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ACCELERATED COMMUNICATION

Homomeric GluR1 Excitatory Amino Acid Receptors Expressed in *Xenopus* Oocytes

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SUMMARY

The GluR1 cDNA clone encodes a functional excitatory amino acid receptor (Hollmann et al., *Nature* **342:** 643–648 (1989)). We have studied the pharmacological properties of this homomeric (single subunit) receptor expressed in *Xenopus* oocytes and compared these properties with those of receptors encoded by rat forebrain mRNA. (*RS*)-α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionate, quisqualate, and glutamate were partial agonists at both GluR1 and forebrain non-*N*-methyl-D-aspartate (-NMDA) receptors. The potency of the agonists kainate, domoate, and glutamate was higher, and that of the antagonists 6-cyano-7-nitro-quinoxalinedione and 6,7-dichloro-3-hydroxy-2-quinoxaline carboxylic acid lower, for GluR1 receptors as compared with forebrain non-NMDA receptors. The GluR1 receptor differed strikingly from forebrain-derived non-NMDA receptors, however, in that it exhibited slow, calcium-dependent desensitization. Thus, most properties of the GluR1 receptor are similar but not identical to those of non-NMDA receptors expressed from forebrain mRNA. These results indicate that the ligand recognition sites on GluR1 homomeric receptors are subtly different from those of non-NMDA receptors expressed from a mixture of forebrain mRNA.

Glutamate receptors are mediators of fast synaptic transmission at the majority of excitatory synapses in the mammalian brain. These receptors have been classified on functional grounds into at least two types, the NMDA and "non-NMDA" receptors. Domoate and kainate are full agonists at non-NMDA receptors on brain neurons, whereas glutamate, AMPA, and quisqualate may be partial agonists, because they produce a small steady state response but greatly decrease responses of the full agonists. Although different binding sites for [^{3}H] kainate and [^{3}H]AMPA can be identified in brain membranes (1), the functional evidence for separate kainate and AMPA subtypes of non-NMDA receptors is currently weak (2–4). On the other hand, given the variety of $GABA_A$ and nicotinic receptors identified by the molecular cloning approach, it is likely that the diversity of amino acid receptors far outstrips the ability of current pharmacological tools to distinguish among them (reviewed in Ref. 5).

Recently, cDNA clones that encode kainate-binding proteins have been isolated from rat, chick, and frog brain (6–8). Only one of these clones, GluR1, produced functional kainate-responsive receptors in the *Xenopus* oocyte translation system (6), and several sequences related to GluR1 have subsequently been isolated by low stringency screening of rat brain cDNA libraries (9, 10). It thus appears likely that a non-NMDA receptor family exists, analogous to the nicotinic and $GABA_A$ receptor families previously identified (11, 12). Within each family, different receptors are presumably composed of different combinations of subunits and possibly carry out somewhat different functions. It is now important to compare the properties of the receptors formed by the cloned subunits with those of "native" receptors.

Hollman *et al.* (6) demonstrated that *Xenopus* oocytes expressing GluR1 receptors respond to kainate and domoate with an inward current, but they did not carry out extensive pharmacological characterization. We have used the *Xenopus* oocyte expression system (13, 14) to compare in more detail the pharmacological properties of receptors encoded by GluR1 mRNA with those of receptors encoded by mRNA isolated from rat forebrain. The oocyte allows the comparison of receptors in the same cell type that may normally be expressed *in situ* by different neurons. Use of the oocyte thus minimizes possible differences in receptor properties due to different membrane environments and allows quantitative measurement of several distinguishing pharmacological features.

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ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; AMPA, (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; CNQX, 6-cyano-7-nitroquinoxalinedione; 6,7-diCl-HQC, 6,7-dichloro-3-hydroxy-2-quinoxaline carboxylic acid; GABA, γ-aminobutyric acid; HEPES, 4-(2-hydroxethyl)-1-piperazineethanesulfonic acid; DRG, dorsal root ganglion.

