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Different characteristics of AMPA receptor agonists acting at AMPA receptors expressed in *Xenopus* oocytes [1]

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Abstract

A series of (*RS*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) analogues were evaluated for activity at homo-oligomeric glutamate$_1$-flop (Glu_1-flop) receptors expressed in *Xenopus* oocytes, using the two-electrode voltage clamp technique. (*RS*)-2-Amino-3-(3-carboxy-5-methyl-4-isoxazolyl)propionic acid (ACPA) (EC_{50}, 2.4 μM), a homologue of AMPA having a carboxyl group as the terminal acidic functionality, was five times more potent than AMPA (EC_{50}, 12 μM) and 20 times more potent than kainate (EC_{50}, 46 μM). (*RS*)-2-Amino-3-(3-hydroxy-5-trifluoromethyl-4-isoxazolyl)propionic acid (Tri-F-AMPA), in which an electronegative trifluoromethyl group is substituted for the methyl group on the isoxazole ring in the AMPA structure, was three times more potent than AMPA, whereas (*RS*)-3-hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-5-carboxylic acid (5-HPCA), a bicyclic analogue of AMPA with highly restricted conformational flexibility was 10 times less potent than AMPA. The limiting slope of log-log plots of Glu_1-flop receptor currents versus low agonist concentrations had a value of 1.7 for ACPA and kainate compared to 1.5 for Tri-F-AMPA and 1.3 for 5-HPCA and AMPA. The amplitude of responses evoked by near saturating concentrations of the agonists varied more than 7-fold. The sequence of efficacy was ACPA = kainate > Tri-F-AMPA > AMPA > 5-HPCA. Moreover, when saturating concentrations of Tri-F-AMPA and kainate were co-applied, the response was significantly greater than when each of the agonists was applied separately. The potency of the antagonist 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(*f*)quinoxaline (NBQX) (estimated K_B, ~ 200 nM), to block currents mediated by Glu_1-flop receptors was similar for all of the agonists tested in this study. These results indicate that relatively minor changes in the molecular structure of AMPA are associated with marked effects on potency and efficacy. In particular, it is suggested that the acidity of the terminal group plays a major role in determining the degree of receptor activation in the steady state.

Keywords: Glu_1-flop receptor; AMPA receptor agonist; NBQX (2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(*f*)quinoxaline); (Pharmacology)

1. Introduction

Fast synaptic transmission in the mammalian central nervous system (CNS) is primarily mediated by L-glutamic acid (Glu) receptors of the (*RS*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid/kainate (AMPA/kainate) subtypes. The AMPA/kainate receptor channel(s) require cooperative binding of two or three agonist molecules for activation (Patneau and Mayer, 1990; Vodyanoy et al., 1993) and is permeable mainly to sodium and potassium ions (Mayer and Westbrook, 1987). AMPA and kainate produce physiological responses with different characteristics, e.g. the elementary conductance induced by AMPA is higher than that induced by kainate (Cull-Candy and Usowicz, 1989; Jonas and Sakmann, 1992). AMPA receptors form channels which exhibit rapidly desensitizing responses to AMPA and glutamate and maintain steady state responses to kainate (Patneau and Mayer, 1991). Kainate receptors, on the other hand, desensitize profoundly in the presence of both glutamate and kainate (Egebjerg et al., 1991; Partin et al., 1993). Data from studies using recombinant AMPA/kainate receptors have suggested that kainate and AMPA bind to different sites within the substructure of the AMPA receptor (Stein et al., 1992).

