Br. J. Pharmacol. (1992), **106**, 367–372

Pharmacological characterization of non-NMDA subtypes of glutamate receptor in the neonatal rat hemisected spinal cord *in vitro*

Sophie Zeman & [1]David Lodge

Department of Veterinary Basic Sciences, Royal Veterinary College, Royal College Street, London NW1 0TU

1 A grease-gap technique was used to record depolarizing responses to α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA), kainate and N-methyl-D-aspartate (NMDA) in the hemisected spinal cord of the neonatal rat. The pharmacology of non-NMDA subtypes of glutamate receptor was investigated with the novel quinoxalinedione, 2,3-dihydroxy-6-nitro-7-sulphamoyl-benzo (F)-quinoxaline (NBQX) and with a series of barbiturates.

2 NBQX antagonized AMPA- and kainate-, but not NMDA- induced depolarizations. The near parallel shifts of the major part of the dose-response curves for AMPA and kainate by NBQX gave pA_2 values (± s.e.) of 6.7 ± 0.2 and 6.8 ± 0.2 respectively, consistent with a common site of action for these two agonists.

3 Below the 50% level at which these pA_2 values were calculated, however, an NBQX-resistant plateau was seen within the kainate, but not the AMPA, dose-response curve.

4 In decreasing order of potency, methohexitone, secobarbitone, thiopentone, pentobarbitone and phenobarbitone preferentially reduced kainate-, rather than AMPA- and NMDA-, induced depolarizations. Methohexitone was also the most selective with IC_{50}s against kainate, AMPA and NMDA of 31 ± 7, 172 ± 47 and >200 μM respectively.

5 The NBQX-resistant plateau seen within the kainate dose-response curve was reduced by methohexitone. Kainate antagonism by methohexitone was not reduced by 50 μM picrotoxin.

6 We conclude that, while mixed agonist actions may hamper demonstration of antagonist selectivity, depolarizations induced by the non-NMDA ionotropic agonists, AMPA and kainate, are mediated in part via distinct receptors.

Keywords: Glutamate receptors; kainate; α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA); 2,3-dihydroxy-6-nitro-7-sulphamoyl-benzo-(F)-quinoxaline; barbiturates; rat spinal cord *in vitro*

Introduction

On the basis of agonist and antagonist studies, the post-synaptic ionotropic receptors in the mammalian central nervous system for the excitatory amino acid transmitter, L-glutamate, are now divided into N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-isoxazole-4-proprionate (AMPA, formerly quisqualate) and kainate subtypes (Watkins *et al.*, 1990).

The NMDA receptor subtype is clearly a discrete entity, with well-documented selective antagonists, modulators and channel blockers, (Watkins & Collingridge, 1989). Pharmacological differentiation between responses to kainate and AMPA has, however, proved difficult. Early electrophysiological studies supported the concept of separate receptors since glutamate diethyl ester (GDEE) inhibited AMPA but not kainate-induced responses in the cat and rat spinal cord *in vivo* (Krogsgaard Larsen *et al.*, 1980; McLennan & Liu, 1982) whereas γ-D-glutamyl-aminomethyl-sulphonate (GAMS) had the reverse selectivity (Davies & Watkins, 1985). The lack of potency of these antagonists and their poor selectivity *in vitro* have, however, both limited their usefulness and been taken as evidence for a single receptor class mediating the effects of AMPA and kainate. Initial kinetic studies showing different rates of desensitization for AMPA and kainate were also taken as evidence for separate receptors (Kiskin *et al.*, 1986; Mayer & Westbrook, 1987) but the more recent observations of cross-desensitization between these agonists indicate a common site of action (Kiskin *et al.*, 1990; Patneau & Mayer, 1991). Such results from hippocampal neurones in culture, however, do not exclude the possibility of separate subclasses of receptors at other neuronal sites.

Studies with [^{3}H]-AMPA and [^{3}H]-kainate indicate high and low affinity binding sites for each agonist with distinct structure activity requirements at the high affinity sites (Foster & Fagg, 1984; Honore & Drejer, 1988; Monaghan *et al.*, 1989). Anatomical distribution of these AMPA and kainate binding sites in brain is also quite different (Greenmayre *et al.*, 1983; Monaghan *et al.*, 1983). Despite this evidence for distinct binding sites, kainate readily displaces high affinity [^{3}H]-AMPA binding whereas the reverse is not the case (Krogsgaard-Larsen *et al.*, 1980; Honore & Drejer, 1988).

Other biochemical studies support the idea of a common receptor subtype mediating some of the actions of AMPA and kainate; for example, quisqualate and AMPA reduce kainate-induced [^{3}H]-aspartate and [^{3}H]-GABA release from cultured cerebellar granule and striatal neurones respectively (Gallo *et al.*, 1990; Pin *et al.*, 1989).

The cloning of glutamate receptor subunits and their expression in *Xenopus* oocytes initially supported the idea of a unitary non-NMDA receptor (Hollmann *et al.*, 1989). More recently, however, with the identification of more glutamate receptor genes and their expression both singly and in various combinations, AMPA- or kainate-preferring receptor channel complexes have been described (Keinanen *et al.*, 1990; Egebjerg *et al.*, 1991; for review see Dingledine, 1991).

