been pooled together into what has been called the non-NMDA receptor subtype. For instance, AMPA has a negligible effect on recombinant kainate

TABLE 1. Agonist sensitivity of recombinant kainate receptors

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<th>GluR5</th>
<th>GluR5/KA</th>
<th>GluR6</th>
<th>GluR6/KA</th>
<th>GluR5/GluR6</th>
<th>GluR7</th>
<th>GluR7/KA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate</td>
<td>+ D</td>
<td>+ D</td>
<td>+ D</td>
<td>+ D</td>
<td>+ D</td>
<td>+ D</td>
<td>+ D</td>
</tr>
<tr>
<td>Kainate</td>
<td>+ D</td>
<td>+ D</td>
<td>+ D</td>
<td>+ D</td>
<td>+ D</td>
<td>+ D</td>
<td>+ D</td>
</tr>
<tr>
<td>Domoate</td>
<td>+ PD</td>
<td>+ PD</td>
<td>+ PD</td>
<td>+ PD</td>
<td>+ ?</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AMPA</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+ PD</td>
<td>+ D</td>
<td>-</td>
<td>+ D</td>
</tr>
<tr>
<td>(S)-5-IW</td>
<td>+ D</td>
<td>+ D</td>
<td>-</td>
<td>+ ND</td>
<td>+ PD</td>
<td>-</td>
<td>+ D</td>
</tr>
<tr>
<td>Me-Glu</td>
<td>+</td>
<td>+</td>
<td>+ D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATPA</td>
<td>+</td>
<td>+ D</td>
<td>-</td>
<td>+ ND</td>
<td>+ PD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con A</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Modest</td>
<td>+</td>
</tr>
</tbody>
</table>

+, Sensitive; --, insensitive; D, desensitizing response (>90%); PD, partially desensitizing response (<80%); ND, nondesensitizing response. AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; ATPA, (RS)-s-amino-3(3-hydroxy-5-tert-butylisoxazol-4-yl)propanoic acid; Con A, concanavalin A; (S)-5-IW, (S)-5-iodowillardiine; Me-Glu, (2S,AR)-4-methylglutamate.
receptors (40, 66). It is inactive in GluR6 and GluR7 and has an EC50 of 3 mM for the GluR5 subunit (160). Similarly, AMPA does not activate native kainate receptors in cultured hippocampal neurons or only at high concentrations in DRG cells (71, 94, 194). In contrast, kainate, albeit showing a clear preference for kainate receptors, has a very significant effect on AMPA channels at relatively low doses. The difference in EC50 between the two receptors lies around a mere 5- to 30-fold higher affinity for kainate receptors (29, 71, 94, 188). At the same time, the classic non-NMDA receptor antagonists, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 6,7-dinitroquinoxaline-2,3-dione (DNQX), and 6-nitro-7-sulfamoylbenzoquinoxaline-2,3-dione (NBQX) (69), show at best a three- to fivefold selectivity between AMPA and kainate receptors (12, 98, 188). Thus the realization that kainate could activate both receptor subtypes, and that the classic battery of competitive antagonists was also unable to distinguish between them, led the field to a stagnation that lasted until the mid-1990s, a state that, fortunately, has begun to change.

1. Agonist pharmacology

The affinity of kainate for its different receptor subunits is very variable (Table 2). As mentioned earlier, radioligand binding assays have identified two subclasses of kainate receptors with different affinity. The high-affinity subunits KA1 and KA2 have a dissociation constant (Kd) of 4–15 nM (66, 191). This figure is one order of magnitude higher than for GluR5–7, the low-affinity subunits (11, 160) (Kd50 ~100 nM). However, functional studies on recombinant receptors have revealed that the actual effective concentrations of kainate are much higher and vary depending on the subunit. Thus the EC50 for the GluR5 subunit (160) is 33.6 μM, 299 μM for the GluR6 subunit (164) (although other values have been reported) (78), and 1.8 mM for the GluR7 subunit (152). A similar situation has been found for glutamate, the endogenous agonist (Table 2). These figures illustrate that kainate receptors do not have a high affinity for their prototypical activators. This conclusion can be extended to the native channels because the EC50 values for kainate and glutamate measured in hippocampal and DRG neurons are similar to those obtained on some recombinant receptors (94, 124, 189).

In addition to kainate and glutamate, several molecules have been shown to activate kainate receptors with a certain degree of specificity. Domoate, one of the first to be identified, is 20- to 25-fold more effective than kainate by 10.220.33.6 on October 18, 2017 http://physrev.physiology.org/ Downloaded from http://physrev.physiology.org/ by 10.220.33.6 on October 18, 2017

### Table 2. Agonist pharmacology of native and recombinant kainate receptors

<table>
<thead>
<tr>
<th></th>
<th>EC50, μM</th>
<th>R5/R6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hippocampal neurons</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamate</td>
<td>310–330a,b</td>
<td></td>
</tr>
<tr>
<td>Kainate</td>
<td>22–23c,e</td>
<td></td>
</tr>
<tr>
<td>Domoate</td>
<td>0.7f</td>
<td></td>
</tr>
<tr>
<td>(S)-5-W</td>
<td>0.14j</td>
<td></td>
</tr>
<tr>
<td>SYM2081</td>
<td>0.6g/i,p</td>
<td></td>
</tr>
<tr>
<td>ATPA</td>
<td>Not activec,d</td>
<td></td>
</tr>
<tr>
<td>AMPA</td>
<td>260–520a,e</td>
<td></td>
</tr>
<tr>
<td><strong>GluR6</strong></td>
<td>631h</td>
<td></td>
</tr>
<tr>
<td><strong>GluR7</strong></td>
<td>5,900n</td>
<td></td>
</tr>
<tr>
<td><strong>R5/R6</strong></td>
<td>1,338q</td>
<td></td>
</tr>
</tbody>
</table>

Determinations carried out after concanavalin A treatment are not included except for ATPA and (S)-5-W in dorsal root ganglion (DRG) cells.