Studies of the molecular biology of AMPA/kainate receptors have uncovered a growing number of sequence-related subunits (Gasic and Hollmann, 1992; Hollmann et

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al., 1989, 1991; Keinänen et al., 1990; Boutler et al., 1990; Seeburg, 1993). These subunits can be grouped into three classes according to sequence characteristics: Glu_{1-4} receptors which can be activated by both AMPA and kainate; Glu_{5-7}, of which Glu_5 and Glu_6 assemble into homomeric channels activated by kainate, but not AMPA; and $kainate_1$ and $kainate_2$ receptors which do not generate functional homomeric channels. Subunit-specific differences in functional properties of Glu_{1-4} channels have been reported. The rectification properties and the calcium ion permeability of Glu_2 receptors on one hand and of Glu_1, Glu_3, Glu_4 receptors on the other hand are different (Hollmann et al., 1991; Verdoorn et al., 1991). The Glu_{1-4} receptor subunits have been shown to exist in two alternatively spliced versions termed flip and flop, which impart different pharmacological and kinetic properties to currents evoked by AMPA and kainate (Sommer et al., 1990; Partin et al., 1994). Whereas AMPA and kainate evoke steady state currents of similar amplitude in flip-expressing cells, kainate-evoked currents are much larger than those produced by AMPA in flop-expressing cells. The expression patterns for the flip and flop versions in rat brain have been shown to be developmentally regulated. Thus, AMPA receptors in the young rat brain do not contain the flop version, which appears to be present only in mature receptor forms. In contrast, flip versions remain largely invariant during postnatal brain development (Monyer et al., 1991).

Detailed structure/activity studies of AMPA/kainate receptors are needed to clarify the relationship between native and recombinant receptors and can be used to determine the structural requirements for activation of receptor subtypes. A number of AMPA receptor agonists have been described and tested using receptor binding techniques and electrophysiological assays in different mammalian systems, e.g. in vivo on cat spinal neurons and in vitro on rat cortical neurons (Hansen and Krogsgaard-Larsen, 1990; Krogsgaard-Larsen et al., 1992). Such studies have shown AMPA and a number of its structural analogues to be potent and selective agonists at AMPA receptors (Krogsgaard-Larsen et al., 1985; Lauridsen et al., 1985; Madsen and Wong, 1992; Madsen et al., 1992). These compounds include (*RS*)-3-hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-5-carboxylic acid (5-HPCA), with highly restricted conformational flexibility, (*RS*)-2-amino-3-(3-hydroxy-5-trifluoromethyl-4-isoxazolyl)propionic acid (Tri-F-AMPA), in which an electronegative trifluoromethyl group has been substituted for the methyl group on the isoxazole ring in the AMPA structure, and (*RS*)-2-amino-3-(3-carboxy-5-methyl-4-isoxazolyl) propionic acid (ACPA) which is a homologue of AMPA with a carboxyl group as the terminal acidic functionality. AMPA and these three analogues of AMPA with different structural characteristics (Fig. 1) have been chosen for pharmacological characterization at Glu_1-flop receptors. In the present study, homomeric Glu_1-flop receptors were expressed in *Xenopus* oocytes, and the pharmacological properties of the AMPA receptor agonists described were investigated. This expression system has previously been shown to be well suited for quantitative pharmacological studies of AMPA receptors (Stein et al., 1992; Wahl et al., 1992). *Xenopus* oocytes injected with rat brain mRNA express functional AMPA/kainate receptors that have electrical and pharmacological properties similar to those of neuronal receptors (Verdoorn and Dingledine, 1988). In this report, responses to the above mentioned series of AMPA analogues were characterized, and the results indicate that changes in molecular structure within certain limits are associated with marked differences in agonist potency and efficacy. These responses were further characterized using the competitive AMPA/kainate antagonist, 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(*f*)quinoxaline (NBQX) (Sheardown et al., 1990).

2. Materials and methods

2.1. RNA preparation

Glu_1 cDNA in pBluescript SK-plasmid, a gift from Dr S. Heinemann and Dr J. Boutler (Salk Institute, La Jolla, CA, USA), was amplified in JM 109 cells (Promega, Madison, WI, USA), purified with Qiagen Plasmid Kit (Chatsworth, CA, USA). For transcription of RNA, plasmid was linearized with the restriction enzyme, *XhoI* (Promega), and cRNA was synthesized by means of an mRNA Capping Kit (Stratagene, La Jolla, CA, USA). The cRNA transcripts were extracted twice with phenol/chloroform (1 : 1) and chloroform/isoamylalcohol (49 : 1) and purified by three precipitations in ammonium acetate and ethanol. RNA was washed twice with 70% ethanol, dried, and dissolved in diethyl pyrocarbonate-treated water at a final concentration of about 0.4 ng/nl and stored at −80°C. The concentration of synthesized cRNA was estimated by agarose gel electrophoresis and ethidium bromide staining.

