Comparative Patch Clamp Studies on the Kinetics and Selectivity of Glutamate Receptor Antagonism by 2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX) and 1-(4-Amino-phenyl)-4-methyl-7,8-methylendioxy-5H-2,3-benzodiazepine (GYKI 52466)

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Summary—The glutamate antagonistic effects of NBQX [2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline] and GYKI 52466 [1-(4-amino-phenyl)-4-methyl-7,8-methylendioxy-5H-2,3-benzodiazepine] were compared on inward current responses of cultured superior collicular and hippocampal neurones with the whole cell patch clamp technique. Both NBQX (8 PM) and GYKI 52466 (33 PM) selectively reduced responses to AMPA [(S)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, 50 PM] and kainate (50 μM) whilst having little effect on responses to NMDA (N-methyl-D-aspartate, 100 μM). The effects of the two antagonists on the kinetics of AMPA (50 PM) responses were, however, very different—NBQX dramatically slowed the rise time of responses so that peak currents (IC50 60.4 ± 4.2 nM) were markedly more effected than desensitized plateau currents (IC50 706 ± 99 nM) whereas GYKI 52466 antagonized plateau responses (IC50 4.44 ± 0.21 μM) somewhat more than peak responses (IC50 6.87 ± 0.46 μM) and had only marginal effects on kinetics. In fact, low concentrations of NBQX (50-250 nM) actually potentiated plateau AMPA responses—an effect likely to be due to a reduction in the degree of AMPA-induced desensitization. Similar effects on response kinetics, were seen with kainate such that the IC50s for NBQX in antagonizing initial and plateau components of current responses to kainate 400 μM were 18.1 ± 2.9 nM and 298 ± 27 nM respectively whereas the IC50s for GYKI 52466 against kainate 50 μM were 17.3 ± 1.8 μM and 15.5 ± 3.3 μM respectively. These differences are likely to be due to the different modes of action of the two antagonists—NBQX shifted kainate concentration responses curves to the right in a parallel fashion indicative of competitive antagonism whereas the effects of GYKI 52466 were largely noncompetitive. There was, however, some indication for a small allosteric influence of GYKI 52466 on the affinity of the glutamate recognition site of the AMPA/kainate receptor. Estimation of Kd using the Cheng–Prussoff relationship revealed little difference in the affinity of NBQX in antagonizing plateau responses to AMPA (Kd 23.2 nM) and kainate (Kd 57.1 nM) and indicate that the effects of these two agonists are mediated at a common receptor under the experimental conditions used. Moreover, the differential effects of NBQX on peak and plateau components of AMPA (50 μM) responses was associated with a desensitization-induced, paradoxical increase in the agonist affinity and was probably not due to any change in the affinity of NBQX. As GYKI 52466 decreases plateau AMPA responses somewhat more than peak responses it may have more influence on prolonged pathological than transient physiological activation of AMPA receptors. The reverse was true for NBQX which might therefore be predicted to produce more side effects by blocking normal synaptic transmission.

Key words—NMDA, AMPA, kainate, GYKI 52466, NBQX, patch clamp, hippocampus, superior colliculus, culture, kinetics, concentration clamp, desensitization.

Glutamate is probably the major excitatory transmitter in the central nervous system (CNS) but is also likely to be involved in many pathological processes. As such there is a great deal of interest in the development of glutamate antagonists for therapeutic use. Until recently, most research effort was concentrated on selective antagonists for the N-methyl-D-aspartate (NMDA) subtype of ionotropic glutamate receptor due to a lack of selective antagonists for non-NMDA ionotropic glutamate receptors i.e. (S)-α-amino-3-hydroxy-5-methyl-4-
isoxazolopropionic acid (AMPA) and kainate receptors. However, with a few exceptions (for review e.g. see Rogawski, 1993) such antagonists have proven to have poor therapeutic indices and often produce memory deficits, ataxia and psychotomimetic side effects, both in animal models and man, thus excluding their long term use. Moreover, although such side effects would be less important for the acute use of NMDA antagonists in global ischaemia, the therapeutic time window is probably too short to make this feasible. Thus, the discovery of the potent, selective, competitive AMPA/kainate antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo-(F)quinoxaline (NBQX, Sheardown et al., 1990) was rapidly followed by extensive research on its effects in various animal models of glutamate pathology.