The major breakthrough in differentiating NMDA receptors from other glutamate receptor subtypes was the elucidation of selective antagonists (Watkins & Evans, 1981). Thus

[1] Author for correspondence.

the search for potent and selective non-NMDA antagonists assumes extreme importance in resolving the possible differentiation between AMPA and kainate receptor subtypes. The selectivity of the 6,7-dinitro- and 6-cyano-7-nitroquinoxalinediones (DNQX and CNQX respectively) for [^{3}H]-AMPA, rather than [^{3}H]-kainate, binding has parallels in some functional studies, e.g. depolarization of cortical slices (Fletcher *et al.*, 1988) and release of GABA from cortical cultures (Honore *et al.*, 1988), but not in others, e.g. excitation of spinal neurones *in vivo* (Honore *et al.*, 1988) or *in vitro* (Birch *et al.*, 1988a). Since the maximum selectivity achieved with CNQX and DNQX of approximately 5 fold is clearly insufficient to be very useful particularly in functional studies, the demonstration that a new quinoxalinedione, 2,3-dibydroxy-6-nitro-7-sulphamoyl-benzo(F)quinoxaline(NBQX) displaced [^{3}H]-AMPA binding 30-times more potently than that of [^{3}H]-kainate binding was thus of considerable interest (Sheardown *et al.*, 1990). In our experiments, responses to AMPA and kainate were not, however, distinguished by this antagonist when tested on rat spinal cord and brainstem neurones *in vivo*, but, on rat neocortical slices *in vitro*, NBQX was about 30 fold more potent as an antagonist of AMPA than of kainate (Lodge *et al.*, 1991). We have therefore used an *in vitro* spinal cord preparation (Birch *et al.*, 1988b) to investigate this anomaly.

In addition to their well documented facilitatory action at γ-aminobutyric acid (GABA) receptors, barbiturates are also known to reduce glutamatergic neurotransmission (for review see Simmonds & Horne, 1988). Pharmacological studies have largely focussed on barbiturate antagonism of the non-NMDA agonist, quisqualate. For example, a series of barbiturates produced rightward shifts of quisqualate dose-response curves consistent with a competitive action at low doses and non-competitive at high doses (Horne & Simmonds, 1986), although earlier studies on agonist stimulated sodium flux showed no specificity of barbiturates for quisqualate relative to kainate (Teichberg *et al.*, 1984). A report by Frandsen *et al.* (1990) that kainate-induced cytotoxicity in culture was selectively reduced by phenobarbitone indicated the possible value of reassessing the effects of barbiturates on subtypes of glutamate receptor.

Some of the data discussed here have been presented elsewhere in preliminary form (Zeman & Lodge, 1991a, b).

Methods

Dissection and tissue preparation

Following the methods of Birch *et al.* (1988b), 2–7 day-old Sprague Dawley or Wistar rats were decapitated and the spinal cords exposed from the ventral side by removal of overlying internal organs and vertebral bodies. By gentle lifting at the rostral end, while the ventral and dorsal roots were cut, the cords were freed and placed in Krebs solution (of composition (mM): NaCl 118, $NaHCO_3$ 25, glucose 11.1, KCl 4.7, KH_2PO_4 1.2, $CaCl_2$ 1.25, equilibrated with 95% O_2/5% CO_2 to give pH 7.4 at room temperature) in a Petri dish, any remaining roots trimmed off and a midline hemisection made.

The hemicord was placed in a two compartment bath (Harrison & Simmonds, 1985) with a greased barrier across the thoraco-lumbar region. The rostral end of the cord was kept immersed in Krebs solution, all drug additions being made to the continuously (2 ml min^{-1}) superfused caudal end, which was less likely to have suffered damage during the excision procedure and furthermore contained a large number of neurones in the lumbar enlargement.

Pharmacological investigation and recording of responses

In preliminary experiments, unlike Birch *et al.* (1988b), we did not find that semi-cumulative dose cycles (3 min agonist pulses with 1.5 min intervals) produced repeatable responses or that their amplitudes were as large as those seen when longer intervals elapsed between agonist applications. Agonists were, therefore, applied to the lumbar cord at 20 min intervals as 5 ml aliquots of the superfusion medium. Tetrodotoxin had no effect on the nature or amplitude of responses to depolarizing agonists and so was not routinely included in the superfusate.

Agonist-induced depolarizations were detected as changes in d.c. potential across the grease-seal via Ag/AgCl electrodes in each compartment, displayed continuously on a chart recorder following amplification (× 100) and filtering (d.c.-5 Hz) and measured as peak amplitudes.

Full agonist dose-response curves (AMPA 316 nM–20 μM; kainate 316 nM–30 μM; NMDA 1–40 μM) or individual agonist doses (AMPA 10 μM; kainate 5 μM or 10 μM; NMDA 10 or 20 μM) were tested before the addition of antagonists to the superfusion medium, after which an equilibration period of at least 20 min was allowed before agonists were retested. After 1 h wash, recovery was assessed.