2.2. Expression in Xenopus oocytes

Oocytes from *Xenopus laevis* (Xenopus I, Ann Arbor, USA) were injected with 50 nl of cRNA solution and incubated in Barth's medium with gentamycin (0.1 mg/ml) at about 17°C. Two days after injection, the oocytes were treated with collagenase (0.5 mg/ml) to remove the enveloping follicular cells (Miledi and Woodward, 1989), and recordings were performed 6–12 days after injection. The oocytes were voltage-clamped with a two-electrode voltage clamp (Dagan) having a virtual ground, and both microelectrodes were filled with 3 M KCl (Kusano et al., 1982; Miledi, 1982). Recordings were made while the oocyte was continuously superfused with frog Ringer's solution (115 mM NaCl/2 mM KCl/1.8 mM $CaCl_2$/5 mM Hepes, pH 7.0) in a small Plexiglas chamber, and all

drugs were added to the Ringer's solution. Unless otherwise stated, all recordings were made at room temperature and at a holding potential of −100 mV.

Agonist dose-response curves were made by measuring the maximal current induced by increasing concentrations of agonist. Data from individual oocytes were fitted to the logistic equation (De Lean et al., 1978):

$$I = I_{max}/\left[1 + (EC_{50}/\text{agonist})^{n}\right] \quad (1)$$

were I is the steady state current produced by the agonist. The parameters I_{max} (maximal current at infinite agonist concentration), n (the Hill coefficient), and EC_{50} (concentration of agonist producing 50% of I_{max}) were determined by an iterative least squares fitting routine. IC_{50} values were determined by fitting the data to eq. 1, except that EC_{50} was replaced by IC_{50}, and n was negative.

2.3. Drugs

5-HPCA, Tri-F-AMPA, AMPA and ACPA were synthesized as previously described (Krogsgaard-Larsen et al., 1985; Lauridsen et al., 1985; Madsen and Wong, 1992; Madsen et al., 1992). Kainate was from Sigma (St. Louis, MO, USA). NBQX was a generous gift from Dr. Tage Honoré (Novo-Nordisk A/S, Denmark). Drugs were dissolved in Ringer's solution and the pH was adjusted to 7.0.

3. Results

Oocytes expressing homomeric Glu_1-flop receptors responded reproducibly to bath applications of kainate, AMPA, 5-HPCA, Tri-F-AMPA, or ACPA (Fig. 1). The effects depicted in Fig. 1 are all obtained with near saturating concentrations of agonists, thus representing maximum responses. The currents observed in the oocytes represent the steady-state component of agonist responses, and the peak current elicited upon rapid agonist application was not seen because of slow solution changes around oocytes relative to the kinetics of desensitization. The potencies of

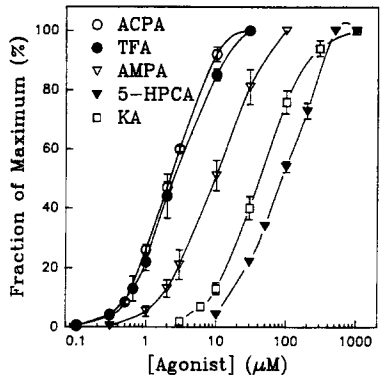

Fig. 2. Dose-response analysis for Glu_1-flop receptor activation by AMPA receptor agonists. Data points are the means ± S.E.M. expressed as a fraction of the response to the highest concentration of agonist tested. The curves were drawn using the logistic equation.