NBQX has indeed proven to be effective in numerous models of both focal (Sheardown et al., 1990; Buchan et al., 1991b; Gill et al., 1992) and global ischaemia (Buchan et al., 1991a; Balchen and Diemer, 1992; Lepellet et al., 1992) and selectively reduces the toxic effects of focal injections of AMPA and kainate (Moncada et al., 1991; Bisaga et al., 1993). Thus, in vitro antagonism at AMPA/kainate receptors (Lodge et al., 1991) is likely to underly the beneficial effects of NBQX seen in animal models of epilepsy (Chapman et al., 1991; Smith et al., 1991; McAllister, 1993), spasticity (Turski et al., 1992), dystonia (Richter et al., 1993) and possibly Morbus Parkinson (Klockgether et al., 1991; Loschmann et al., 1991; Wachtel et al., 1992; but see Luquin et al., 1993). Moreover, the therapeutic time window in ischaemia may be somewhat better for non-NMDA, than that for NMDA, antagonists (Buchan et al., 1991b; Judge et al., 1991; Diemer et al., 1992; Nellgard and Wieloch, 1992; Sheardown et al., 1993).

Low concentrations of NBQX also block synaptic transmission in hippocampal slices (Randle et al., 1992; Goldstein and Litwin, 1993). Due to the wealth of glutamatergic synapses in the CNS it might be expected that a competitive AMPA/kainate antagonists such as NBQX should also produce numerous side effects in vivo and it is therefore not surprising that NBQX also produces ataxia. As such, there is also considerable interest in the biological effects of 1-(4-amino-phenyl)-4-methyl-7,8-methyl-endoxioyl-5H-2,3-benzodiazepine (GYKI 52466) which is a selective, non-competitive AMPA/kainate receptor antagonist (Ourdouz and Durand, 1991; Donevan and Rogawski, 1993). GYKI 52466 also reduces AMPA/kainate toxicity in vitro (May and Robinson, 1993) and in vivo (Moncada et al., 1991; Bisaga et al., 1993) and is effective in animal models of focal ischaemia (Smith and Meldrum, 1992) and epilepsy (Chapman et al., 1991; Smith et al., 1991; Yamaguchi et al., 1993). It is now clear that NBQX and GYKI 52466 have very different mechanisms of antagonistic action at AMPA/kainate receptors and it is hoped that GYKI 52466 may be a more promising lead for the development of therapeutic agents directed at AMPA/kainate receptors due to its noncompetitive nature. Unfortu-

nately, present data indicate that GYKI 52466 also blocks fast synaptic transmission in vitro (Iarnawa et al., 1992) and in vivo (Engberg et al., 1993) and produces similar ataxia to that seen with NBQX (Smith et al., 1993; Yamaguchi et al., 1993).

To date, there has been no systematic electrophysiological study comparing the selectivity and kinetics of glutamate antagonism by NBQX and GYKI 52466 in vivo. Moreover, only the effects of GYKI 52466 have been adequately assessed on primary cultures of mammalian central neurones. We used the patch clamp technique and a fast superfusion system to characterize further the selectivity, kinetics and concentration-dependency of ionotropic glutamate receptor blockade by NBQX and GYKI 52466 with the aim of highlighting possible differences in the mechanism of blockade which could, theoretically, have important functional consequences for AMPA/kainate receptor blockade in vivo.

METHODS

Patch clamp recordings were made from cultured hippocampal and/or superior collicular neurones (10–14 days in vitro) in whole cell mode at room temperature (20–22°C) with the aid of an EPC-7 amplifier (List). Most recordings were made at a membrane potential of −70 mV. Patch clamp electrodes were pulled with a horizontal puller (DMZ) and had an internal tip diameter between 1.0 and 1.2 μM and a tip resistance of 4 to 8 MΩ. Cells were continuously superfused via one of eight channels of a fast superfusion system (Konnerth et al., 1987). Test substances then were applied by rapidly switching channels. Complete exchange of the superfused solution was achieved within 10–20 msec—confirmed by the speed of block of voltage-activated calcium channels when switching to solutions containing cadmium. The applications of solutions and the synchronized on-line electronic acquisition of data were controlled by the IBM program PCLAMP. Subsequently, AUTESP for IBM (Garching Instruments) was utilized to analyze the data semi-automatically off-line. Only results from stable cells were accepted for inclusion in the final analysis, i.e. following recovery of responses to AMPA or kainate by at least 80% of their depression by NBQX and/or GYKI 52466.