Materials and Methods

The GluR1 cDNA was kindly provided in pBluescript SK− plasmid (pGluR-K1 of Ref. 6) by Drs. Michael Hollmann and Stephen Heinemann (The Salk Institute). Plasmid DNA was amplified in *Escherichia coli*, purified by CsCl gradient centrifugation, and linearized with *Xho*I, which digests the DNA at the 3′ end of the GluR$_A$-coding sequence. The *m*CAP RNA transcription kit (Stratagene) was used to synthesize mRNA containing a 5′-7MeGpppG cap analogue. After transcription, the mixture was treated with RNase-free DNase, followed by phenol-chloroform extraction and ethanol precipitation in 1 M ammonium acetate. RNA synthesized by T3 RNA polymerase ran as a discrete band on nondenaturing 1% agarose gels. RNA was dissolved in water (0.2–0.5 μg/μl) and stored in single-use aliquots (6 μl) at −70°.

The methods for isolation of poly(A)$^+$ RNA from rat forebrain, microinjection into defolliculated *Xenopus* oocytes, and voltage-clamp recording from these cells have been described (14). The composition of the bathing medium was (in mM): 88 NaCl, 1.0 KCl, 2.4 $NaHCO_3$, 10 HEPES, 0.82 $MgSO_4$, 0.33 $Ca(NO_3)_2$, 1.9 $CaCl_2$, pH 7.2. To test for NMDA responsiveness, Mg^{2+} was omitted and 3 μM glycine was included in the bathing medium. All voltage-clamp experiments were carried out with a holding potential of −60 mV. Drugs were applied by perfusion, with a dead time of 5–10 sec and time to peak response of 15–30 sec from response onset. The fitting of agonist dose-response curves to a logistic equation and Schild analysis of antagonist potency were carried out as described (3, 14). Variability is expressed as standard error or 95% confidence intervals.

Results

Agonists. Injection of GluR1 mRNA into *Xenopus* oocytes led to the formation of receptors that could be activated by kainate, domoate, glutamate, AMPA, and quisqualate, but not NMDA plus glycine. Smooth inward currents evoked by kainate were dependent on the amount of mRNA injected and the postinjection incubation time. In one experiment, the application of 1 mM kainate to oocytes injected with 1, 10, or 25 ng of GluR1 RNA produced 13 ± 3.1 nA (n = 4), 201 ± 24 nA (n = 26), and 484 ± 67 nA (n = 10) of inward current on day 3–5 after injection. In another experiment, oocytes were injected with 10 ng of GluR1 RNA; the mean current amplitude evoked by 1 mM kainate increased from 118 ± 29 nA on day 3 to 160 ± 25 nA on day 4 to 329 ± 40 nA on day 5 (n = 3 at each time point). The largest steady state current produced by kainate (1 mM) was 942 nA. The potency of agonists and antagonists was not obviously dependent on the amount of mRNA injected or the time after injection at which experiments were performed.

The magnitude of kainate-evoked currents at GluR1 receptors was similar to that at receptors translated from a complex mixture of rat forebrain mRNAs (3, 14). Because forebrain mRNA must contain only a small proportion of mRNA encoding non-NMDA receptors, these results suggest that the functional expression of homomeric GluR1 receptors is not very efficient. As reported by Hollmann *et al.* (6), the culture of GluR1-injected oocytes in 50 μg/ml Actinomycin D did not eliminate receptor expression, indicating that the GluR1 cDNA does not likely encode a transcriptional regulator of excitatory amino acid receptor genes in *Xenopus*.

Several quantitative differences between GluR1 receptors and the non-NMDA receptors expressed from rat forebrain mRNA were apparent. Kainate was 2.9-fold more potent in activating GluR1 receptors than receptors expressed by forebrain mRNA (Fig. 1A, Table 1). Likewise, domoate and glutamate, two other agonists for this receptor (Fig. 1B), were 10.8-fold and 4.1-fold more potent, respectively, at GluR1 receptors (Table 1). The EC_{50} valves for kainate and domoate acting on GluR1 receptors were similar to those reported by Hollmann *et al.* (6). Additionally, the dose-response curves for all three agonists were less steep for activation of homomeric GluR1 than forebrain non-NMDA receptors (Fig. 1B, Table 1). Finally, glutamate had higher efficacy on GluR1 receptors than on forebrain non-NMDA receptors (Table 1), which could reflect a lower degree of rapid desensitization at GluR1 receptors (15–17). The explicit comparison of the responses of the two receptor types to agonists in the same batch of oocytes was not necessary, because drug potency in oocytes is highly reproducible for the agonists and antagonists tested here. In parallel experiments with oocytes injected with brain mRNA, the EC_{50} of glutamate was 11 μM and that of kainate was 106 μM.[1] These values are in good agreement with those reported in Table 1.