AMPA and its derivatives were determined by making concentration-response curves spanning a 2.5 log unit concentration range for each agonist. EC_{50} values spanned a 50-fold range of potency, with ACPA being the most potent agonist ($EC_{50} = 2.4 \pm 1.6$ μM) and 5-HPCA the least potent ($EC_{50} = 119 \pm 19$ μM). The potency rank order was ACPA > Tri-F-AMPA > AMPA > kainate > 5-HPCA (Fig. 2). As shown in Fig. 3, a plot of EC_{50}s determined in voltage clamp experiments versus IC_{50}s for displacement of [^{3}H]AMPA binding exhibits a linear correlation over a 2.5 log unit scale of agonist potency. However, the agonist potency generally was about 30 times lover for voltage-clamp studies as compared to receptor binding IC_{50}s. The Glu_1-flop receptor concentration-response curve was steeper than predicted by a single binding site isotherm for all agonists, suggesting cooperative activation by agonist binding to more than one subunit of the receptor complex. This was confirmed by examining the limiting slope for the concentration-response relationship for low doses of agonist plotted on a log-log scale. The limiting slope of the log-log plots was steepest for kainate (1.7 ± 0.03) and ACPA (1.6 ± 0.05) as compared with 1.5 ± 0.09 for Tri-F-AMPA, 1.3 ± 0.09 for AMPA,

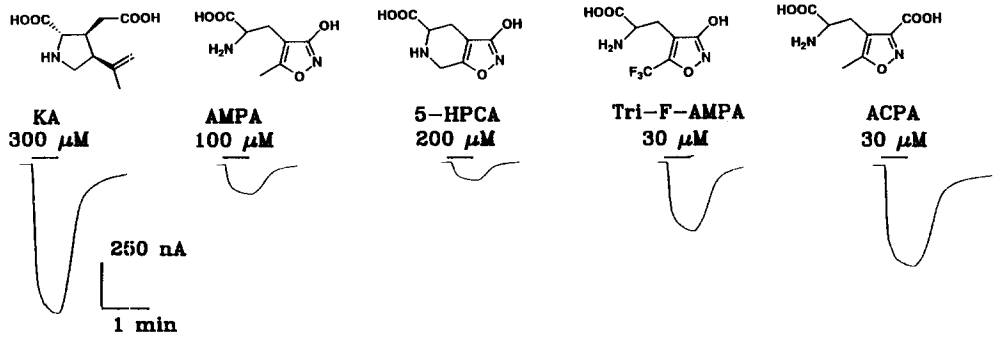

Fig. 1. Inward currents activated by saturating concentrations of kainate, AMPA, Tri-F-AMPA, and ACPA and submaximal concentration of 5-HPCA were recorded from a single oocyte injected with 20 ng Glu_1-flop receptor cRNA. Drug applications are indicated by bars and, unless otherwise stated, the holding potential in this and all following experiments was −100 mV. Structures of the AMPA receptor agonist used in this study are shown above the sample records.

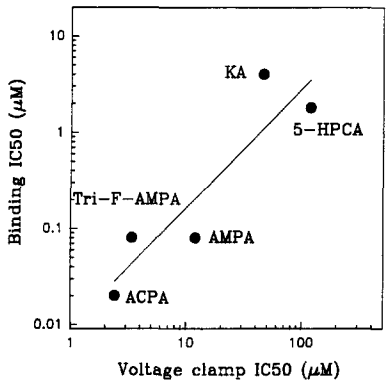

Fig. 3. The EC_{50}s estimated for steady state responses at Glu_1-flop receptors, using the logistic equation, are plotted versus IC_{50}s estimated from displacement of [^{3}H]AMPA binding to rat cortical membranes. The fit is by linear regression. Binding data from Madsen et al. (1990) and Madsen and Wong (1992).