### Table 3. Antagonist pharmacology of kainate receptors

<table>
<thead>
<tr>
<th>Native Kainate Receptors</th>
<th>IC50, μM</th>
<th>pKb, μM</th>
<th>IC50, μM</th>
<th>IC50, μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNQX</td>
<td>6.1(Hip)d</td>
<td>6.5(DRG)b</td>
<td>GluR5</td>
<td>0.02d</td>
</tr>
<tr>
<td>NBQX</td>
<td>2.9(DRG)a</td>
<td>6–7(DRG)b</td>
<td>GluR6</td>
<td>0.16a</td>
</tr>
<tr>
<td>NS-192</td>
<td>2.2(Hip)d</td>
<td>5(DRG)b</td>
<td>GluR7</td>
<td>&gt;30a</td>
</tr>
<tr>
<td>LY204486</td>
<td>0.6(DRG)a</td>
<td>3.9f</td>
<td>AMPA receptors</td>
<td></td>
</tr>
<tr>
<td>LY203558</td>
<td>1(DRG)a</td>
<td>2.5 (6.2)a</td>
<td>Inactive at 100 μM*</td>
<td>0.47 (5.4)a</td>
</tr>
<tr>
<td>LY382884</td>
<td>1(DRG)b</td>
<td>6.2(DRG)a</td>
<td>Kᵢ = 6.9a</td>
<td>Inactive</td>
</tr>
<tr>
<td>SYM2206</td>
<td>20% at 100 μM</td>
<td>1a</td>
<td>Inactive at 100 μM*</td>
<td>4.8 8m</td>
</tr>
<tr>
<td>GYKI53405 (LY203606)</td>
<td>Inactive at 100 μM</td>
<td></td>
<td>Inactive at 100 μM*</td>
<td>0.9–1.5j</td>
</tr>
<tr>
<td>GYKI53655 (LY300168)</td>
<td>Inactive at 100 μM</td>
<td></td>
<td>Inactive at 100 μM*</td>
<td>Inactive</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate the corresponding pKb values when available. The type of cells from which the data were obtained is also indicated. Kᵢ, inhibitory constant. a Ref. 12; c Ref. 188; e Ref. 124; f Ref. 125; g Ref. 157; h Ref. 173; i Paternain and Lerma, unpublished data; j Ref. 135; k Ref. 123; l Ref. 187; m Ref. 14; n Ref. 142; o Ref. 13.
on DRG cells and on recombinant GluR5 subunits (71, 160), although it is inactive on GluR7 homomers (152). It induces a slowly desensitizing response, and it shows a 50-fold preference for kainate over AMPA receptors (71). This selectivity has been now largely surpassed by a new generation of agonists among which (RS)-5-amino-3(3-hydroxy-5-tert-butyloxazol-4-yl)propanoic acid (ATPA) is the prime example (89). The EC50 of ATPA is 0.6 μM for native kainate receptors in DRG neurons and 2.1 μM for recombinant GluR5 subunits (29), an order of magnitude more effective than kainate. At the same time, its affinity for AMPA receptors is about 500 times lower (340 μM in cerebellar Purkinje cells). Nevertheless, these figures must be interpreted with caution because they have been measured from receptors treated with concanavalin A, a molecule that reduces the desensitization of the receptor and artificially increases its affinity (see below). In addition, it must also be noted that ATPA has been hitherto regarded as a GluR5-specific agonist. It is inactive on GluR6 homomeric channels, and it exhibits a potency 1,000-fold weaker for displacing [3H]kainate from GluR7 than from GluR5 in binding assays (29). However, it has been recently shown (122) that ATPA can also act on GluR5/KA2 (EC50 = 6.3 μM), GluR6/KA2 (EC50 = 84 μM), and GluR5/GluR6 heteromers (EC50 = 21 μM). Furthermore, each of these assemblies exhibits a different desensitization profile in response to the agonist (Fig. 4). These findings may lead us to revisit previous findings where the selective activation of GluR5 is presumed to be responsible for the presynaptic effects of hippocampal kainate receptors, as discussed later.

Some derivatives of willardiine have also been advanced as selective kainate receptor agonists. Among them, (S)-5-trifluoromethylwillardiine is the most effective (EC50 = 74 nM on DRG cells), but its selectivity for kainate over AMPA receptors is only 50-fold, a relatively low figure (194). On the other hand, (S)-5-iodowillardiine shows a ~130-fold selectivity for kainate receptors, but its affinity is lower (194) (EC50 = 140 nM). As is the case for ATPA, (S)-5-iodowillardiine produces desensitizing currents on GluR5 but not on GluR6 or GluR7 homomers. In fact, it has been demonstrated that a single amino acid in the loop between M3 and M4 confers GluR5 with this differential sensitivity (166). However, this observation fails to explain why GluR6/KA2 heteromers are also responsive to this molecule (166). Finally, it is noteworthy that replacing iodine for fluoride transforms this drug into an AMPA receptor agonist with a 50-fold selectivity over kainate receptors (61, 194).

SYM 2081, the (2S-4R) diastereomer of 4-methylglutamate, is one of the latest additions to the list of specific kainate receptor agonists. It displays a selectivity three orders of magnitude larger for kainate than for AMPA receptors both in binding and in functional assays, but the selectivity of this molecule for kainate over NMDA receptors is significantly lower, only 200-fold (60). Although its pharmacological profile is incomplete, SYM 2081 does not seem to show the subunit specificity observed for ATPA and for (S)-5-iodowillardiine, as it elicits rapidly desensitizing currents on GluR5 and GluR6 homomeric channels (38, 78, 189, 198). Lastly, SYM 2081 has also been used as a functional antagonist of the kainate receptors (96). The activation and desensitization curves of GluR6 channels in response to this agonist (78) and to glutamate (124) show a minimal overlap. Therefore, its presence at low concentrations drives the receptor to an inactive state, preventing its subsequent opening by other agonists.

2. Antagonist pharmacology

The quest for specific kainate receptor antagonists has not been as successful as the search for agonists. As mentioned above, the first generation of non-NMDA receptor blockers such as CNQX, DNQX, and NBQX cannot distinguish between kainate and AMPA receptors. The molecule 5-nitro-6,7,8,9-tetrahydrobenzo[glindole-2,3-dione-3-oxime (NS-102) was then proposed as a selective kainate receptor antagonist based on its ability to block low-affinity [3H]kainate binding to cortical membranes (76). However, functional assays have yielded contradictory results regarding its specificity for kainate over AMPA receptors. Thus it was first shown that NS-102 blocked recombinant GluR6 and kainate receptors at a concentration 20 times lower than required for AMPA channels (173). However, it was later found that the IC50 values of this antagonist for kainate and for AMPA receptors in cultured hippocampal neurons was very similar (2.2 and 4.1 μM, respectively). More importantly, ~30% of the current through either channel subtype remained unaffected at the solubility limit of NS-102, a fact that has greatly constrained its usage (125, 188).