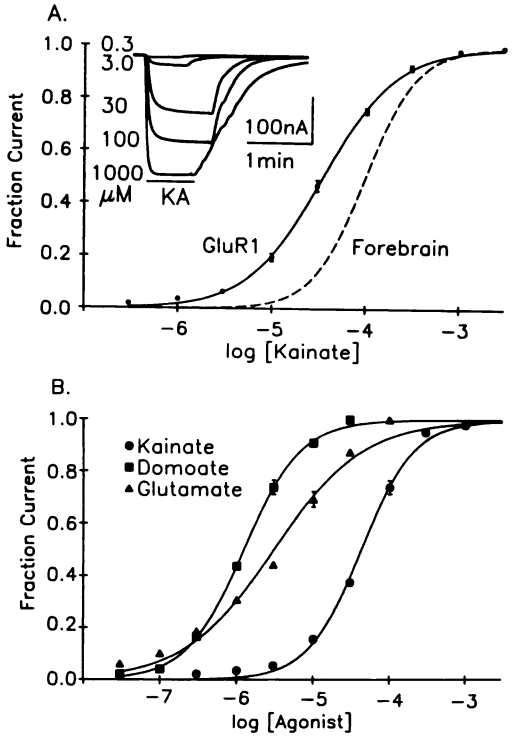

Fig. 1. Concentration-dependent activation of GluR1 receptors. A, *Inset*, current traces obtained from one oocyte held at −60 mV during the successive application of 0.3–1000 μM kainate (*KA*). The graph shows similar data from 17 cells, plotted as a concentration-response curve. Each *point* shows the mean ± standard error of the current produced by a given concentration of kainate, normalized to the maximum current produced by 1 or 3 mM kainate in each cell. For comparison, the concentration-response curve for kainate acting at non-NMDA receptors translated from forebrain mRNA is shown as a *dashed line* (n = 13 from Ref. 14). B, Concentration-response curves for glutamate, domoate, and kainate obtained in the same population of three oocytes. Each current is expressed relative to the maximum current evoked by each agoinst.

AMPA and quisqualate reduce the kainate response in a variety of neuron types (15, 16) and in oocytes injected with rat forebrain mRNA (14, 18). Hollmann *et al.* (6) originally reported that quisqualate failed to activate the GluR1 receptor, although in more recent experiments quisqualate proved active (9, 19). The reason for this discrepency is unclear. In our hands both AMPA and quisqualate reliably activated the GluR1 re-

[1] M. Curras and R. Dingledine. Manuscript in preparation.

TABLE 1

Comparison of GluR1 receptors to forebrain non-NMDA receptors

The data for forebrain non-NMDA receptors were taken from Refs. 14 and 28. The EC_{50} (mean and 95% confidence interval), slope factor n (mean ± standard error), and efficacy (mean ± standard error) relative to that of kainate were determined by fitting dose response curves to the equation

$$\frac{\text{Drug response}}{\text{Maximum kainate response}} = \frac{\text{efficacy}}{1 + (EC_{50}/[\text{drug}])^n}$$

The dose ratios of 6,7-diCl-HQC represent the fold rightward shift in the kainate dose-response curve, and pA_2 values were determined by Schild analysis.

Drug	GluR1	Forebrain
Kainate		
EC_{50} (μM)	34 (33–36)	98 (76–125)
Slope factor	1.14 ± 0.01	1.58 ± 0.13
Efficacy	1.00	1.00
Domoate		
EC_{50} (μM)	1.3 (0.6–2.6)	14 (13–15)
Slope factor	1.17 ± 0.03	1.47 ± 0.07
Efficacy	0.60 ± 0.035	ND
Glutamate		
EC_{50} (μM)	3.4 (2.1–4.7)	14 (8.4–23)
Slope factor	0.72 ± 0.07	1.14 ± 0.05
Efficacy	0.14 ± 0.023	0.05 ± 0.01
CNQX, pA_2	6.21 ± 0.08	6.53 ± 0.03
6,7-di Cl-HQC (1 μM), Dose ratio	2.4	10.8
AMPA/quisqualate	Partial agonist	Partial agonist
Ca^{2+}-dependent desensitization	Yes	No