All studies were performed on at least two different days and four hemicords.

Source of compounds

AMPA and quisqualate were obtained from Tocris Neuramin, NBQX from Novo Nordirsk, thiopentone from C-Vet, methohexitone from Eli Lilly Co. and all other compounds from Sigma Chemical Co. Ltd.

Results

Depolarizations evoked by agonist superfusion of the hemisected cord were reproducible and of similar amplitude to those seen in the cortical wedge preparation (Harrison & Simmonds, 1985; Fletcher *et al.*, 1988). As with the cortical wedge, NMDA, AMPA and kainate were approximately equipotent and high doses of each agonist led to irreversible reductions in amplitude of all subsequent agonist responses, which is presumably a reflection of their excitotoxic properties (Olney, 1969). For this reason, maximum concentrations of agonist used in control conditions were AMPA 20 μM, kainate 30 μM and NMDA 40 μM. Depolarizing response amplitudes obtained from these doses of agonist were used as the 100% value for the normalization of dose-response curves.

Antagonism of AMPA, kainate and NMDA by NBQX

NBQX (1 μM) reduced responses to AMPA and kainate while those to NMDA were largely unaffected. Dose-dependent and reversible rightward shifts of the AMPA and kainate dose-response curves by 100 nM to 3.16 μM NBQX were seen (Figure 1). These shifts were near parallel for the AMPA curves but, for kainate, although the upper two thirds of the curve appeared to be shifted in parallel, there was also an NBQX-resistant component within the dose-response curve (Figure 1b). Schild plot slopes, calculated at the 50% maximum response level, were near unity for both AMPA (0.95 ± 0.29) and kainate (0.97 ± 0.26) and the resultant pA_2 values (± s.e.) were 6.7 ± 0.2 and 6.8 ± 0.2 for NBQX against AMPA and kainate respectively. Such results are consistent with competitive antagonism by NBQX at a single receptor subtype shared by AMPA and, in the main, by kainate.

The NBQX-resistant component of the kainate dose-response curve produced a plateau at about 33% of the normalized control response (Figure 1b). This is most obvious in the presence of 3.16 μM NBQX, the highest concentration used, when 40–320 μM kainate produced a stable level of depolarization. This part of the dose-response curve is at least 10 times less sensitive to NBQX than the

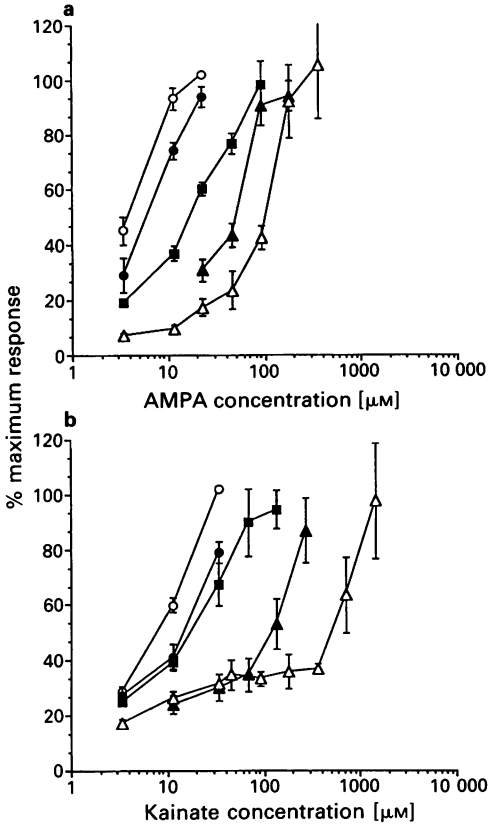

Figure 1 Effects of 100 nM to 3.16 μM 2,3-dihydroxy-6-nitro-7-sulphamoyl-benzo(F)-quinoxalone (NBQX) on dose-response curves of the neonatal rat spinal cord to α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA) and kainate. In (a) the control AMPA curve (○) is shifted to the right by NBQX at 100 nM (●), 316 nM (■), 1 μM (▲) and 3.16 μM (△). In (b) rightward shifts of the control kainate dose-response curve (○) are produced by NBQX at 100 nM (●), 316 nM (■), 1 μM (▲) and 3.16 μM (△). An NBQX-resistant component is clear in the presence of 3.16 μM NBQX (△), yielding a plateau at about 33% of the normalized maximum response. Ordinate scale: amplitude of depolarizing response expressed as a percentage of the response to (a) AMPA 20 μM and (b) kainate 30 μM. Abscissa scale: agonist concentration in μM.

remainder. No such plateau was seen within the AMPA dose-response curve (Figure 1a).

Antagonism of AMPA, kainate and NMDA by barbiturates

Following the report from Frandsen *et al.* (1990), we initially tested 10 and 100 μM phenobarbitone against single responses to NMDA (20 μM), AMPA (10 μM) and kainate (10 μM) and found a preferential reduction of that to kainate (Figure 2). The response to kainate recovered after 1 h superfusion in drug-free medium.