The development of a new series of decahydroxyisoquinoline carboxylates appears to indicate the path to follow in the development of highly specific kainate receptor antagonists. The molecule LY293558 [(3S,4aR,6R,8aR)-6(2-(tetrazol-5-yl)ethyl)decahydroxyisoquinoline-3-carboxylate] is a noncompetitive antagonist of AMPA receptors (IC50 = 0.5 μM), also blocks GluR5-mediated responses (IC50 = 2.5 μM) with no effect on GluR6 homomers (13) (IC50 >300 μM). A related derivative, LY294486 [(3SR,4aR,6SR,8aR)-6-(((tetrazol-5-yl)methyl)oxy)methyl)-1,2,3,4,4a,5,6,7,8a-decahydroxyisoquinoline-3-carboxylate], has a significantly higher selectivity for GluR5 than for AMPA receptors (IC50 = 4 μM and 30–100 μM, respectively) and no effect on GluR6 or GluR7 homomeric channels (29). Lastly, the compound LY382884 [(3S,4aR,6S,8aR)-6-(4-carboxyphenyl)methyl-1,2,3,4,4a,5,6,7,8,8a-decahydroxyisoquinoline-3-carboxylate] has an even higher selectivity for GluR5 over the other kainate receptor subunits and, more importantly, over AMPA receptors (14, 157).
Despite this progress, the real breakthrough in the pharmacological distinction between AMPA and kainate receptors has been the advent of a series of 2,3-benzodiazepines that prevent the activation of AMPA receptors in a selective and noncompetitive manner (178). Among them, GYKI 53655 (also known as LY300168) and its active stereoisomer LY303070 are the most effective and specific antagonists (123, 187). The IC50 of GYKY 53655 for AMPA receptors is $\sim 1 \mu M$, and it has no effect on kainate receptors at concentrations as high as 100 $\mu M$. As a result, the use of this molecule has made it possible to unmask the kainate receptors present in neurons, advancing very significantly the study of their physiological role. Other molecules related to GYKI 53655, such as GYKI 52466 and GYKI 53405, have been also used to discriminate between the two receptor subtypes (12, 39, 123). Although the affinity of these derivatives for AMPA receptors is comparable to the affinity of GYKI 53655, high concentrations can reduce the activity of kainate receptors, a fact that has limited their applicability. Similarly, SYM 2206, another diastereomer of 4-methylglutamate, has recently been used as a selective blocker of AMPA receptors (96, 128, 142), but the detailed pharmacological characterization of this molecule has not been completed (Table 3).

One final pharmacological tool that has been greatly used during the study of kainate receptors is the lectin concanavalin A. This molecule binds to a series of N-glycosylated residues present in this receptor subtype (43, 44), leading to a reduction of the fast desensitization observed upon ligand binding (71, 118, 193). It is active in native as well as in recombinant receptors, although the sensitivity of GluR7 is lower compared with the rest of the subunits (152). However, its practical use is limited because of several reasons. First, concanavalin A binds nonspecifically to sugars in cell membranes (43, 44), a fact that restricts its accessibility into more physiologically relevant preparations such as brain slices. Second, in addition to its effect on desensitization, concanavalin A seems to increase the affinity of kainate receptors by one or two orders of magnitude (78, 124), a fact that may lead to confusions about the actual biophysical and pharmacological properties of the channels. It has been proposed (92, 124) that the lectin could uncover quiescent receptors in a high-affinity, previously desensitized state that become conductive after the binding of concanavalin A. However, the actual mechanism underlying this increase in affinity remains to be elucidated. Third, it has been observed that other subtypes of glutamate receptors can also be modulated by treatment with concanavalin A. For instance, the currents through homomeric channels composed of GluR1 or NMDAR1–4a subunits are increased in the presence of the lectin (44).

IV. PHYSIOLOGICAL ROLES OF KAINATE RECEPTORS

The first evidence of a role for kainate receptors in synaptic function was obtained in the peripheral nervous system. Early studies had shown that peripheral dorsal root nerves from immature rats, specifically C fibers, were depolarized by kainate (1, 35). Subsequent studies demonstrated that kainate application could elicit rapidly desensitizing responses on acutely dissociated DRG cells (71), an effect markedly different from the nondesensitizing currents mediated by the kainate activation of AMPA receptors in central neurons. The realization that kainate induced a desensitizing response in recombinant kainate receptors (66, 160) and that the mRNA of the different subunits were expressed by a population of DRG cells (10, 171) led to the notion that kainate receptors were responsible for the response of the DRG neurons. This provided the first example of a physiological role for this kind of receptors, and recent studies have explored in more detail the actual molecular composition of the DRG kainate receptors. The pharmacological and biophysical profile of the channels resembles more closely the one observed for homeric channels made of GluR5(Q) subunits: they are sensitive to LY293558, a GluR5 antagonist (13), and exhibit the same subconductance levels and desensitization rate (194) resolved for this subunit in heterologous expression systems (164, 166).

It is illustrative to remember that glutamate is released by C fibers at their termination site in the dorsal horn (75). This fact raises the possibility that the kainate receptors present at the DRG cells act as autoreceptors, modulating the release of the amino acid upon peripheral stimulation (see NOTE ADDED IN PROOF). In addition to this, it has been found that high-intensity stimulation of the primary afferent fibers evoke an excitatory postsynaptic current (EPSC) mediated by kainate receptors in the spinal cord (96), suggesting that this receptor subtype may be directly involved in nociception. Further support for this idea comes from the observation that several kainate receptor antagonists have shown analgesic activity in a variety of animal models of pain, as discussed in section ivA.