Superior colliculi for cell culture were isolated from rat embryos (E20–E21) and were then transferred to calcium and magnesium free Hank's buffered salt solution (Gibco) on ice. Cells were mechanically dissociated in 0.05% DNAase/0.3% ovomucoid (Sigma) following a 15 min preincubation with 0.66% trypsin/O.1% DNAase (Sigma). The dissociated cells were then centrifuged at 18 g for 10 min, resuspended in minimum essential medium (Gibco) and plated at a density of 150,000 cells cm−2 onto poly-L-lysine (Sigma)-precoated plastic petri dishes (Falcon). The cells were nourished with NaHCO3/HEPES-buffered minimum essential medium supplemented with 5% foetal calf serum and 5% horse
serum (Gibco) and incubated at 37°C with 95% O₂/5% CO₂. The medium was exchanged completely following inhibition of further glial mitosis with cytosine-β-D-arabinofuranoside (10 μM, Sigma) for 48 h, after about 7 days in vitro. Thereafter, the medium was exchanged partially twice weekly. The protocol for the preparation of hippocampal cells was essentially similar to that utilized for superior colliculus except for the omission of trypsin pre-incubation and the use of younger (E17) rat embryos.

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**Fig. 1.** Selective antagonistic effects of NBQX 8 μM on current responses of a single hippocampal neurone to AMPA and kainate. NMDA (100 μM, for 2.5 sec every 35 sec), kainate (50 μM, for 2.5 sec every 35 sec) or AMPA (50 μM, for 350 msec every 10 sec) were applied in the continuous presence of glycine 1 μM and at a constant membrane potential of −70 mV. The left and right panels show control and recovery responses to the agonists tested. The middle panels show equilibrium agonist responses in the continuous presence of NBQX 8 μM. NB: different time scales.
RESULTS

NBQX 8 μM almost fully antagonized responses of hippocampal neurones to both AMPA 50 μM and kainate 50 μM whilst having no effect on responses to NMDA 100 μM (Figs 1 and 2). This profile of selectivity was somewhat different for GYKI 52466 33 μM which reduced hippocampal neuronal current response to AMPA somewhat more than responses to kainate. Responses to NMDA were practically unaffected by this concentration of GYKI 52466 (Figs 3 and 4).

Further experiments to test the concentration-dependency of the effects of NBQX and GYKI 52466 on AMPA responses were performed on superior colliculus neurones as these cells are smaller and circumvent some of the problems of buffered diffusion reported to occur with large hippocampal neurones. Control responses to AMPA 50 μM rose relatively sharply (τ on = 7.2 ± 1.3 msec) to a pronounced peak and then desensitized rapidly (τ desens = 42.9 ± 2.1 msec) by around 65% to a stable plateau current (n = 52). NBQX (50–250 nM) concentration-dependently reduced peak responses to AMPA but, surprisingly, potentiated plateau responses in an inverse concentration-dependent manner i.e. the lowest concentration of 50 nM potentiated the most [Figs 5 and 8(a)]. This unexpected result was not due to artifacts associated with the perfusion system as responses to coapplied AMPA and NBQX, i.e. without preincubation of the antagonist, were similar to control responses. However, higher concentrations of NBQX (0.5–8 μM) did, as expected, progressively antagonize both the peak and plateau components of the current response to AMPA (Figs 6 and 8). In this regard the “peak” of responses in the presence of NBQX was
quantified by measuring the current at the same time point as that of the true peak of the control response. The antagonism of AMPA plateau responses was accompanied by a NBQX concentration-dependent, dramatic slowing in the rate of onset of this response (Table 1). Closer examination of AMPA response kinetics in the presence of various concentrations of NBQX suggests that this effect was due the rapid association of AMPA to receptors not occupied by NBQX—the proportion of which should be dependent on the NBQX concentration—and a slower association subsequent to the dissociation of NBQX, which should be antagonist concentration-independent. This is particularly apparent for data with NBQX 250 nM (Fig. 5) where the rise times could best be described by a double exponential fit (\( \tau \) ons of 17.0 ± 2.1 and 508 ± 117 msec).