Five other barbiturates were tested similarly to ascertain their potency and selectivity relative to phenobarbitone. Table 1 shows the results obtained; with each barbiturate, responses to kainate were more sensitive than those to AMPA. Methohexitone was the most potent and selective, and hence was used for all further pharmacokinetic studies of responses to AMPA and kainate. It should be noted that NBQX has the reverse selectivity to barbiturates against these concentrations of AMPA and kainate.

Methohexitone, 10, 31.6 and 100 μM caused dose-dependent rightward shifts of the kainate dose-response curve, while smaller and less dose-dependent shifts of the AMPA dose-response curve were seen with 31.6 and 100 μM methohexitone (Figure 3). The antagonism was not clearly

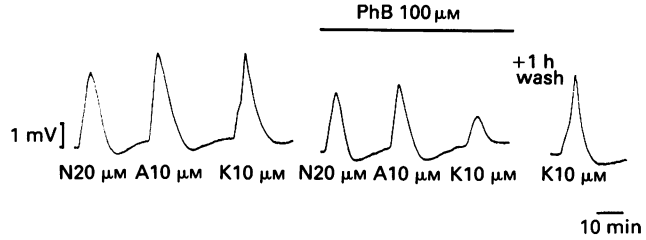

Figure 2 Selective effect of phenobarbitone (PhB, 100 μM) on responses to kainate (K, 10 μM), rather than those to α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (A, 10 μM) or N-methyl-D-aspartate (N, 20 μM) in the neonatal rat spinal cord. Phenobarbitone was superfused for 20 min before the start of the middle trace, and artificial CSF for 60 min before the right hand trace. Calibration: amplitude in mV; time in min.

competitive, with the rightward shifts being non-parallel, although the Schild plot slopes were near unity. Thus, apparent pA_2 values for methohexitone, calculated at the 25% and 50% of maximum response levels were, respectively, 4.5 and 3.3 against kainate and 2.8 and 3.1 against AMPA. Consistent with the selectivity study showing phenobarbitone to only weakly antagonize NMDA (Figure 2), methohexitone (31.6 μM) did not greatly affect the full dose-response curve to this agonist (not illustrated).

Picrotoxin (50 μM) did not reverse the effect of methohexitone on the kainate dose-response curve which, in conjunction with the observation that methohexitone application did not change the basal level of depolarization, strongly suggests the effect of methohexitone to be independent of GABA-potentiation.

Effect of methohexitone on NBQX-insensitive responses to kainate

Demonstration of an NBQX-resistant plateau within the kainate dose-response curve, coupled with the ability of methohexitone to decrease preferentially responses to this agonist, led us to assess the effect of combined application of these antagonists. Figure 4a shows that 31.6 μM methohexitone reduced the NBQX-resistant plateau of the kainate dose-response curve, while in Figure 4b, application of methohexitone to the AMPA dose-response curve already displaced to the right by NBQX did not elicit a further rightward shift.

Discussion

This study was an attempt to investigate the anomaly in our previous results between spinal cord *in vivo* and cerebral cortex *in vitro* with respect to the selectivity of NBQX as an antagonist at the non-NMDA subtypes of glutamate receptors (Lodge *et al.*, 1991). On the one hand, the present results on the spinal cord *in vitro*, showing that, for the major part of the dose-response curves, the depolarizing actions of kainate and AMPA are equally sensitive to NBQX, is in agreement with the failure of NBQX to distinguish between responses to these two agonists on spinal neurones *in vivo*. The similarity in calculated NBQX pA_2 values against kainate and AMPA suggests that the major action of these two agonists on the hemisected cord is mediated by a common receptor subtype. On the other hand, the finding that part of the kainate dose-response is more resistant to NBQX is reminiscent of the previous results from cortical slices *in vitro* and is consistant with there being a further, distinct, receptor subtype via which kainate is able to produce depolarization in both these tissues. This interpretation of our data is supported by the observation that barbiturates selectively affect that part of the kainate dose-response curve

Table 1 Barbiturate IC_{50}s *vs.* responses to α-amino-3-hydroxy-5-methyl-osoxazole-4-propionate (AMPA) 10 μM and kainate 5 μM

Barbiturate	*IC_{50} vs. AMPA*	*IC_{50} vs. kainate*	*Selectivity ratio*
Methohexitone	171.5 ± 47.4	31.1 ± 7.2	5.5
Secobarbitone	114.6 ± 25.6	54.2 ± 8.8	2.1
Thiopentone	102.5 ± 13.0	63.5 ± 11.6	1.6
Pentobarbitone	213.1 ± 38.9	80.4 ± 14.9	2.7
Phenobarbitone	882.7 ± 319	188.3 ± 42.5	4.7
NBQX	≈200 nM	≈3.16 μM	

IC_{50} values are μM ± s.d. The drugs are listed in descending order of potency against responses to kainate and the selectivity ratio indicates the degree of differential sensitivity of kainate and AMPA to antagonism. NBQX: 2,3-dihydroxy-6-nitro-7-sulphamoyl-benzo(F)-quinoxalone.