In contrast to what is observed in the DRG cells, AMPA receptor activation masks the existence of kainate receptors in essentially every central neuron. This fact made it difficult at first to unequivocally demonstrate their existence beyond the peripheral nervous system. However, a series of early observations clearly pointed to their presence throughout the brain. For instance, it was well known that kainate could act as a potent convulsive agent when administered in vivo (6, 31) and more importantly, that the ingestion of contaminated mussels and the ensuing intoxication with domoate, a more selective agonist, also led to the appearance of seizures and produced am-
nesia (168). It had also been established that certain brain areas such as the CA3 and the CA1 regions of the hippocampus were particularly sensitive to the depolarizing and neurotoxic effects of kainate (25, 116, 138, 186). Thus concentrations of kainate unsuitable for activating AMPA receptors were nevertheless able to induce epileptiform activity (138). In addition, autoradiographic studies had revealed a discrete band of high-affinity kainate binding sites along stratum lucidum, the site of termination of the mossy fibers (49, 113). When the granule cells were destroyed, there was a parallel reduction in the number of binding sites (137) and a decrease in the susceptibility to the toxicity (36) and epileptogenic activity induced by kainate (53). These observations suggested that the putative kainate receptors were localized presynaptically, a hypothesis further supported by some ultrastructural evidence (23, 132) and by early studies showing that kainate could stimulate transmitter release from slices from a variety of brain regions (46, 47).

Despite this significant body of evidence, the ambiguity associated with a possible effect of kainate on AMPA receptors did not allow any definitive conclusion about the actual involvement of legitimate kainate receptors. It was only in 1993 that pure kainate receptor responses were recorded from embryonic hippocampal neurons in culture by predesensitizing AMPA channels (94). Under these conditions, it was observed that kainate elicited a small, rapidly desensitizing current that showed strong inward rectification, akin to what had been reported for homomeric GluR6(Q) receptors (40, 66). Indeed, the subsequent analysis of the kainate receptor mRNA content of individual neurons revealed that most hippocampal cells in culture expressed GluR6 and, furthermore, that there was a correlation between the GluR6(Q)/GluR6(R) ratio and the degree of rectification observed (144). Later studies have confirmed and extended these observations by demonstrating the existence of kainate receptors in several brain cell types including glial progenitors (127), cerebellar (129) and trigeminal ganglion neurons (146), and postnatal hippocampal cultures (189). It is interesting to note that, in the latter case, the properties of the kainate receptors are significantly different from those found in cultures from embryonic neurons: the currents do not desensitize fully, and no inward rectification is observed. These features resemble the characteristics of GluR5(R) ensembles and, in principle, be explained by the age-related changes in the levels of expression of this receptor subunit (4) and by the developmental shift in the editing at the Q/R site (9).

A. Involvement of Kainate Receptors in Excitatory Synaptic Transmission

The demonstration that pure kainate receptors did exist in nerve cells prompted the obvious question about their involvement in synaptic transmission. The advent of GYKI 53655 has made it possible to explore directly whether the stimulation of excitatory neurons is accompanied by the postsynaptic activation of kainate receptors in several brain areas. The initial observations made in hippocampal cell cultures seemed to indicate that this was not the case. Despite the fact that these neurons expressed functional kainate receptors, no EPSC could be recorded in the presence of AMPA and NMDA receptor antagonists (93). However, subsequent studies performed in brain slices have identified a collection of synapses that possess kainate receptors and have begun to reveal certain aspects of their physiological role (51).

The mossy fiber pathway, the connection between the dentate gyrus granule cells and the CA3 pyramidal neurons of the hippocampus, was the first synapse shown to employ kainate receptors (Fig. 7). The repetitive stimulation of the mossy fiber pathway is accompanied by a kainate receptor-mediated excitatory postsynaptic current (EPSC) resistant to GYKI 53655. This current is not observed upon repetitive stimulation of the associational-commissural fibers or of the mossy fibers in a GluR6 knockout mouse (C). [B from Castillo et al. (22). Reprinted from Neuron with permission of Elsevier Science. C from Muller et al. (115). Reprinted by permission from Nature, Macmillan Magazines Ltd.]
choice of the NMDA receptor subtype, was associated with the appearance of a current with slow rise and decay time constants that disappeared when CNQX was added to the bath (21, 176). In addition to its slow kinetic properties (a feature common to most kainate receptor synapses hitherto described), several characteristics of this current are noteworthy. First, the need for repetitive stimulation to open the receptor channels has been interpreted by some groups as evidence for their putative extrasynaptic (and not postsynaptic) location (8, 90, 108). In this model, the glutamate accumulated after tetanic stimulation would spill away from the synapse, acting on receptors located far from the release site. Nevertheless, the observation that the kinetic features of the kainate receptor-mediated current are not affected by blocking the uptake of glutamate would seem to argue against this possibility (111). It should be kept in mind, however, that glutamate uptake is very sensitive to temperature variations (180). As the aforementioned experiments were performed at room temperature, it is conceivable that the glutamate carriers were already inactive, consequently explaining the lack of effect of the antagonists. In any case, if the kainate receptors activated by the mossy fibers are extrasynaptic, then they must be located rather close to the release site. In favor of this idea, the observation that the kinetic features of the kainate receptor-mediated current are not affected by blocking the uptake of glutamate would seem to argue against this possibility (111). It should be kept in mind, however, that glutamate uptake is very sensitive to temperature variations (180). As the aforementioned experiments were performed at room temperature, it is conceivable that the glutamate carriers were already inactive, consequently explaining the lack of effect of the antagonists. In any case, if the kainate receptors activated by the mossy fibers are extrasynaptic, then they must be located rather close to the release sites. In favor of this idea, it has been observed that reducing the diffusion of glutamate by increasing the viscosity of the extracellular medium increases the size of the observed current (111).

Second, the activation of kainate receptors is not observed at other excitatory connections received by the CA3 neurons (21, 176). For instance, the repetitive activation of the associational-commissural pathway, axons that interconnect ipsi- and contralateral CA3 cells, generates an EPSC that is completely blocked by GYKI 53655 and APV (Fig. 7B). This result is in agreement with the early localization data (49, 113), and it indicates that the targeting of kainate receptors to the synapse is not unspecific within a given cell but that it is regulated by a mechanism yet to be discovered.

Third, the kainate receptor that mediates the synaptic current is probably heteromeric. On the one hand, the GluR5-specific antagonists LY293558 and LY294486 block the kainate-mediated response (174). On the other hand, the activation of the mossy fiber pathway in GluR6 knockout mice is not accompanied by a residual EPSC in the presence of AMPA and NMDA receptor blockers (115) (Fig. 7C). The recent observation that GluR5 and GluR6 can form heteromeric channels provides a plausible explanation to unify these disparate observations (14, 32, 122).