![Image](image.png)

**Fig. 3.** Selective antagonistic effects of GYKI 52466 33 \( \mu \)M on current responses of a single hippocampal neurone to NMDA, AMPA and kainate. Agonists were applied as in Fig. 1.
This preferential antagonism of peak responses to AMPA 50 \( \mu M \) by NBQX contrasts strongly with the effects of GYKI 52466 which concentration-dependently antagonized plateau somewhat more than peak-currents (Fig. 7) and only slowed the rise time of AMPA responses to a small degree (Table 1). This effect of GYKI 52466 was not associated with a consistent change in the rate of AMPA receptor desensitization (Table 1). For comparison, the pooled data on the concentration-dependent antagonism of AMPA current responses by NBQX and GYKI 52466 have been presented in Fig. 8. The IC\(_{50}\)s for NBQX in antagonizing peak and plateau components of current responses to AMPA 50 \( \mu M \) were 60.4 \( \pm \) 4.2 nM and 706 \( \pm \) 99 nM respectively whereas for GYKI 52466 these values were 6.87 \( \pm \) 0.46 \( \mu M \) and 4.44 \( \pm \) 0.21 \( \mu M \) respectively.

The unexpected potentiation of plateau AMPA responses by the lowest concentration of NBQX led us to investigate further whether AMPA receptor desensitization is involved in this effect. As can be seen from Fig. 9(A) the relative degree of AMPA receptor desensitization was concentration-dependent, e.g. current responses to AMPA 3.3 \( \mu M \) were very small but only desensitize by around 17% whereas responses to AMPA 800 \( \mu M \) rose very rapidly (\( \tau \) on 5.4 \( \pm \) 1.9 msec) to a large peak and then decayed rapidly (\( \tau \) desen 19.7 \( \pm \) 1.6 msec) by 85% to a new stable plateau current. The onset rate and desensitization rate were concentration-dependent, both becoming faster with increasing concentrations of AMPA and were best described by single exponential fits. However, a plot of 1/\( \tau \) (sec) against AMPA concentration failed to reveal the expected linear relationship for either parameter [Fig. 10(A)] suggesting that the speed of solution exchange became the rate limiting step at higher AMPA concentrations. Further analysis of these data revealed that the desensitization is accompanied by a change in AMPA/kainate receptor affinity for AMPA but that, paradoxically, the desensitized plateau component reflects activation of the receptor in its high affinity state. Only AMPA concentrations up to 12.5 \( \mu M \) could be used to determine the AMPA EC\(_{50}\) value for plateau responses of 1.7 \( \pm \) 0.93 \( \mu M \) as the absolute magnitude of the desensitized response became smaller at intermediate AMPA concentrations. In contrast, the whole of the concentration responses curve was used to calculate the corresponding EC\(_{50}\) value for AMPA in evoking the peak response of 38.9 \( \pm \) 9.4 \( \mu M \) [Fig. 9(B)]. The EC\(_{50}\) for AMPA to induce AMPA/kainate receptor desensitization of 11.5 \( \pm \) 1.7 \( \mu M \) was between these two values [Fig. 10(B)].

Use of these data together with the Cheng–Prussoff equation to calculate \( K_a \) values revealed that the receptor affinity of NBQX was in fact similar for both peak (\( K_a \) 26.4 nM–95% confidence limits: [95% c.l.] 20.9–31.7 nM) and plateau components of AMPA current responses (\( K_a \) 23.2 nM–95% c.l. 9.2–40.2 nM) and implies that the differential blockade seen with low NBQX concentrations rather reflects a lower receptor occupancy by AMPA 50 \( \mu M \) during the peak of the response. It therefore seems plausible that whilst control responses to AMPA 50 \( \mu M \) normally desensitize to a large degree, low concentrations of NBQX prevent rapid activation and as such, decrease the degree of desensitization. This could in turn result in the observed increase in the size

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**Fig. 4.** Selective antagonistic effects of GYKI 52466 33 \( \mu M \) on pooled hippocampal neuronal current responses to NMDA, AMPA and kainate. Agonists were applied as in Fig. 1. Analysis and presentation of data as in Fig. 2.
of the plateau response as it does not desensitize to the same degree.