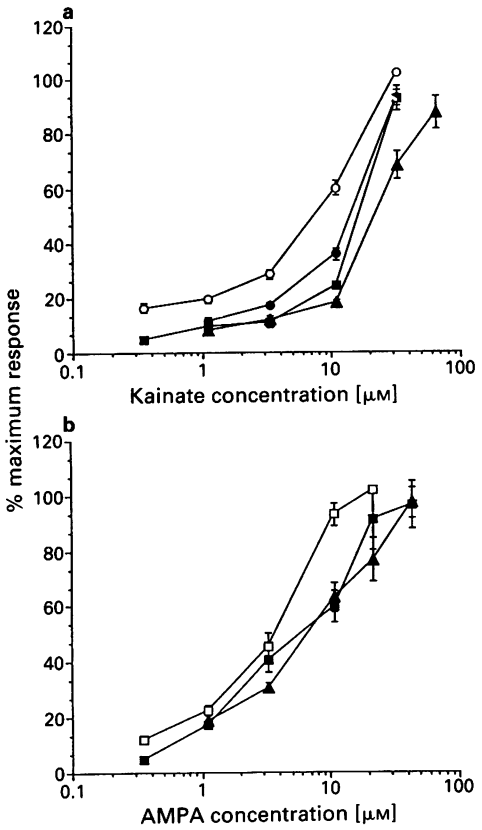

Figure 3 Effects of methohexitone (10–100 μM) on the dose-response curves to kainate and α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA) in the neonatal rat spinal cord. (a) The control kainate dose-response curve (○) is dose-dependently and reversibly shifted to the right by methohexitone at 10 μM (●), 31.6 μM (■) and 100 μM (▲). (b) The shift of the control AMPA dose-response curve (○) by methohexitone at 31.6 μM (■) and 100 μM (▲) is less marked and dose-dependency less apparent. Ordinate scale: amplitude of depolarizing response expressed as a percentage of the response to kainate 30 μM or AMPA 20 μM. Abscissa scale: agonist concentration in μM.

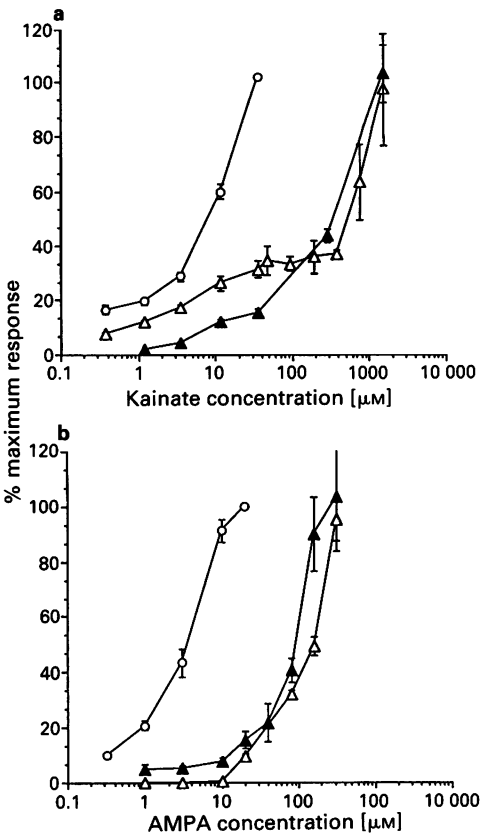

Figure 4 Effects of application of 3.16 μM 2,3-dihydroxy-6-nitro-7-sulphamoyl-benzo(F)-quinoxalone (NBQX) alone, and in combination with 31.6 μM methohexitone, against dose-response curves to kainate and α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA) in the neonatal rat spinal cord. In (a) the upper part of the control curve for kainate (○) is shifted to the right in the presence of 3.16 μM NBQX (△), with no further effect of 31.6 μM methohexitone (▲). The NBQX-resistant plateau at the lower end of the dose-response curve is uniquely sensitive to antagonism by methohexitone (31.6 μM). In (b) the control AMPA dose-response curve (○) is shifted to the right by 3.16 μM NBQX (△). Co-application of methohexitone (31.6 μM) did not elicit a marked further rightward shift (▲). Ordinate scale: amplitude of depolarizing response expressed as a percentage of that to (a) kainate 30 μM and (b) AMPA 20 μM. Abscissa scale: agonist concentration in μM.

which is resistant to NBQX and have little effect on responses in this tissue to AMPA. To account for the above data, we suggest that low doses of kainate activate a methohexitone-sensitive receptor subtype while higher doses activate an NBQX-sensitive receptor not dissimilar to that activated by AMPA.

The idea that kainate actions are mediated by receptor subtypes some of which are shared with AMPA and some of which are unique is consistent with data from binding, functional and molecular biology studies as discussed in the Introduction.