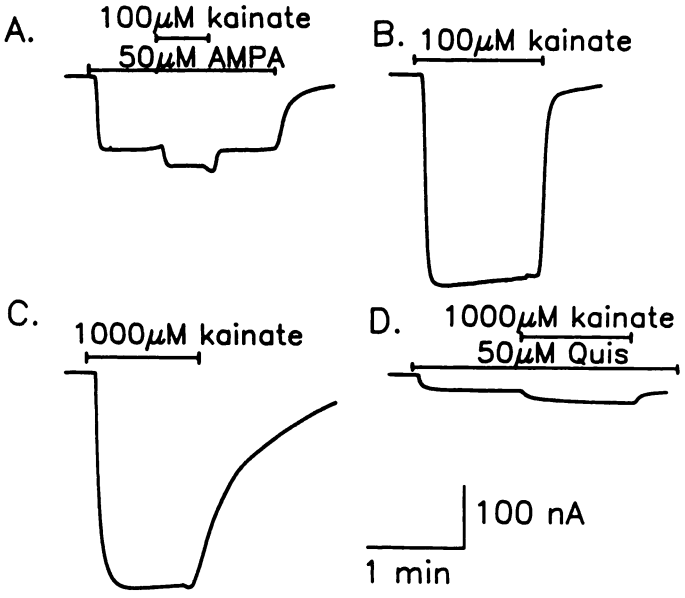

Fig. 2. Activation and block of homomeric GluR1 receptors by AMPA and quisqualate. A, AMPA (50 μM) perfusion induced an inward current of approximately 120 nA and reduced the current evoked by 100 μM kainate. B, Subsequent response to 100 μM kainate alone in the same cell. In another cell, a maximum response to 1000 μM kainate (C) was greatly reduced by 50 μM quisqualate (*Quis*) (D).

ceptor and appeared to act as partial agonists, because they reduced the response to the full agonist kainate (Fig. 2). Although full concentration-response curves were not generated for AMPA and quisqualate, these agonists appeared to have higher efficacy at GluR1 receptors than at forebrain non-NMDA receptors.

Slow desensitization by kainate. The response to both kainate and domoate in cortical neurons (20) and in oocytes injected with hippocampal or cortical mRNA (14) is nondesensitizing. In contrast, large responses of homomeric GluR1 receptors to kainate in many but not all cells slowly faded over a period of tens of seconds. As illustrated in Fig. 3A, slow desensitization was most marked at high kainate concentrations and/or when kainate response amplitude was high (typically >300 nA). Interestingly, the currents produced by domoate did not show desensitization. Desensitization was eliminated when $[Ca^{2+}]_o$ was reduced from 1.0 to 0.1 mM, and the steady state amplitude of kainate currents was also decreased in low $[Ca^{2+}]_o$ (Fig. 3B). Although we have never observed a similar fade of the kainate response in oocytes injected with forebrain or the kainate response in oocytes injected with forebrain or hippocampal mRNA, Rassendren *et al.* (21) reported a slow fade in the kainate-evoked current in oocytes injected with mRNA isolated from whole brain (including cerebellum).

The fade of GluR1 responses was not mediated through conventional NMDA receptors, because elimination of Mg^{2+} at a holding potential of −60 mV had no effect on the kainate currents (Fig. 3C). The fade of kainate currents was also not due to a loss of voltage control in the two-electrode clamp. We considered whether the calcium-dependent fade of kainate currents might reflect the transient activation of endogenous calcium-dependent Cl^- channels in the oocyte membrane (see Ref. 22). The Cl^- equilibrium potential is near −25 mV in oocytes; activation of these channels at a holding potential of −60 mV typically produces a transient inward current not dissimilar in appearance from that shown in Fig. 3B. Reduction of $[Cl^-]_o$ from 92 to 31 mM would cause a 27-mV depolarizing shift in the Cl^- equilibrium potential that should increase the amplitude of any Cl^- currents, a prediction we have verified for the calcium-dependent chloride current.[2] This procedure, however, enhanced neither steady state kainate currents at GluR1 receptors nor the degree of desensitization (Fig. 3D; $n = 3$). Instead, lowering of $[Cl^-]_o$ reduced the peak kainate-evoked current at GluR1 receptors without affecting desensitization. This obser-