One final issue regarding the existence of kainate receptors at the mossy fiber pathway is their involvement in long-term potentiation of transmission at this synapse. Early findings had shown that the induction of this plastic change was resistant to CNQX and kynurenic acid (22, 74). However, the more selective kainate receptor blocker LY382884 has been recently shown to prevent the synaptic potentiation at this pathway (14). The reason for the discrepancy among these experiments is unknown. Similarly, it remains to be determined whether LY382884 exerts its action by blocking pre- or postsynaptic kainate receptors, a problem with an additional complication: the uncertainty about the locus of induction of mossy fiber long-term potentiation (99).

The search for postsynaptic kainate receptors in the pyramidal cells of CA1 has been unsuccessful (17, 50, 93). However, it has been demonstrated that the interneurons present in strata radiatum and oriens of this hippocampal...
region contain a population of postsynaptic kainate receptors at the connection they receive from the principal cells (30, 50) (Fig. 8). In contrast to what has been observed at the mossy fiber pathway, the activation of the interneuron receptors does not require stimulation of the afferent axons at high frequency (30). Instead, individual stimuli elicit a slow EPSC sensitive to LY293558, suggesting that the activated channels contain GluR5 subunits. The impact of these receptors in synaptic transmission is unknown, but it has been proposed that their activation is associated with an overinhibition of the CA1 pyramidal cells (50). This idea is based largely on the observation that bath application of kainate induces a large increase in the firing rate of the interneurons. However, it is hard to accommodate considering the known epileptogenic effects of kainate (6, 31). This idea is based largely on the observation that bath application of kainate induces a large increase in the firing rate of the interneurons. However, it is hard to accommodate considering the known epileptogenic effects of kainate (6, 31). In addition, the activation of the primary afferent sensory fibers produces a kainate receptor-mediated EPSC on the spinal neurons of the dorsal horn (96). As is the case for the hippocampal currents, the onset and decay time constants of the spinal EPSC are slow compared with the AMPA receptor-mediated response in the same cells. A remarkable feature of this current is that it can only be elicited upon the stimulation of the afferent axons at an intensity strong enough to activate the high-threshold Aδ and C fibers, raising the possibility that the kainate receptor subtype may be exclusively involved in nociception at this level (96). This hypothesis has received significant support from experiments showing that the application of opiate agonists can reduce the amplitude of the kainate receptor EPSC (96), and from the observation that this receptor subtype is present at synapses on identified spinothalamic projection neurons (96). Moreover, several kainate receptor antagonists possess analgesic activity in a number of animal models of pain. For instance, SYM 2081, presumably acting as a functional antagonist, increases the latency of escape in the hot-plate and the tail-flick tests (96), whereas
LY382884 decreases the frequency of paw licking induced by the subcutaneous injection of Formalin (157).

Postsynaptic kainate receptors are commonly assumed to coexist with other glutamate receptor subtypes, in particular with AMPA receptors and, in fact, there is indirect evidence that this is actually the case for some synapses (50). However, it has been clearly demonstrated that certain synaptic contacts solely contain kainate receptors. In this regard, the connection between the cones and the off-center bipolar cells of the squirrel retina is a prime example (37). The depolarization of an individual cone leads to the appearance of a fast EPSC in this class of bipolar cells (Fig. 9). This EPSC is not affected by the addition of GYKI 53655 and APV, but it is blocked by CNQX, clearly indicating its dependence on kainate receptors. In contrast to what has been observed at all other kainate receptor synapses, the kinetic properties of the EPSC resemble those described for recombinant and for somatodendritic, extrasynaptic channels (94, 124): fast activation and desensitization time constants, an essentially complete inactivation in the continuous presence of the agonist, and a slow recovery from the desensitized state. It has been proposed that these properties of the receptors have profound implications for retinal function, controlling the gain of the bipolar cell output (37). Thus, in the dark, the cones release glutamate relentlessly, but the desensitization of the kainate channels prevents the continuous depolarization and the saturation of the off-center cell. The hyperpolarization of the cones upon illumination would interrupt transmitter release, allowing for the slow recovery of the receptors from the inactive state. If darkness were reestablished before the recovery is complete, the depolarization of the bipolar cell would be smaller depending on the duration of the illumination period (37). This mechanism for the transmission of graded, phasic signals in response to tonic release constitutes an elegant counterpart by an ionotropic receptor to the well-understood action of metabotropic glutamate receptors on on-center bipolar cells (106, 117).

One final example of synapses purely containing kainate receptors is found in the cerebral cortex (Fig. 10). The stimulation of thalamocortical axons elicits an EPSC that consists of a fast AMPA receptor-mediated component and a slower one, mediated by the activation of kainate receptors (85). However, the spontaneous EPSC recorded from cortical neurons never exhibit the two components but, instead, they fall into two separate classes corresponding to the activation of each of the different receptor subtypes (85). In other words, despite the fact that a given cortical neuron can express both AMPA and kainate receptors, they never seem to colocalize at synaptic sites (Fig. 10, B and C). Furthermore, there is a dynamic switch that governs during development the number of synapses of each class (85). Thus the occurrence of the critical period for activity-dependent plastic changes in the thalamocortical pathway coincides with a reduction in the number of synapses that possess kainate receptors and with a parallel increase in the abundance of AMPA receptor-containing contacts (Fig. 10E). A similar phenomenon has been observed after the induction of long-term potentiation at this pathway (Fig. 10D), indicating that the redistribution can occur within minutes (85). These results highlight once more the necessity to identify what synaptic proteins interact with the kainate receptors to help us understand the mechanisms that control their function and their regulation. At the same time, the existence of such a rapid switch for modifying the receptor subtype present at the synaptic contact is reminiscent of what has been observed at the so-called silent synapses (103), contacts devoid of AMPA channels that only pos-
sess NMDA receptors. The functional implications of those thalamocortical kainate receptor-only synapses remain unresolved.