It is uncertain, however, why an NBQX-resistant component of kainate's action is not seen in most electrophysiological recordings from single neurones, thereby preventing a selective action of this antagonist being demonstrated *in vivo*. It may indicate that methohexitone-sensitive kainate receptors mediate only a small part of kainate's

action or that they are not expressed at all on the soma and proximal dendrites of the neurones being studied. Since other elements, such as glia and neuronal terminals, are also likely to play a role both in depolarizations seen in grease-seal preparations and in excitotoxic pathogenesis, the possibility of such kainate receptors being sited on these structures needs consideration. While glial cells can support both kainate and AMPA mediated conductances (Wylie *et al.* 1991), Teichberg *et al.* (1990) have reported a predominance of kainate binding proteins on some glia. It seems unlikely, however, that an action on glia makes a major contribution to the NBQX-resistant component of kainate-induced depolarization, since preliminary studies in a preparation where results are consistent with those described here (Palmer *et al.*, 1991) show that responses to AMPA and kainate decrease in parallel following exposure of neocortical slices to the gliotoxin, L-α-aminoadipate (Palmer, personal communication). It thus seems possible that some part of the signal produced by kainate is due directly to afferent terminal depolarization. The presence of kainate receptors on afferent fibres (Agrawal & Evans, 1986) and nerve terminals (see Stone, 1990). and the calcium permeability which this agonist may activate (Iino *et al.*, 1990; Egebjerg *et al.*, 1991) would allow such receptors to function as positive feedback autoreceptors, facilitating release of excitatory transmitter (Collins *et al.*, 1983; Potashner & Gerard, 1983) and contributing to excitotoxicity (Campochiaro & Coyle, 1978; Okazaki & Nadler, 1988).

The possibility that some part of the action of kainate is mediated via presynaptic receptors remains consistent with our proposal that some of the neuronal receptor complexes activated by this agonist are pharmacologically distinct from those at which AMPA acts. It will be interesting to see whether any of the recently described kainate-sensitive receptor subunit isolated from rat cerebellum, (Egebjerg *et al.*, 1991) and with a binding pattern akin to that of [^{3}H]-kainate, can eventually be resolved to presynaptic neuronal sites.

The present finding that barbiturates, and methohexitone in particular, block the NBQX-resistant component of the kainate response on the hemisected spinal cord has been repeated in rat cortical slices (Palmer *et al.*, 1991), although most previous reports of interactions between barbiturates and excitatory amino acids have largely been concerned with antagonism of quisqualate/AMPA responses (Simmonds & Horne, 1988). Since the presently described kainate-specific antagonism by methohexitone is unaffected by picrotoxin at a concentration known to inhibit GABA-mediated events (Evans, 1978), it is unlikely to be related to the well known GABA-potentiating effects of barbiturates, although some earlier reports have suggested the possibility of an interaction between GABA and kainate (e.g. Stone & Javid, 1980; Turski *et al.*, 1990). A more likely mode of action is a voltage-dependent block of channels opened by kainate (Miljkovic & MacDonald, 1986).

Consistent with the idea of barbiturate-sensitive presynaptic kainate receptors (as discussed above) are several reports of reduced release of neurotransmitters by barbiturates (e.g. Weakley, 1969; Cutler & Young, 1979) including that stimulated by kainate (Potashner & Gerard, 1983).

In summary, we propose that the profiles of AMPA and kainate antagonism by NBQX and the barbiturates indicate pharmacologically distinct sites via which these agonists act to depolarize the hemisected spinal cord preparation. The precise cellular distributions and the functions of such receptors remain to be elucidated.

We wish to thank Dr T. Honore of NovoNordisk for supplies of NBQX.

References

AGRAWAL, S.G. & EVANS, R.H. (1986). The primary afferent depolarizing effect of kainate in the rat. *Br. J. Pharmacol.*, **87**, 345–355.

BIRCH, P.J., GROSSMAN, C.J. & HAYES, A.G. (1988a). Kynurenate and FG9041 have both competitive and non-competitive antagonists actions at excitatory amino acid receptors. *Eur. J. Pharmacol.*, **156**, 177–180.

BIRCH, P.J., GROSSMAN, C.J. & HAYES, A.G. (1988b). Pharmacology of excitatory amino acid receptors in the rat hemisected spinal cord. *Neurol. Neurobiol.*, **46**, 71–74.

CAMPOCHIARO, P. & COYLE, J.T. (1978). Ontogenic development of kainate neurotoxicity: correlates with glutamatergic innervation. *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 2025–2029.

COLLINS, G.G.S., ANSON, J. & SURTEES, L. (1983). Presynaptic kainate and NMDA receptors regulate excitatory amino acid release in the olfactory cortex. *Brain Res.*, **265**, 157–159.

CUTLER, R.W.P. & YOUNG, J. (1979). Effect of barbiturates on release of endogenous amino acids from rat cortex slices. *Neurochem. Res.*, **4**, 319–329.

DAVIES, J. & WATKINS, J.C. (1985). Depressant action of γ-D-glutamylaminomethyl sulfonate (GAMS) on amino acid-induced and synaptic excitation in the cat spinal cord. *Br. Res.*, **327**, 113–120.

DINGLEDINE, R. (1991). New wave of non-NMDA excitatory amino acid receptors. *Trends Pharmacol. Sci.*, **12**, 360–362.