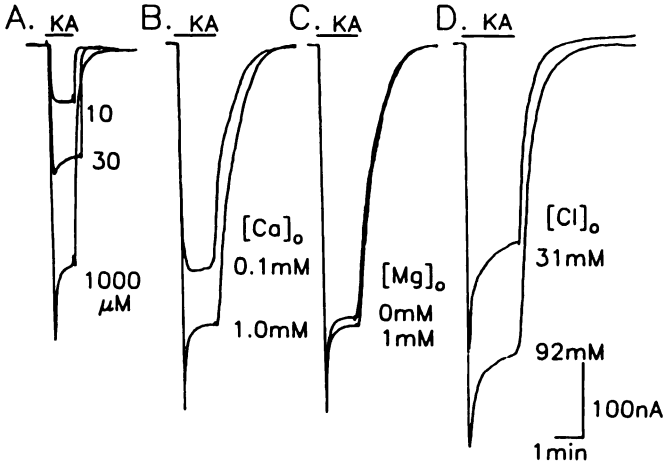

Fig. 3. Slow desensitization of kainate currents through GluR1 receptors. A, Currents evoked by increasing concentrations of kainate (*KA*) showed a progressively larger degree of fade. B, In another cell, reduction of $[Ca^{2+}]_o$ eliminated fade and decreased the steady state current. C, Changing of $[Mg^{2+}]_o$ had no effect on kainate-evoked current in the same cell. D, Partial replacement of NaCl with sodium methanesulfonate did not change the degree of fade but reduced the overall magnitude of the kainate response. Kainate was applied at 1 mM in B–D.

[2] N. Kleckner and R. Dingledine, unpublished observations.

vation indicates that activation of the calcium-dependent chloride current is unlikely to play a major role in the fade of kainate currents. In oocytes injected with rat forebrain or hippocampal mRNA, replacement of $[Cl^-]_o$ by methanesulfonate also reduces kainate-evoked currents by causing a small negative shift in reversal potential.[3] The reduction of whole-cell GluR1 kainate currents in low calcium suggests that this divalent cation may permeate the GluR1 channel. Preliminary measurements of the reversal potential of kainate currents in different $[Ca^{2+}]_o$ supports this hypothesis.[4] We presume that the slow fade of the kainate current is secondary to the build-up of intracellular calcium, but the subsequent steps leading to fade of the kainate current are unknown. The variability of this phenomenon (compare Figs. 1A and 3) is not unexpected for a metabolic process.

Antagonists. CNQX and certain quinoxaline derivatives are competitive antagonists of non-NMDA receptors in rat brain neurons (23) and in oocytes injected with cortical or forebrain mRNA (3). CNQX also blocked kainate-evoked currents at GluR1 receptors (Fig. 4A). Addition of 1 μM CNQX resulted in a 3.3-fold parallel shift to the right in the kainate dose-response curve at GluR1 receptors (Fig. 4B), compared with a 4.4-fold rightward shift at non-NMDA receptors encoded by forebrain mRNA (3).

A Schild analysis was performed to explore more fully the competitive nature of the CNQX block. Raising the concentration of CNQX caused a progressively larger rightward shift in the kainate dose-response curve at GluR1 receptors (Fig. 4C). The Schild plot of these data (Fig. 4D) had a slope of 1.09 ± 0.12, consistent with competitive antagonism. The K_B of CNQX was 519 nM at GluR1 receptors (Fig. 4D), compared with 295 nM at forebrain non-NMDA receptors (Table 1; Ref. 3). Thus, CNQX was less potent at blocking kainate responses at GluR1 receptors than at forebrain-derived receptors. Another quinoxaline derivative, 6,7-diCl-HQC (15 μM), exerted a competitive block of GluR1 receptors (Fig. 5) and was also less potent at GluR1 than at forebrain non-NMDA receptors (Table 1).