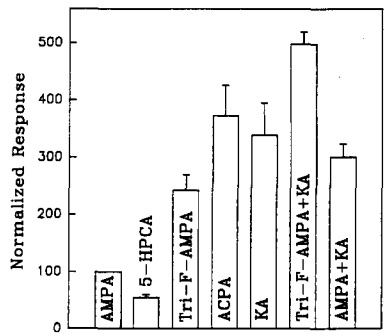

Fig. 4. Comparison of agonist efficacy for responses at Glu_1-flop receptors. Responses were evoked by saturating concentrations of agonists, as in Fig. 1, and normalized with respect to the response evoked by 100 μM AMPA. The bar graph summarizes data ± S.E.M. from 8 oocytes (2 different donors) injected with 20 ng Glu_1-flop cRNA 10 days prior to recordings. The amplitude of the response to Tri-F-AMPA plus kainate was significantly greater than that to kainate alone ($P < 0.001$, paired t-test).

and 1.3 ± 0.09 for 5-HPCA (Table 1). These findings may indicate subtle differences in the stoichiometry of the receptor binding, depending on the nature of the agonist molecule. However, mechanistic interpretations are problematic, because a desensitized state of the receptor is being measured.

The results illustrated in Fig. 1 reveal marked differences in the efficacy of AMPA and its analogues. This was examined in more detail by measuring responses evoked by near saturating concentrations of agonists from individual oocytes. In order to control for differences in receptor expression between oocytes, measurements of responses evoked by AMPA and its analogues were normalized to the response evoked by a saturating concentration of AMPA (100 μM). Responses evoked by the agonists varied more than 7-fold in amplitude, 5-HPCA being the least efficacious agonist (55 ± 5.5%) and ACPA (373 ± 53%) and kainate (339 ± 56%) the most efficacious agonists. The sequence of efficacy was ACPA = kainate > Tri-F-AMPA > AMPA > 5-HPCA (Fig. 4). AMPA and quisqualic acid are known to reduce the kainate response in neurons and in

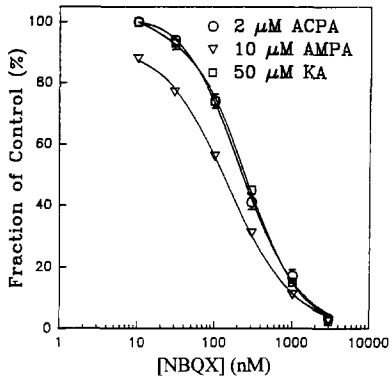

Fig. 5. Concentration-response curves for NBQX of the currents activated by kainate (50 μM), AMPA (10 μM), or ACPA (2 μM). Responses were normalized as fractions of the response activated in the absence of NBQX. Each point represents the mean ± S.E.M. of three to four experiments.

oocytes expressing homomeric or heteromeric Glu receptor subunits (Stein et al., 1992; Dawson et al., 1991). Interestingly, when saturating doses of Tri-F-AMPA and kainate

Table 1
EC_{50} values for AMPA receptor agonists and potency of NBQX

	EC_{50} (μM)	Limiting slope	IC_{50} (nM)	K_B or K_I (nM)	pK_{AI} (amino acid)	pK_{AIII} (distal)	pK_{AII}
AMPA	12 ± 3	1.26 ± 0.03	164 ± 28	197	2.5	10.0	4.8
Kainate	46 ± 6	1.73 ± 0.03	251 ± 12	231	–	–	–
ACPA	2.4 ± 0.2	1.62 ± 0.05	219 ± 28	263	< 2	9.3	2.2
Tri-F-AMPA	3.4 ± 0.4	1.49 ± 0.05	–	186	< 2	9.3	3.4
5-HPCA	119 ± 19	1.27 ± 0.09	–	–	2.2	8.1	4.7

EC_{50} values were determined from analysis of 3–5 dose-response curves per agonist using the logistic equation; values are means ± S.E.M. The limiting slope was determined from double-logarithmic plots at low concentrations of agonist. IC_{50} values were derived from inhibition curves obtained with NBQX and K_I estimates were derived for kainate, AMPA and ACPA using the formula $K_I = IC_{50} \times (EC_{50}/\text{agonist})$ (Cheng and Prusoff, 1973). The K_B value for Tri-F-AMPA was culculated as the negative logarithm of the pA_2 value from the result of the Schild analysis. pK_A values from Madsen et al. (1986) (AMPA and 5-HPCA) and Madsen et al. (1992) (Tri-F-AMPA). The pK_A values for ACPA were determined by titration as described (Madsen et al., 1986). –: not determined.