EGEBJERG, J., BETTLER, B., HERMANS-BORGMEYER, I. & HEINEMANN, S. (1991). Cloning of a cDNA for a glutamate receptor subunit activated by kainate. *Nature*, **351**, 745–748.

EVANS, R.H. (1978). The effects of amino acids and antagonists on the isolated hemisected spinal cord of the immature rat. *Br. J. Pharmacol.*, **62**, 171–176.

FLETCHER, E.J., MARTIN, D., ARAM, J.A., LODGE, D. & HONORE, T. (1988). Quinoxalinediones selectively block quisqualate and kainate receptors and synaptic events in rat neocortex and hippocampus and frog spinal cord *in vitro*. *Br. J. Pharmacol.*, **95**, 585–597.

FOSTER, A.C. & FAGG, G.E. (1984). Acidic amino acid binding sites in mammalian membranes: their characteristics and relationship to synaptic receptors. *Brain Res. Rev.*, **7**, 103–164.

FRANDSEN, A., QUISTORFF, B. & SCHOUSBOE, A. (1990). Phenobarbital protects cerebral cortex neurones against toxicity induced by kainate but not by other excitatory amino acids. *Neurosci. Lett.*, **111**, 233–238.

GALLO, V., GIOVANNINI, C. & LEVI, G. (1990). Modulation of non N-methyl-D-aspartate receptors in cultured cerebellar granule cells. *J. Neurochem.*, **54**, 1619–1625.

GREENMAYRE, J.T., YOUNG, A.B. & PENNEY, J.B. (1983). Quantitative autoradiography of L-[^{3}H]-glutamate binding to rat brain. *Neurosci. Lett.*, **37**, 155–160.

HARRISON, N.L. & SIMMONDS, M.A. (1985). Quantitative studies on some antagonists of N-methyl-D-apartate in slices of rat cerebral cortex. *Br. J. Pharmacol.*, **84**, 381–391.

HOLLMANN, M., O'SHEA-GREENFIELD, A., ROGERS, S.W. & HEINEMANN, S. (1989). Cloning by functional expression of a member of the glutamate receptor family. *Nature*, **342**, 643–648.

HONORE, T., DAVIES, S.N., DREJER, J., FLETCHER, E.J., JACOBSEN, J., LODGE, D. & NIELSEN, F.E. (1988). Quinoxalinediones: Potent, competitive non-NMDA glutamate receptor antagonists. *Science*, **241**, 701–703.

HONORE, T. & DREJER, J. (1988). Binding characteristics of non-NMDA receptors. In *Excitatory Amino Acids in Health and Disease*. ed. Lodge, D. pp. 91–106. London: John Wiley & Sons.

HORNE, A.L. & SIMMONDS, M.A. (1986). Antagonism of quisqualate responses by barbiturates. *Br. J. Pharmacol.*, **89**, 776P.

IINO, M., OZAWA, S. & TSUZUKI, K. (1990). Permeation of calcium through excitatory amino acid receptor channels in cultured rat hippocampal neurones. *J. Physiol.*, **424**, 151–165.

KEINANEN, K., WISDEN, W., SOMMER, P., WERNER, P., HERB, A., VERDOORN, T.A., SAKMANN, B. & SEEBURG, P.H. (1990). A family of AMPA-selective glutamate receptors. *Science*, **249**, 556–560.

KISKIN, N.I., KRISHTAL, O.A. & TYSNDRENKO, A.Y. (1986). Excitatory amino acid receptors in hippocampal neurons: kainate fails to desensitize them. *Neurosci. Lett.*, **63**, 225–230.

KISKEN, N.I., KRISHTAL, O.A. & TYSNDRENKO, A.Y. (1990). Cross-desensitization reveals pharmacological specificity of excitatory amino acid receptors in isolated hippocampal neurones. *Eur. J. Neurosci.*, **2**, 461–470.

KROGSGAARD-LARSEN, P., HONORE, T., HANSEN, J.J., CURTIS, D.R. & LODGE, D. (1980). New class of glutamate agonist structurally related to ibotenic acid. *Nature*, **284**, 64–66.

LODGE, D., JONES, M.G. & PALMER, A.J. (1991). Excitatory amino acids: New tools for old stories. *Can. J. Physiol. Pharmacol.*, **69**, 1123–1128.

MAYER, M.L. & WESTBROOK, G. (1987). The physiology of excitatory amino acids in the vertebrate CNS. *Prog. Neurobiol.*, **28**, 197–296.

McLENNAN, H. & LIU, J.-R. (1982). The action of six antagonists of the excitatory amino acids on neurones of the rat spinal cord. *Exp. Brain Res.*, **45**, 151–156.

MILJKOVIC, Z. & MACDONALD, J.F. (1986). Voltage-dependent block of excitatory amino acid currents by pentobarbital. *Brain Res.*, **376**, 365–402.

MONAGHAN, D.T., BRIDGES, R.J. & COTMAN, C.W. (1989). The excitatory amino acid receptors. *Annu. Rev. Pharmacol. Toxicol.*, **29**, 365–399.