Another unresolved issue regarding the synaptic activation of kainate receptors is the slow kinetic properties of the response. With the exception of the channels found at the squirrel retina (37), the time constants determined for the activation and desensitization of kainate receptors in cultured cells and in heterologous systems predict synaptic responses markedly different from those actually measured. One possible explanation for these differences is that the composition of the synaptic receptors is dissimilar to any of the combinations of recombinant subunits hitherto analyzed or, alternatively, that postsynaptic kainate receptors contain additional, unidentified subunits capable of altering the kinetic features of the response. Similarly, the interaction of the receptors with other synaptic proteins (54), as discussed above, is likely to have an effect on the properties of the channel. Furthermore, the kainate receptor subunits possess consensus sites for the action of a series of protein kinases and have been shown to undergo phosphorylation in a variety of systems (55, 136, 172, 181, 196). The effects of such covalent modification on the behavior of the native channels remain largely unexplored. Lastly, the prolonged duration of the response could be the manifestation of the window current predicted by the overlap between the activation and the desensitization curves of the kainate receptors (124). It is conceivable that the concentration of glutamate that reaches the channels is high enough to open a few of them and, at the same time, insufficient to cause complete desensitization. This possibility would be even more appealing if the receptors were not directly underneath the release site but if, instead, glutamate had to spill to the periphery of the synapse to reach them. The determination of the exact localization of the kainate receptors at the synapse will help to answer this question, although indirect observations suggest that diffusion of glutamate far from the release site is unlikely to account for the slow kinetic properties of the response (18, 111).

### B. Presynaptic Kainate Receptors

In addition to their postsynaptic role, kainate receptors have been found to modulate transmitter release by a presynaptic mechanism. As mentioned above, a number of early experiments pointed to a possible presynaptic
Localization of this receptor subtype, although the lack of specific pharmacological agents made it unfeasible to establish the actual identity of the receptors under analysis. The use of GYKI 53655 has largely eliminated these ambiguities, making it possible to revisit and extend those original experiments. As a result, it is now largely accepted that kainate receptors are present at a subset of both excitatory and inhibitory terminals, where they affect release by a series of mechanisms that are beginning to be unraveled.

Studies on the effect of kainate receptor activation on glutamate release from synaptosomes have yielded disparate results. On the one hand, a GYKI 52466-resistant, kainate-induced increase in transmitter released from cortical synaptosomes has been reported (130), an effect mimicked by the addition of kainate or domoate to synaptosomal preparations enriched for CA3 terminals (104, 105). On the other hand, observations made on CA1 synaptosomes have revealed no effect of kainate on glutamate release but only an intriguing effect on the release of aspartate (199). Finally, other reports have revealed a kainate-mediated decrease in the influx of calcium in the CA3 region (2) and a decrease of glutamate release from hippocampal synaptosomes (28) in the presence of GYKI 52466. Thus, although the evidence seems to indicate that kainate receptors are present on isolated synaptic terminals, it is not clear whether they stimulate or inhibit transmitter release, although it appears that the effect is synapse specific.

Experiments performed on brain slices have provided additional evidence for the participation of kainate receptors on the regulation of excitatory synapses of the Schaffer collateral (28, 80, 175) and mossy fiber pathways (81, 154, 175). It has been observed that the administration of kainate to hippocampal slices in the presence of GYKI 52466 is accompanied by a biphasic modulation of the NMDA receptor-mediated EPSC on CA1 neurons (28). After an initial period of enhanced release, the agonist induced a reversible reduction of the EPSC amplitude. This reduction was sensitive to LY294486, suggesting the involvement of GluR5-containing receptors in the phenomenon (175). This biphasic behavior can be explained by proposing an early depolarization of the terminal and the subsequent reduction of calcium influx due to the inactivation of the calcium channels involved in the release process (28, 80). Nevertheless, the observed decrease of the excitatory drive is a surprising observation considering the known epileptogenic effects of kainate. Interestingly, lower concentrations of the agonist were effective in inducing only the increase of the synaptic response (28). Therefore, the exogenous application of kainate will not suffice to obtain a definitive answer on the modulation of excitatory neurotransmission by this receptor subtype. Indeed, subsequent studies should aim to establish the actual concentration of glutamate that reaches the receptors during normal and excessive synaptic excitation and, as recently shown for the mossy fiber pathway (154), test whether endogenous glutamate can cause a kainate receptor-mediated reduction of glutamate release.

The modulation of GABA release by the activation of kainate receptors has also been reported in the hippocam-
pus (29, 30, 50, 52, 110, 140) (Fig. 11) and in the hypothalamus (97). In the case of the hippocampus, the literature is more homogeneous about the effect, and all of the available studies agree that the application of kainate in the presence of GYKI 53655 causes a reversible decrease of the evoked IPSC amplitude (29, 30, 50, 52, 110, 140). Unfortunately, despite this general agreement, the exact role of kainate receptors on its action is a matter of dispute.

Three lines of evidence lent initial support to the idea that kainate receptors were acting directly on the presynaptic terminal in the hippocampus (140). First, kainate increased the failures of transmission between pairs of hippocampal neurons in culture. Second, kainate reduced the frequency of miniature IPSC in hippocampal slices (Fig. 11, E and F), an effect, however, not observed by other groups (50, 52). Third, the coefficient of variation of the synaptic responses changed in parallel to the reduction of the IPSC amplitude (Fig. 11 D). These results were placed in doubt after the realization that inhibitory interneurons possessed postsynaptic kainate receptors (30, 50). It was then argued that the massive activation of this cell type caused an activity-dependent depression of the evoked IPSC, a depression that had no relationship to a direct action of kainate on its putative presynaptic receptors. Although the presence of kainate does induce a marked increase in the spontaneous interneuronal firing rate (30, 50), a phenomenon known for more than 15 years (48), further studies have shown that the reduction of the evoked IPSC amplitude is independent of this phenomenon. On the one hand, the use of different kainate receptor agonists has allowed for the dissociation of the two events (142). Thus glutamate, the endogenous ligand, produces a decrease of the IPSC amplitude without affecting the spontaneous activity of the interneurons. In contrast, low concentrations of the kainate-receptor agonist ATPA increase the firing rate of the GABAergic cells but do not induce any decrease of the IPSC amplitude (Fig. 12). Moreover, the direct stimulation of the interneurons by high-frequency electrical stimulation is not accompanied by a reduction of the IPSC size comparable to what is observed after the application of kainate (142). Finally, it has been possible to record directly from synaptically connected interneuron-pyramidal cell pairs. Under these conditions, kainate continues to induce a decrease of the unitary IPSC amplitude in the absence of repetitive firing of the presynaptic neuron in culture (140) and in slices (52).