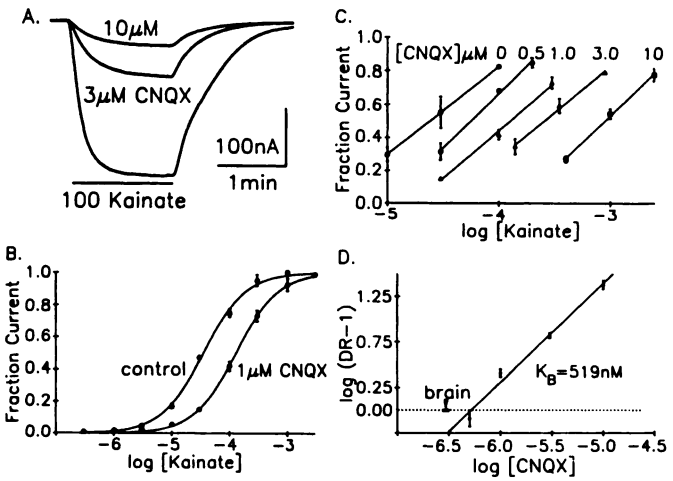

Fig. 4. Competitive block of GluR1 receptors by CNQX. A, Currents evoked by 100 μM kainate (*bottom trace*) are reduced by 3 and 10 μM CNQX. B, Concentration-response curve for kainate acting on GluR1 receptors in the absence and presence of 1 μM CNQX. Each *point* represents the mean ± standard error of the current, normalized to the maximum current produced in the absence of CNQX (n = 3). C, Partial kainate dose-response curves in the presence of increasing concentrations of CNQX (indicated above each curve). Each *point* is the mean ± standard error of measurements from three separate experiments. D, Schild regression derived from experiments illustrated in B and C. Each *point* represents the mean ± standard error of three determinations. The correlation for the Schild regression was 0.954. The average slope over three experiments was 1.09 ± 0.12, and the pA_2 was 6.21 ± 0.08. For comparison the mean ± standard error for the pA_2 of CNQX at forebrain receptors (3) is indicated by the *arrow*.

Discussion

Identity of the GluR1 non-NMDA receptor. The GluR1 subunit forms a functional homomeric receptor in oocytes that can be activated by kainate, AMPA, and several other agonists. From the following considerations, we suggest that GluR1 cDNA may encode a subunit of an AMPA receptor in the brain. The pharmacological properties of homomeric GluR1 receptors in the present study were similar but not identical to those of the non-NMDA receptors expressed from a forebrain mRNA population. Rat forebrain mRNA directs the expression in oocytes of kainate-activated non-NMDA receptors that have many pharmacological properties expected of [^{3}H]AMPA binding sites (3). Thus, in both electrophysiological and binding experiments the rank order of agonist potency is domoate ≥ glutamate > kainate, the affinity of the competitive antagonist CNQX measured by Schild analysis and Scatchard plots is nearly identical, and the agonists occlude one another's actions. Fletcher *et al.* (2) and Sheardown *et al.* (4) have also suggested that the non-NMDA receptor of electrophysiological studies corresponds to a [^{3}H]AMPA binding site. This conclusion is supported by the finding of Keinanen *et al.* (10) that the combined expression of GluR1 and a related sequence (GluR2) in a mammalian cell line results in receptors responsive to both kainate and AMPA.

Although GluR1 receptors resembled in many respects the non-NMDA receptors encoded by forebrain mRNA, there were several notable differences. Agonists were more potent and antagonists less potent on GluR1 receptors than on the population of receptors expressed by rat forebrain mRNA. Moreover, the agonist dose-response curves were less steep at GluR1 receptors than at forebrain non-NMDA receptors. This recalls the situation for cloned $GABA_A$ receptors, in which the apparent cooperativity (24) and potency (25) of GABA depend on the subunit composition. The subtle differences observed between GluR1 and forebrain non-NMDA receptors invite the

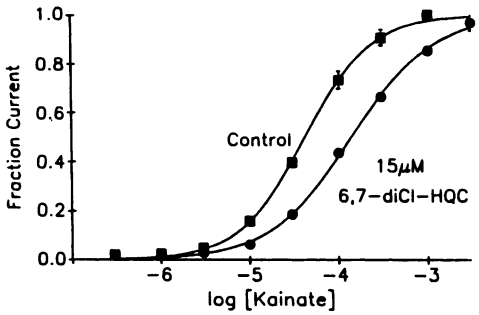

Fig. 5. Competitive block of GluR1 receptors by 6,7-diCl-HQC. A concentration-response curve for kainate was determined with and without 15 μM 6,7-diCl-HQC. Each *point* represents the mean ± standard error from three cells.