MONAGHAN, D.T., HOLETS, V.R., TOY, D.W. & COTMAN, C.W. (1983). Anatomical distribution of four pharmacologically distinct ^{3}H-L-glutamate binding sites. *Nature*, **306**, 176–179.

OKAZAKI, M.M. & NADLER, J.V. (1988). Protective effects of mossy fibre lesions against kainic acid induced seizures and neuronal degeneration. *Neurosci.*, **26**, 763–781.

OLNEY, J.W. (1969). Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate. *Science*, **164**, 719–721.

PALMER, A.J., ZEMAN, S. & LODGE, D. (1991). Pharmacological separation between AMPA and kainate responses of rat cortical slices by a 2,3-benzodiazepine, methohexitone and nickel. *Br. J. Pharmacol.*, **104**, 380P.

PATNEAU, D.K. & MAYER, M.L. (1991). Kinetic, analysis of interactions between kainate and AMPA: evidence for activation of a single receptor in mouse hippocampal neurons. *Neuron*, **6**, 785–799.

PIN, J.P., BERNARD, J.V.-V. & BOCKAERT, J. (1989). Complex interactions between quisqualate and kainate receptors as revealed by measurement of GABA release from striatal neurons in primary culture. *Mol. Pharmacol.*, **172**, 81–91.

POTASHNER, S.J. & GERARD, D. (1983). Kainate enhanced release of D-[^{3}H]-aspartate from cerebral cortex and striatum: reversal by baclofen and pentobarbitone. *J. Neurochem.*, **40**, 1548–1557.

SHEARDOWN, M.J., NIELSEN, E.O., HANSEN, A.J., JACOBSEN, P. & HONORE, T. (1990). 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F) quinoxaline: a neuroprotectant for cerebral ischemia. *Science*, **247**, 571–574.

SIMMONDS, M.L. & HORNE, A.L. (1988). Barbiturates and excitatory amino acid interactions. In *Excitatory Amino Acids in Health and Disease*. pp. 219–236. ed. Lodge, D. London: John Wiley & Sons.

STONE, T.W. (1990). Sensitivity of hippocampal neurones to kainic acid, and antagonism by kynurenate. *Br. J. Pharmacol.*, **101**, 847–852.

STONE, W.E. & JAVID, M.J. (1980). Effects of anticonvulsants and glutamate antagonists on the convulsive action of kainic acid. *Arch. Int. Pharmcodyn.*, **243**, 56–65.

TEICHBERG, V.I., ESHHAR, N., MAOZ, I., MANO, I., ORNSTEIN, D., ORTEGA, A. & GREGOR, P. (1990). Molecular characterization, ultrastructural localization and gene cloning of the chick cerebellar kainate receptor. *Adv. Exp. Med. Biol.*, **268**, 73–78.

TEICHBERG, V.I., TAL, N., GOLDBERG, O. & LUINI, A. (1984). Barbiturates, alcohols and the CNS excitatory neurotransmission: specific effects on the kainate and quisqualate receptors. *Brain Res.*, **291**, 285–292.

TURSKI, L., NIEMANN, W. & STEPHENS, D.R. (1990). Differential effects of antiepileptic drugs and β-carbolines on seizures induced by excitatory amino acids. *Neurosci.*, **39**, 799–807.

WATKINS, J.C. & COLLINGRIDGE, G.L. (ed.) (1989). *The NMDA receptor*. Oxford: IRL Press.

WATKINS, J.C. & EVANS, R.H. (1981). Excitatory amino acid transmitters. *Annu. Rev. Pharmacol. Toxicol.*, **21**, 165–204.

WATKINS, J.C., POOK, P.K.C., SUNTER, D.C., DAVIES, J. & HONORE, T. (1990). Experiments with kainate and quisqualate agonists and antagonists in relation to the sub-classification of 'non-NMDA' receptors. In *Excitatory Amino Acids and Neuronal Plasticity*. pp. 49–55. ed. Ben Ari, Y. New York: Plenum Press.

WEAKLEY, J.N. (1969). Effects of barbiturates on 'quantal' synaptic transmission in spinal motoneurones. *J. Physiol.*, **204**, 63–77.

WYLLIE, D.J.A., MATHIE, A., SYMONDS, C.J. & CULL-CANDY, S.G. (1991). Activation of glutamate receptors and glutamate uptake in identified macroglial cells in rat cerebellar cultures. *J. Physiol.*, **432**, 235–258.

ZEMAN, S. & LODGE, D. (1991a). 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)-quinoxaline (NBQX) as an excitatory amino acid antagonist on neonatal rat hemisected spinal cords. *Br. J. Pharmacol.*, **102**, 284P.

ZEMAN, S. & LODGE, D. (1991b). Barbiturates selectively reduce depolarizing responses to kainate rather than those to AMPA in neonatal rat spinal cord *in vitro*. *Br. J. Pharmacol.*, **104**, 335P.

(Received December 9, 1991
Revised February 7, 1992
Accepted February 13, 1992)