The observation that the spontaneous activity of GABAergic neurons is strongly increased by the presence of kainate has prompted a second alternative explanation for the inhibitory effect of this agonist on the IPSC amplitude: the involvement of GABA receptors as the real mediators of the phenomenon (52). In this scenario, kainate would depolarize the interneurons causing them to fire repeatedly and to release an excessive amount of GABA. The resultant increase in the extracellular concentration of this amino acid would then have two interrelated actions. First, it would lead to the activation of presynaptic GABA$_B$ receptors, molecules known to downregulate release from inhibitory terminals (109, 170, 195). Although initial experiments had shown that the presence of GABA$_B$ receptor blockers had no effect on the kainate-mediated inhibition (50, 141), subsequent studies (52)
have revealed a partial blockade of the effect of kainate by the GABA_B receptor antagonist SCH50911. Second, an excess of extracellular GABA would also lead to the opening of the GABA_A channel subtype and to a concomitant decrease in the input resistance of the CA1 neuron. Indeed, it has been observed that kainate induces a decrease in the input resistance of the CA1 neurons, a change that reduces the half-width of the synaptic responses and the amplitude of GABA-induced somatic currents (52). The addition of GABA_A receptor antagonists prevents this change in the passive properties of the pyramidal cell membrane, suggesting that a fraction of the effect of kainate has a postsynaptic locus and is indirectly mediated by the activation of this GABA receptor subtype (52).

In principle, the sum of these two actions of the inhibitory transmitter could account for the effect of kainate on the IPSC amplitude. However, it has been found that the synaptic activation of excitatory pathways can mimic the effect of bath-applied kainate (110). Under these conditions, there is no massive surge of GABA release but, nevertheless, the size of the evoked IPSC is significantly reduced after a brief, high-frequency train of pulses to the Schaffer collaterals in the presence of GYKI 52466 and APV (Fig. 13). Furthermore, this depression is not affected by the application of GABA_A receptor antagonists, and it is abolished by kynurenic acid, a wide-spectrum glutamate receptor blocker (110). These findings demonstrate that, under more physiologically relevant conditions, kainate receptor activation has a real effect on release from inhibitory terminals, which is independent of the possible action of GABA. In this regard, it is important to remark that the net effect of low concentrations of kainate in the in vivo preparation is an increase of tissue excitability. In fact, one of the clearest electrophysiological evidences of kainate action in vivo is a reduction of GABAergic inhibition, as judged by paired pulse stimulation (140) (Fig. 14).

In summary, a significant body of evidence, pioneered by the study of Fisher and Alger (48), indicates that kainate reduces the efficacy of the inhibitory connections in the hippocampus. Although some indirect actions of the agonist may contribute to its global effect, it can be concluded that the activation of kainate receptors located at the GABAergic terminal is also involved in the genesis of the reduction. What is the molecular composition of the receptors responsible for this modulatory action? On the one hand, the observations that LY294486 blocks the action of kainate (29), and that a large number of presumptive interneurons express the GluR5 mRNA (122), point to the involvement of this subunit in the phenomenon. On the other hand, the reduction of the evoked IPSC as well as the large increase in interneuronal firing are still observed in mice lacking the GluR6 subunit (17), suggesting that this subunit does not participate in the presynaptic modulation of GABAergic neurotransmission.

Finally, it is necessary to consider the mechanisms whereby a cationic channel can exert an inhibitory effect on the release process. It has been proposed that the depolarization induced by an ionotropic receptor could lead to the inactivation of voltage-gated sodium or calcium channels present at the terminal (100, 109). Indeed, as mentioned above, evidence for this type of mechanism has already been

![FIG. 14. The intrahippocampal infusion of kainate in vivo is accompanied by a decrease in recurrent inhibition. A: kainate is delivered via a dialysis fiber located near the recording extracellular electrode. B: two pulses 40 ms apart are delivered to the Schaffer collaterals. In the absence of kainate, the population response to the second pulse is significantly reduced due to the polysynaptic activation of inhibitory, recurrent fibers. In the presence of kainate, the amplitude of the second response increases with time, an indication of a reduction in the hippocampal inhibitory drive. An additional effect of kainate is the generation of double population spikes (bottom right record in B), which are reminiscent of status epilepticus. In fact, interictal epileptic spikes can also be seen in the hippocampal electrical activity (C). [B and C are from Rodríguez-Moreno et al. (141).]
obtained for the activation of kainate receptors at excitatory synapses in the hippocampus (80). Nevertheless, it has been found that the effect of kainate is sensitive to the presence of pertussis toxin (141), pointing to the involvement of G proteins in this regulatory process (Fig. 15). Moreover, protein kinase C (PKC) inhibitors can also block the action of kainate, further delineating the signaling pathway involved in the modulation of inhibitory neurotransmission (141). It is worthwhile to emphasize that the recruitment of the PKC pathway is not secondary to the depolarization of the terminal since reducing the influx of sodium ions does not affect the action of kainate (141). Therefore, it has been proposed that there is a physical link between kainate receptors and the G protein involved in the process, a link that could be either direct or through an intermediary molecule. A similar type of association between an ionotropic receptor and the signal transduction machinery has been documented for the AMPA receptor (62, 83, 131, 182, 183). In addition, a goldfish kainate binding protein with significant homology to the rat kainate receptor subunits has been shown to interact with a pertussis toxin-sensitive G protein, an interaction that modifies the affinity of the binding proteins for the ligand (190, 200). Lastly, the binding of SYM 2081 decreases after incubation of hippocampal membranes with pertussis toxin (33), and a similar effect of the toxin has been observed on the kainate-mediated inhibition of GABA release from synaptosomes (34), further suggesting a direct coupling between kainate receptors and G proteins in the mammalian brain.