Neither NBQX nor GYKI 52466 showed any indication of use-dependency in their antagonism of current responses to AMPA. Thus, the peak and plateau responses of the first AMPA application in the presence of NBQX 1 μM (peak 5.2 ± 1.6% of control, plateau 71.0 ± 10.9% of control) were antagonized to a similar degree as the fourth such response (peak 7.6 ± 1.5% of control, plateau 71.3 ± 11.5% of control). The same was true for GYKI 52466 4 μM where these values were respectively (1st peak 65.0 ± 2.7%, 1st plateau

Fig. 5. Concentration-dependent effects of NBQX on AMPA responses of a single superior colliculus neurone. AMPA 50 μM was applied as in Fig. 1. The 1st and 4th columns show control and recovery responses to AMPA respectively. The 2nd column, from top to bottom, show equilibrium current responses to AMPA in the continuous presence of 50, 100 and 250 nM NBQX, respectively. The 3rd column shows current responses to coapplication of AMPA and the same concentrations of NBQX i.e. without preincubation of the antagonist.
55.3 ± 2.5%) and (4th peak 65.6 ± 2.8%, 4th plateau 51.9 ± 3.2%). Furthermore, neither antagonist was voltage-dependent (data not shown).

Due to the above mentioned complications in the interpretation of the pharmacology of AMPA-induced currents, experiments to evaluate the competitive or non-competitive nature of the antagonistic effects of NBQX and GYKI 52466 were performed with kainate, which shows a classical concentration–response relationship.

The kainate concentration–response curve was shifted to the right in a parallel and concentration-dependent
manner by NBQX. Thus, the EC<sub>50</sub> for kainate-induced plateau currents of 94.9 ± 18.3 μM was increased by NBQX 0.5 and 1 μM to 705 ± 51 μM and 1574 ± 93 μM respectively, indicative of a competitive mechanism of action [Fig. 11(A)]. Moreover, in agreement with the above data for AMPA, NBQX slowed the rise time of kainate 400 μM responses so that initial currents assessed 50 msec after the beginning of the response were antagonized more than the plateau currents [Fig. 11(B)]. Although the NBQX concentration-dependence of this effect on response kinetics was not strong as that seen with AMPA (Table 1), data obtained in the presence of

![Concentration-dependent effects of GYKI 52466 on AMPA responses of a single superior colliculus neurone. AMPA 50 μM was applied as in Fig. 1. The left and right panels show control and recovery responses to AMPA respectively. The middle panels, from top to bottom, show equilibrium current responses to AMPA in the continuous presence of 4, 16 and 33 μM GYKI 52466 respectively.](image_url)
NBQX 50 nM were also best described by a double exponential fit with τ ons of 33.2 ± 2.8 and 415 ± 30 msec which were similar to the association kinetics of control kainate responses (τ ons of 21.2 ± 1.9 msec, n = 50) and the dissociation kinetics of NBQX (τ on of 583.4 ± 47.9 msec, n = 7) respectively.

In contrast, the kainate concentration–response curve was shifted to the right in a non-parallel manner by GYKI 52466. Thus, whilst the EC₅₀ for kainate-induced plateau currents of 66.6 ± 4.3 μM was also increased by GYKI 52466 10 and 33μM to 76.2 ± 8.9 μM and 113.0 ± 6.4 μM respectively, this effect was marginal compared to the more pronounced decrease in the maximal effect of kainate [Fig. 12(A)]. Moreover, no concentration of GYKI 52466 showed any selectivity for initial as compared to the plateau components of responses to kainate 50 μM [Fig. 12(B)]. Finally, although GYKI 52466 also showed the rise time of kainate responses to some degree, this effect was much less pronounced than that seen with NBQX (Table 1).

The IC₅₀s for NBQX in antagonizing initial and plateau components of current responses to kainate 400 μM were 18.1 ± 2.9 nM and 298 ± 27 nM respectively whereas the IC₅₀s for GYKI 52466 against kainate 50 μM were 17.3 ± 1.8 μM and 15.5 ± 3.3 μM respectively. The Cheng–Prussoff equation was again used to calculate a Kᵦ for NBQX of 57.1 nM (95% c.l. 43.6 ± 71.7 nM) against plateau kainate responses which is close to that for plateau responses to AMPA and lends no support for selectivity of NBQX between responses to AMPA and kainate.