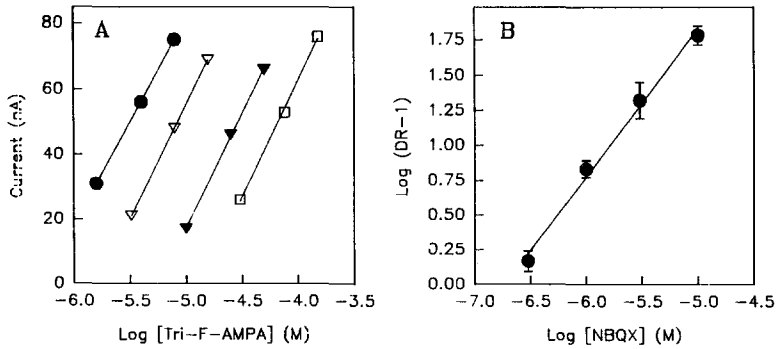

Fig. 6. Competitive block by NBQX of Tri-F-AMPA-evoked currents at Glu_1-flop receptors. (A) The parallel shifts in the concentration-response curve for Tri-F-AMPA (filled circles) and in the presence of 0.3 μM (open triangles), 1.0 μM (filled triangles), and 3 μM (open squares) NBQX. The lines represent linear regressions of these data sets and were used to calculate dose ratios (DR) for Schild analysis. (B) Schild regression derived from experiments illustrated in (A). Each point represents the mean ± S.E.M. of three to four experiments. The slope of the regression is 1.06 ± 0.065 and the pA_2 is 6.73.

were co-applied, the response was significantly greater than the response for kainate alone (Fig. 4). The two responses were, however, not fully additive. Co-application of kainate and AMPA, for example, produced current responses which were clearly not additive (Fig. 4).

NBQX potently inhibited responses evoked by AMPA (10 μM), kainate (50 μM), and ACPA (2 μM) (Table 1 and Fig. 5). A Schild analysis was performed on the blocking action of NBQX on responses evoked by Tri-F-AMPA. NBQX produced an almost parallel shift to the right of the concentration-response curve (Fig. 6A), and linear Schild regression with a slope of 1.06 ± 0.065 was observed, indicating competitive antagonism (Fig. 6B). The K_B of NBQX was 186 nM.

The current-voltage relationship of the AMPA response displayed a pronounced voltage dependence. At potentials between −140 and −70 mV, the relationship of the AMPA-evoked current to voltage was close to linear (Fig. 7). However, at voltages more positive than −30 mV no current was detected and no clear reversal of the current was observed. Currents evoked by Tri-F-AMPA and ACPA displayed a very similar voltage dependence.

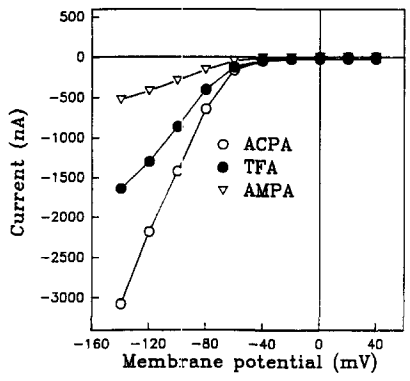

Fig. 7. Current-voltage relationships for the membrane currents elicited by AMPA (10 μM), Tri-F-AMPA (2 μM), and ACPA (2 μM) at Glu_1-flop receptors. Current-voltage relations were obtained by holding the membrane potential at −40 mV and applying pulses (3-s duration) to the various potentials indicated. Points show agonist-activated currents after substraction of 'passive' currents obtained before agonist application.