V. PATHOLOGICAL ACTIONS

The epileptogenic action of kainate has been well known for decades, but the actual involvement of its specific receptors has just begun to be unraveled. The first indication that the activation of kainate receptors could elicit epileptiform discharges in vitro came from studies where low concentrations of the agonist, insufficient to activate AMPA receptors, could induce repetitive discharges in the hippocampus (48, 158, 186). In contrast, the results obtained after the systemic or the intracerebral administration of kainate in vivo have been more difficult to interpret, and the evidence on the participation of kainate receptors has only accrued during the last 10 years. As mentioned above, one of the earliest observations in this regard was that the accidental ingestion of domoate, an agonist with a 50-fold preference for kainate over AMPA receptors, produces seizures and amnesia (168). More recently, it has been discovered that certain allelic variants of the human GluR5 subunit (GRIK1) (149), but not of GluR6 (GRIK2) (150), confer an increased susceptibility to the development of juvenile absence seizures. In addition, the level of kainate receptor mRNA seems to be altered in the hippocampus of patients suffering from temporal lobe epilepsy (107). In addition to this body of circumstantial evidence, the putative role of kainate receptors on epilepsy has received more direct support from two experimental
and any of these diseases is unknown. The actual relationship, if any, between kainate receptors and the age of onset of Huntington disease (101, 145). The correlation between some genotype variants of the GRIK2 gene and schizophrenia (133) and a moderate linkage between some genotype variants of the GRIK2 gene and familial amyotrophic lateral sclerosis also maps to this chromosome (21). Moreover, a mutant gene associated with a form of Down’s syndrome (26, 57). (42, 134), a finding that has prompted the suggestion of a possible role for GluR5 in Down’s syndrome (26, 57). A reduction of GABA release. The possibility that its antagonists can be used in the treatment of certain forms of chronic pain. In addition, chromosome 21 harbors the locus of the GRIK1 gene (42, 134), a finding that has prompted the suggestion of a possible role for GluR5 in Down’s syndrome (26, 57). Moreover, a mutant gene associated with a form of familial amyotrophic lateral sclerosis also maps to this chromosome (42), but any linkage between this and the GRIK genes remains to be discovered. Lastly, a series of correlative studies have shown a reduction of the GluR6 and KA2 mRNA in the hippocampus of patients with schizophrenia (133) and a moderate linkage between some genotype variants of the GRIK2 gene and the age of onset of Huntington disease (101, 145). The actual relationship, if any, between kainate receptors and any of these diseases is unknown.

VI. FUTURE DIRECTIONS

Our understanding of kainate receptors trails significantly behind our insights on the workings of the other ionotropic glutamate receptor subtypes. We have just begun to explore the structural determinants of kainate receptor function, a field where the detailed dissection of the AMPA receptor subtype has had a profound heuristic value. In this regard, it is surprising to observe the limited mutational analysis performed on kainate receptors, which in most cases has been aimed simply at extending findings previously obtained in AMPA receptors. However, several important questions still await an answer. Why can AMPA receptors bind kainate but kainate receptors cannot bind AMPA? Discovering the structural elements involved in this discrimination should assist in the design of kainate receptor-selective drugs. Why are KA1 and KA2 not able to form functional channels on their own and yet the conductance of heteromeric GluR5/KA2 assemblies is almost twice as large as for homomeric GluR5 channels? What regions of the molecule mediate intersubunit interactions in heteromeric channels? Is it possible that the binding site is actually formed at the interface between two subunits as is the case for acetylcholine receptor (82)? These issues have been hitherto neglected despite their relevance for the understanding of the function of this receptor subtype.

Similarly, there are many unsolved questions about the physiological role of kainate receptors. Why are the properties of the postsynaptic kainate currents so different from the characteristics observed in recombinant receptors? We have already speculated in this regard, but the actual answer still eludes us. Clearly, the use of paired recordings and the analysis of unitary KAR-mediated postsynaptic currents will be instrumental in determining the actual properties of these receptors at synaptic sites. Another intriguing fact about the kainate receptor-mediated component of the EPSC is their small amplitude. If the synaptic current through the AMPA receptors is so much larger, what is the significance of such a small kainate receptor EPSC? Its longer duration and its concomitantly longer integration time provide a clue, but is this the whole answer? Could it be that AMPA and kainate receptors never coexist, as in the case of the thalamocortical synapses (85), and we have not paid close attention? Furthermore, as presynaptic kainate receptors act through the activation of a G protein and the recruitment of PKC in inhibitory neurons (141), why could a similar phenomenon not occur at the postsynaptic membrane to amplify the effect of their activation? We have also considered the idea that the kainate receptors are not located exactly under the release site but in the periphery of the synapse, a possibility that would certainly have deep implications for their function.
Regarding presynaptic kainate receptors, one of the problems that will certainly receive more attention in the immediate future is the mechanism of their interaction with the signal transduction machinery of the cell. Does it occur through an intermediary protein? If not, how does the binding of the ligand to the receptor gets transmitted to a G protein? Is this phenomenon exclusive of glutamate receptors or is it shared by other neurotransmitter receptor molecules? The study of kainate receptors may turn into a beacon for the research on a novel area of cellular signaling.

Finally, will kainate receptor-specific antagonists be useful as antiepileptic agents? The fact that the GluR6 knockout mice show reduced susceptibility to seizures induced by low doses of kainate (115) raises the intriguing possibility that during an epileptic crisis, an initial, moderate increase in the extracellular concentration of glutamate has a more profound effect on kainate receptors. Is this the first step on a chain reaction that culminates in the development of a full-blown convolution? Undoubtedly, kainate receptors largely remain terra incognita.

NOTE ADDED IN PROOF

A number of recent publications, which appeared while this review was being considered for publication, have presented convincing additional evidence showing that kainate receptors are also able to regulate the release of glutamate at excitatory synapses. In particular, GluR6 kainate receptor activation markedly depressed the release of glutamate at mossy fibers (29b, 81, 154), where they have been postulated to play a significant role in synaptic plasticity (14, 29a). Similarly, kainate was found to suppress EPSCs evoked by dorsal root fiber stimulation (84a), indicating that presynaptic kainate receptors may also regulate primary afferent sensory transmission. Although the relevance of these processes and the underlying mechanisms have not been established unequivocally, it is worth noting that presynaptic kainate receptors may play a dual role in overall excitability (see Ref. 6a). In addition, a role for RNA editing of kainate receptors has been recently revealed. In mice engineered to lack the GluR6 Q/R editing site, Vissel et al. (177a) found that NMDA-independent LTP could be more easily induced than in normal mice and that these mutant mice were more vulnerable to kainate-induced seizures. This constitutes the first indication that the Q/R site editing of GluR6 RNA may modulate both seizure susceptibility and synaptic plasticity, therefore providing evidence for a critical biological role of kainate receptor editing.

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