**DISCUSSION**

Both NBQX and GYKI 52466 selectively and concentration-dependently antagonized responses of cultured hippocampal and superior collicular neurones to AMPA.
Fig. 9. AMPA concentration-dependent induction of AMPA/kainate receptor desensitization. Various concentrations of AMPA were applied as in Fig. 1. (A) Pooled responses were quantified as peak and plateau current (pA) after subtraction of any leak current and plotted as means against log concentration of AMPA. (B) Pooled responses were quantified as peak and plateau current (pA) after subtraction of any leak current and plotted, after normalization to the maximum current (at 12.5 µM for plateau and 800 µM for peak), as means against log concentration of AMPA. The 4 parameter logistic equation was used to fit the data and to calculate the EC_{50} for AMPA (see results). Error bars represent the SEM (n = 4 for each concentration).

and kainate whilst having little effect on responses to NMDA. The parallel shift of concentration-response curves to kainate by NBQX is in close agreement with recent whole cell data from cultured hippocampal neurones (Donevan and Rogawski, 1993) and for AMPA/kainate receptors expressed in Xenopus oocytes (Randle et al., 1992) and is in line with the proposed mechanism of action of this substance as a competitive antagonist of AMPA/kainate receptors. Similarly, the non-parallel shift of concentration-response curves to kainate by GYKI 52466 is like that seen in cultured hippocampal neurones (Donevan and Rogawski, 1993) and cortical wedges (Lodge et al., 1993) and supports a noncompetitive mode of action for GYKI 52466.

There was however some indication for a decrease in the affinity of both AMPA and kainate in the presence of GYKI 52466 as evidenced by the increased EC_{50} for kainate and the small slowing in the onset kinetics of responses to both AMPA and kainate in the presence of this antagonist. This might indicate a reciprocal coupling between the GYKI 52466 and AMPA/kainate recognition sites similar to that already reported between glycine_{B} and glutamate recognition sites on the NMDA receptor channel complex (Kemp and Priestley, 1991; Lester et al., 1993; Grimwood et al., 1993). Indeed, the GYKI 52466 and glycine_{B} sites share a number of common properties implying a degree of functional homology. Thus, the effects of both GYKI 52466 and glycine_{B} antagonists are voltage-independent and are not use-dependent. Moreover, diazoxide, cyclothiazide and related compounds may act as agonists at the GYKI 52466 site and can prevent rapid AMPA receptor desensitization (Yamada and Rothman, 1992; Zorumski et al., 1993; Palmer and Lodge, 1993; Trussell et al., 1993) in much the same way as glycine prevents fast desensitization of NMDA receptors (see Kemp and Leeson, 1993). Cyclothiazide like substances were not investigated in this study and were evidently not spontaneously released in large quantities from the cell cultures as GYKI 52466 had only moderate effects on the degree of AMPA receptor desensitization. Moreover, the present data provide no evidence that GYKI 52466 can induce desensitization of normally nondesensitizing responses to kainate. It is at present not known if an agonist for the GYKI 52466 site exists in vivo but it is tempting to speculate that such a substance might be released under pathological conditions in much the same way as glycine concentration is increased by ischaemia (Globus et al., 1991). Fast receptor desensitization may represent a physiological process serving as an endogenous control mechanism to prevent long term neurotoxic activation of glutamate receptors—the release of such an agonistic substance at the GYKI 52466 site might then be expected to increase the degree of neurological damage by decreasing the amount of desensitization. GYKI 52466 or substances with a similar mechanism of action could restore normal synaptic transmission under such conditions by increasing AMPA receptor desensitization to its physiological level. Although, data from the present study provide only limited support for an induction of desensitization under "normal" conditions it should be noted that GYKI 52466 was somewhat more potent against desensitizing responses to AMPA than against nondesensitizing responses to kainate.