4. Discussion

Pharmacological studies using recombinant AMPA receptor channels, functionally expressed in *Xenopus* oocytes, are a useful approach to the understanding of the structural requirements for activation of AMPA receptor subtypes at the molecular level. The experiments described in the present paper were performed on oocytes injected with the flop version of Glu_1. In the rat brain, this splice variant seems to be specific for mature receptor forms, since it is not expressed before the initiation of synapse formation (Monyer et al., 1991). Recently it was demonstrated that dentate gyrus basket interneurons predominantly express Glu_1-flop and only small amounts of mRNAs encoding Glu_{2-4} receptor subunits (Geiger et al., 1995). This raises the possibility that interneurons in the CNS regulate Ca^{2+} entry through homomeric Glu_1 receptors.

The purpose of this study was to continue the process of identifying structural characteristics of the molecules of AMPA and AMPA analogues that are important for receptor activation. The discovery that AMPA is a potent and selective agonist at AMPA receptors (Krogsgaard-Larsen, 1980) prompted the development of a series of AMPA analogues with different structural characteristics. The compounds under study, AMPA, 5-HPCA, Tri-F-AMPA and ACPA have previously been found to be full AMPA receptor agonists but with varying potencies. Testing them on homomeric Glu_1-flop receptors revealed a number of pharmacological differences between these agonists. All of the AMPA analogues assayed clearly acted at Glu_1-flop receptors expressed in oocytes, albeit with varying potencies. The observed rank order of potency (ACPA > Tri-F-

AMPA > AMPA > kainate > 5-HPCA) is in full agreement with rank orders measured from electrical recordings in rat cortical slices, and it is likewise similar to the potency sequence obtained in [^{3}H]AMPA binding displacement studies (Madsen et al., 1990, 1992; Madsen and Wong, 1992). The EC_{50} value of AMPA and kainate also corresponds well with values reported previously from experiments with oocytes expressing homomeric Glu_1-flop receptors (Li et al., 1994).

One of the characteristic features of the response to activation of AMPA receptors in CNS neurons and in mammalian cells expressing the cDNA for AMPA receptors is an initial fast peak current followed by a lower steady state current as the result of marked desensitization. This desensitization is evoked by AMPA, but not by kainate. In the present experiments, the steady state current obtained with saturating concentrations of AMPA receptor agonists differed greatly (Fig. 1). The experimental evidence suggests that the marked differences in amplitude produced by AMPA receptor agonists are unlikely to result from activation of distinct receptor subtypes, because oocytes were injected with cRNA coding for only one subunit. Instead it is suggested that the differences in steady state currents are due to variation in the extent of the desensitization. L-Glutamic acid receptor desensitization has been shown to play an important role during normal synaptic transmission (Trussel and Fischbach, 1989), and modification of desensitization is one way by which AMPA receptor responses can be altered by pharmacological agents (Tang et al., 1989; Vyklicky et al., 1991; Patneau et al., 1992). Ranking the agonists in terms of efficacy led to the sequence ACPA = kainate > Tri-F-AMPA > AMPA > 5-HPCA. This efficacy rank order is in good agreement with the order of potencies obtained for these agonists, except for kainate, which shows a very low potency relative to its high efficacy. When comparing the limiting-slope obtained values (Table 1) and the efficacies, a more obvious correlation seems to emerge. The two most efficacious agonists, ACPA and kainate, show the highest limiting slope values, Tri-F-AMPA has lower efficacy and lower slope value, and AMPA and 5-HPCA have both the lowest efficacy and limiting slope values (Table 1). These findings may indicate subtle differences in the stoichiometry of the binding reaction, depending on the nature of the agonist molecule. Obviously, a wide range of efficacies can be obtained for agonists with different structural characteristics. The compounds under study vary in terms of structure and physicochemical properties. The acidity of the terminal acidic functionality varies considerably (Table 1). The two analogues, AMPA and 5-HPCA, containing the 3-hydroxyisoxazole moiety show rather low acidity of the distal acidic group, $pK_A = 4.8$ and 4.7, respectively (Madsen et al., 1986). When compared to Tri-F-AMPA, $pK_A = 3.4$ (Madsen and Wong, 1992) this acidity is significantly enhanced by the electronegative trifluoromethyl group incorporated, and for ACPA, $pK_A = 2.2$, with a carboxylate group as the terminal functionality, acidity is further enhanced. These considerations suggest that the acidity of the terminal group, at least among this group of AMPA agonists, plays a major role in determining the degree of receptor activation in the steady (desensitized) state. Interestingly, results of previous studies on willardine analogues have indicated that introduction of electron-withdrawing groups at ring position 5′ expected to increase the concentration of negatively charged species of agonist, increases agonist affinity for the AMPA/kainate receptors present on mouse embryonic hippocampal neurons. However, the electronegativity sequence of the 5-substituted willardines was only partially correlated with the degree of receptor desensitization (Patneau et al., 1992).