[3]N. W. Kleckner and R. Dingledine. Regulation of hippocampal NMDA receptors by magnesium and glycine during development. Manuscript in preparation.

[4]R. Dingledine and S. J. Myers, unpublished observations.

speculation that the conformation of ligand binding sites may be influenced by subunit-subunit interactions or that the ligand binding sites may be located at the junctures between subunits in the assembled receptor. An alternative explanation would involve the encoding, by the mixture of forebrain mRNAs, of posttranslational modifying enzymes that alter the properties of GluR1 receptors.

Although GluR1 receptors appear similar to AMPA receptors, equating the two is probably an oversimplification. The nomenclature for excitatory amino acid receptors will undoubtedly require modification as additional members are identified by molecular cloning and the properties of cloned receptors are compared with those of native receptors. Indeed, it is likely that multiple non-NMDA receptors are formed in oocytes injected with a mixture of forebrain mRNAs and that the properties measured in oocytes represent a weighted average of several receptor subtypes. This is also likely to be the case in neuronal studies, unless each neuron expresses only one type of non-NMDA receptor. Thus, the identification of "native" non-NMDA receptors for comparison with the cloned receptors is complicated. It is also possible that the subunits encoded by a mixture of forebrain mRNAs assemble in oocytes into unnatural receptors, whose collective properties do not reflect those of any native non-NMDA receptor. This is not likely to be a major problem, because the properties of non-NMDA receptors appear quite similar, whether studied in oocytes or in most neurons isolated from the cortex or hippocampus.

Homomeric Glur1 receptors are probably not formed in high numbers in oocytes injected with rat forebrain mRNA, because agonists have lower potency but steeper dose-response curves when acting on forebrain non-NMDA receptors, compared with GluR1 receptors. This combination is the opposite of that expected if homomeric GluR1 receptors make up a large fraction of the receptors expressed by forebrain mRNA. Our forebrain mRNA contains GluR1 mRNA, as determined by Northern blot analysis[5]; presumably, most receptors made from this forebrain mRNA preparation are heteromeric assemblies of multiple subunits.

Neuronal receptors similar to GluR1. The high agonist potency (Fig. 1), slow calcium-dependent desensitization of kainate currents (Fig. 3), and marked inward rectification of current flow (9, 19)[4] differentiate GluR1 receptors from most non-NMDA receptors in cortical neurons and the majority of non-NMDA receptors encoded by forebrain mRNA. Huettner (20) and Iino *et al.* (26) have recently described kainate-activated receptors in DRG and hippocampal neurons that display some of these features. For example, glutamate, kainate, and domoate are all much more potent in DRG than in cortical neurons (20). Furthermore, the action of kainate but not domoate on DRG neurons is marked by slow desensitization (20), similar in appearance to that occurring at GluR1 receptors, however, in DRG cells the slow fade of the kainate current is calcium-independent,[6] the kainate current-voltage curve is linear, and the rank order of agonist potency is domoate > kainate > glutamate (20). These properties are all different from those of GluR1 receptors.

In a minority of cultured hippocampal neurons, Iino *et al.* (26) found that kainate activated a non-NMDA ("type II") receptor that showed marked inward rectification similar to that found in GluR1 receptors (9, 19). Reduction of $[Ca^{2+}]_o$ reduced the inward current through type II receptors, similar to that shown in Fig. 3B; the behavior of the kainate reversal potential as $[Ca^{2+}]_o$ was changed indicated that type II receptors were much more permeable to calcium than conventional non-NMDA receptors. Murphy and Miller (27) provided evidence that the kainate channel of some striatal neurons may also be permeable to calcium.

These observations raise the possibility that homomeric GluR1 receptors are formed in some neurons or that the properties of some non-NMDA receptors are dominated by the GluR1 or similar subunit. In any event, these studies, together with the results presented here, underscore the variety of non-NMDA receptor types that exist, some of which appear to show appreciable calcium permeability. The pharmacological properties of GluR1 homomeric receptors shown here, when combined with similar data from the functional expression of other non-NMDA receptor subunits (both individually and in multiple combinations), should help determine the structural basis of agonist and antagonist recognition sites on non-NMDA receptors. This information may be useful in the development of drugs selective for different receptor subtypes.

Acknowledgments

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