The inability to show a marked selectivity of NBQX for AMPA compared to kainate is in line with electrophysiological data for competitive non-NMDA receptor antagonists in a variety of models (Yamada et al., 1989; Randle et al., 1992; Zeman and Lodge, 1992) and highlights the need for cautious interpretation of biochemical data showing selective displacement of [3H]AMPA binding (for review see Watkins et al., 1990). It seems more likely that [3H]kainate binds with highest affinity to a presynaptic Ca^{2+}-dependent recognition site (Honore et al., 1986; for review see Barnard and Henley, 1990) which is not involved in the direct postsynaptic
response to exogenously-applied AMPA and kainate which is mediated by a common receptor (Pin et al., 1989; Rassendren et al., 1989; Charpentier et al., 1990; Huettner, 1990; Patneau and Mayer, 1991). This presynaptic site may however be indirectly involved in responses to kainate applied to slices (Lodge et al., 1991; Pook et al., 1993). This situation could well be different for current responses recorded in Xenopus oocytes injected with mRNA from whole rat or mouse brain as these cells can be expected to express both pre- and postsynaptic glutamate receptors (see Stein et al., 1992).

The pronounced effect of NBQX on AMPA and kainate response onset kinetics is similar to that already reported for high affinity competitive NMDA antagonists (Benveniste and Mayer, 1991) where slow antagonist dissociation rates are probably the rate limiting step in governing the speed of agonist/receptor interactions. However, data from the present study indicate that the situation may be more complicated for AMPA responses of non-NMDA receptors. Thus, the AMPA recognition site seems initially to be in a low affinity state for AMPA which then concentration-dependently induced rapid desensitization accompanied by a paradoxical change to a higher agonist affinity state with presumed less efficacious coupling to the receptor channel (see also Patneau and Mayer, 1991). In contrast, the antagonist affinity remains the same as reflected by the $K_d$ for NBQX as assessed by the Cheng–Prussoff equation in this study—a finding which is in close agreement with data from binding studies (Honore et al., 1989).

Taken together, these properties may offer an additional explanation for the differential effects of NBQX on peak and desensitized responses to a fixed concentration of AMPA. The inhibitory effects of NBQX would be expected to be more pronounced against agonist responses of the receptor in the low agonist affinity state i.e. at the peak of the response. Blockade of AMPA-induced desensitization by low concentrations of NBQX might then explain the fact that normally strongly desensitized plateau responses to AMPA 50 $\mu$M were actually potentiated by NBQX 50–250 nM. In this regard it should be noted that, in the absence of antagonist, plateau responses to AMPA 50 $\mu$M were somewhat smaller than their maximum value seen with AMPA 12.5 $\mu$M and were considerably smaller than would be predicted by extrapolation of the lower portion of the AMPA concentration response curve [dotted line in Fig. 9(B)] i.e. higher concentrations of AMPA actually desensitized plateau responses to a lower magnitude. Such observations are in close agreement with data on quisqualate and AMPA-induced AMPA/kainate receptor desensitization (Tang et al., 1989; Thio et al., 1991) and CNQX/DNQX-induced potentiation of plateau responses to quisqualate and AMPA (Bijak et al., 1991; Geoffroy et al., 1991). However, such speculations on the mechanism of action should be viewed with some degree of caution as the classical pharmacological calculations used are normally applied to agonist/antagonist interactions measured under equilibrium conditions: we do not wish to, and are not capable of, producing yet

Fig. 10. AMPA concentration-dependent induction of AMPA/kainate receptor desensitization. Various concentrations of AMPA were applied as in Fig. 1. (A) The rate of onset of AMPA responses and of their subsequent desensitization were plotted as mean reciprocals against the concentration of AMPA. Best fits for the concentration-dependency of the rates of onset and desensitization were achieved with double and single exponential routines respectively. (B) The ratio of plateau over peak current was plotted against concentration of AMPA. The 4 parameter logistic equation was used to fit the data and to calculate the EC<sub>50</sub> for AMPA in inducing desensitization (see results). Error bars represent the SEM ($n = 4$).
Patch clamp studies with NBQX and GYKI 52466