The demonstration that responses to high concentrations of kainate and Tri-F-AMPA are partly additive was unexpected. The antagonism of kainate responses by quisqualic acid, AMPA, and L-glutamic acid has been documented repeatedly for a number of preparations. In oocytes injected with rat brain mRNA or Glu_1-flop cRNA, 50 μM AMPA reduced the response to 100 μM kainate by 70 and 50%, respectively (Verdoorn and Dingledine, 1988; Dawson et al., 1991). The data presented here support the hypothesis that kainate and AMPA interact with the same binding site, but, on the other hand, the data also suggest that the binding sites for kainate and Tri-F-AMPA are not identical because the actions of these agonists are partly additive. These observations may again be related to differences in cooperativity when agonist molecules interact with two or more binding sites. Based on a lower activity of NBQX towards AMPA responses than to kainate responses, Stein et al. (1992) suggested separate agonist binding sites for AMPA and kainate within the substructure of heteromeric $Glu_{1/2}$-flip receptors. However, in agreement with our results with Glu_1-flop receptors, Stein et al. (1992) found that NBQX failed to distinguish between kainate and AMPA responses at homomeric Glu_1-flip receptors.

The difference in efficacy observed for the agonists under study may vary depending on the voltage dependence. The observed *I-V* relationships for AMPA, Tri-F-AMPA, and ACPA clearly showed similar voltage dependence for the different agonists, though with varying efficacy. Moreover, the responses at homomeric Glu_1 receptors exhibited strong inward rectification and no clear reversal potential, a striking property of homomeric Glu receptors (Nakanishi et al., 1990).

The observed pA_2 value of 6.73 for the antagonism by NBQX of Tri-F-AMPA-induced currents at Glu_1-flop receptors is consistent with values obtained for NBQX antagonism of kainate- and AMPA-induced currents at Glu_1-flip receptors (Stein et al., 1992). Moreover, the antagonism of Tri-F-AMPA produced by NBQX was clearly competitive, as suggested by the observed Schild regression slope of unity. NBQX also blocked AMPA-, kainate-, and ACPA-induced currents with similar potency.

This lack of selectivity of NBQX apparently contrasts with the reported 30-fold greater potency of NBQX for [^{3}H]AMPA binding sites (0.15 μM) than for [^{3}H]kainate binding sites (4.8 μM) (Sheardown et al., 1990). The action of kainate at Glu_1-flop receptors may reflect activity at the [^{3}H]AMPA binding site, in agreement with the action of kainate observed in neurons as well as oocytes injected with mRNA from cultured mouse cortical neurons (Wahl et al., 1993) or rat brain (Verdoorn et al., 1989). It is important to note, however, that kainate and AMPA differ in some electrophysiological studies in their sensitivity to NBQX (Sheardown et al., 1990; Pook et al., 1993).

The present results indicate that Glu_1-flop receptors have different sensitivities to a variety of isoxazole amino acids typified by AMPA. Further work with fast agonist application techniques may provide additional insight into the ability of the agonists to produce different levels of receptor activation.

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