Fig. 11. (A) Competitive nature of the antagonism of kainate responses by NBQX on superior colliculus neurones. Kainate was applied as in Fig. 1. Pooled data of responses to kainate in the absence (n = 10) and presence of NBQX 0.5 μM (n = 7) and 1 μM (n = 7) were plotted, after normalization to the maximum effect seen with control kainate 3300 μM, as means against log concentration of kainate. (B) Concentration-dependent effects of NBQX on pooled kainate current responses of superior colliculus neurones. Kainate 400 μM was applied as in Fig. 1. Pooled responses were quantified as initial (50 msec after start of the response) and plateau current (pA) after subtraction of any leak current and plotted, after normalization to control, against log concentration of NBQX. The number of cells tested is shown for each concentration. The 4 parameter logistic equation was used to fit the data and to calculate the EC50 for kainate and IC50 for NBQX. Error bars represent SEM. The insert shows typical data for the effects of NBQX (0.05–0.25 μM) on a single neurone—note the biphasic onset of kainate responses in the presence of the lowest concentration of NBQX.

Another computer "model" of drug receptor interactions.

Regardless of the biophysical mechanisms involved, it seems reasonable to assume that high affinity competitive antagonists would influence fast glutamatergic transmission more than the toxic effects of prolonged pathological exposure to increased levels of glutamate and should be burdened with a variety of side effects. In contrast, one might expect substances such as GYKI 52466 to antagonize the pathological activation of AMPA receptors to the same, or even to a greater, degree than synaptic activation, as peak responses to AMPA were effected somewhat less than plateau responses were effected somewhat less than plateau responses. Unfortunately, the very limited comparative data on the effects of NBQX and GYKI 52466 on synaptic transmission do not lend support to this hypothesis (see however Yamaguchi et al., 1993). However, the lack of published data on the possible effects of GYKI 52466 on nonglutamatergic systems, e.g. classical benzodiazepine receptors, makes it impossible to exclude the possibility that effects unrelated to AMPA/kainate receptor blockade are also involved in the reported effects on synaptic transmission and ataxia. Further studies comparing the effects of GYKI 52466 and NBQX on normal synaptic transmission and long term potentiation in hippocampal slices are presently being performed in our laboratory. The apparent weakness of NBQX in producing e.g. ataxia in animals should, in the opinion of these authors, be viewed with a certain degree of caution—if AMPA/kainate receptors are truly those involved in mediating most excitatory fast synaptic transmission in the CNS, how can their blockade by a competitive antagonist cause no side effects? Numerous studies are outstanding to clarify at what site the in vivo effects of NBQX are mediated and whether metabolites are involved in its effects.

A further point worthy of discussion is the relative potencies of NBQX and GYKI 52466 in vitro and in vivo. Thus, in the present study NBQX was some 6 times more potent than GYKI 52466 against plateau responses to AMPA. This difference was even more pronounced for plateau responses to kainate with NBQX being some 50 times more potent than GYKI 52466. Furthermore, the true difference in affinities of the two substances at their respective recognition sites is likely to be even greater but is obscured somewhat by their differing mechanisms of AMPA/kainate receptor blockade i.e. the blockade by GYKI 52466 was more or less independent of agonist concentration whereas the effects of NBQX are highly-dependent on agonist concentration. In contrast, the two
compounds are approximately equi-effective in most in vivo systems (but see Bisaga et al., 1993; Yamaguchi et al., 1993) which tends to imply that CNS penetration is much better for GYKI 52466, an assumption supported by its higher lipophilicity. However, it remains a possibility that the two substances also have very different activities at subtypes and/or substates of AMPA/kainate receptor which could be differentially involved in various physiological and pathological processes (Sommer et al., 1990; see Barnard and Henley, 1990).

In conclusion, NBQX represents an important lead structure for the development of competitive AMPA/kainate antagonists. Modifications aimed at improving its bioavailability could well result in a decrease in affinity. However, this may be advantageous in view of the fact that high affinity competitive antagonists often slow the onset of kinetics of agonist/receptor interactions and might therefore be predicted to influence fast synaptic transmission more than prolonged pathological activation of AMPA/kainate receptors. GYKI 52466 is the prototypic antagonist at a novel modulatory site associated with AMPA/kainate receptors which may represent a more promising target for therapeutic interventions as GYKI 52466 decreased plateau AMPA responses somewhat more than peak responses. Further studies comparing the effects of NBQX, GYKI 52466, cyclothiazide and related compounds on physiological and pathological activation of AMPA/kainate receptors are necessary to test this hypothesis.

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