A comparison of intravenous NBQX and GYKI 53655 as AMPA antagonists in the rat spinal cord

¹Boris A. Chizh, Michael J. Cumberbatch & ²P. Max Headley

Department of Physiology, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD

1 The effects of intravenous administration of two α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) antagonists were studied on responses of single neurones to iontophoretically applied excitatory amino acids. The tests were performed on spinal neurones in α -chloralose anaesthetized, spinalized rats.

2 Both the quinoxaline, NBQX (2–16 mg kg⁻¹) and the 2,3-benzodiazepine, GYKI 53655 (2–8 mg kg⁻¹) dose-dependently decreased responses to AMPA.

3 Both compounds were short acting, with half-recovery times of 15 min for NBQX and 7 min for GYKI 53655.

4 The selectivity for responses to AMPA over those to N-methyl-D-aspartate (NMDA) was significantly poorer for systemic NBQX than for either systemic GYKI 53655 or iontophoretic NBQX, suggesting that systemic NBQX may be converted to a less selective metabolite.

5 GYKI 53655 is therefore likely to be a more valuable tool than NBQX for the study of AMPA receptor-mediated processes *in vivo*.

Keywords: Excitatory amino acids; AMPA; NBQX; GYKI 53655

Introduction

The pathogenesis of several neurological disorders, such as epilepsy and brain ischaemia, involves neurotoxicity mediated by excitatory amino acids (EAA; Rogawski, 1993). N-methyl-D-aspartate (NMDA) antagonists have been tested clinically in such disorders, but the serious side-effects of these compounds, and their limited effectiveness, have pushed interest towards drugs acting via non-NMDA receptors (Rogawski, 1993). Two classes of selective antagonists for AMPA (α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid) have been developed recently. The first class comprises competitive quinoxalinedione antagonists (Honore *et al.*, 1988) such as NBQX (2,3-dihydroxy-6-nitro-7-sulphamoylbenzo(F) quinoxaline; Sheardown *et al.*, 1990). The second comprise a novel group of 2,3-benzodiazepines such as GYKI 53655 (1-(4'-aminophenyl)-3-methylcarbamoyl-4-methyl-3,4-dihydro-7,8-methylene-dioxy-5H-2,3-benzodiazepine HCl; Tarnawa *et al.*, 1993) that act via an allosteric site (Zorumski *et al.*, 1993). Some of these compounds have been found effective in models of epilepsy and brain ischaemia (Rogawski, 1993). However, the interpretation of these data is currently difficult because little is known about the selectivity of these antagonists for non-NMDA vs NMDA receptors after systemic administration *in vivo*, although the structurally similar benzodiazepine, GYKI 52466, appears to be selective (Engberg *et al.*, 1993). We have now compared the selectivity of NBQX and GYKI 53655, administered intravenously, between the response of spinal neurones to iontophoretically applied AMPA and NMDA.

Methods

Animal preparation

Experiments were performed on 27 male Wistar rats (260–350 g). Details of the experimental methods have been described elsewhere (Headley *et al.*, 1987). Briefly, rats were anaesthetized with halothane in O₂ and tracheal, carotid and

jugular cannulae were inserted. The lumbo-thoracic spinal cord was exposed and cut at T9-T11 and the animal was prepared for extracellular recordings of single dorsal horn neurone action potentials. Anaesthesia after surgery was maintained with α -chloralose (50 mg kg⁻¹, i.v. initially, then 10 mg kg⁻¹ as required). Arterial blood pressure was monitored continuously; systolic pressure remained above 100 mmHg. Core temperature was maintained close to 37°C. Extracellular recordings of single dorsal horn neurone action potentials were made with the central barrel of multibarrel glass micropipettes, filled with 3.5 M NaCl. Counts of evoked spike activity, in epochs related to the stimuli, were analysed on-line with a microcomputer. Cell discharge rates were monitored on a chart recorder. At the end of experiments, the rats were killed with an overdose of pentobarbitone.

Drugs

AMPA, NMDA and NBQX were administered microiontophoretically from the side barrels of the multibarrel pipettes, from solutions of the sodium salts of AMPA (Tocris Neuramin; 10 mM in 200 mM NaCl), NMDA (Tocris Neuramin; 100 mM in 100 mM NaCl) and NBQX (Merz + Co; 1 mM in 50 mM NaCl), all at pH 7.5–8. Another barrel contained 200 mM NaCl for current balancing. AMPA and NMDA were ejected in regular cycles. NBQX, and also GYKI 53655 (Lilly & Co), were administered intravenously.

Antagonist effects are expressed quantitatively as percentages of control EAA responses, where control was taken as the mean of the last 3 pre-drug counts; mean values $\pm$ s.e.mean are indicated. No corrections for spontaneous activity were made. Tests were accepted only if AMPA and NMDA responses recovered by at least 50% of the initial antagonist effect. Statistical analysis was performed with the Wilcoxon matched pairs test on original spike count data (except where indicated).

Results

In preliminary experiments, GYKI 53655 (4 mg kg⁻¹) was found to have a short-lasting depressant effect on neuronal

¹ Present address: Dept of Pharmacology, Moscow Sechenov Medical Academy, 2/6 B. Pirogovskaya St, Moscow 119881, Russia.

² Author for correspondence.

responses to iontophoretic AMPA, with a half-recovery time of approximately 7 min (Figure 1a). We assumed that this was due to drug elimination, so for the rest of the experiments, bolus injections of GYKI 53655 were supplemented with a constant infusion at a rate of half the bolus dose given over 7 min. When tested on dorsal horn neurone responses to AMPA and NMDA, GYKI 53655 (4 and 8 mg kg⁻¹) substantially depressed or completely abolished AMPA responses, while NMDA-evoked firing was less affected (Figure 1b). Pooled data from 14 such experiments show the dose-dependence of GYKI 53655 (2–8 mg kg⁻¹) in depressing responses to AMPA (Figure 2a). NBQX also dose-dependently decreased AMPA-evoked responses following either i.v. (2–16 mg kg⁻¹) or microiontophoretic administration (Figure 2b).

Both antagonists reduced responses to AMPA significantly more than those to NMDA ($P < 0.002$ at all doses, based on a comparison of the percentage data). Nonetheless the results in Figure 2a and b indicate that, following i.v. administration, GYKI 53655 was more selective than NBQX. At the highest doses tested, GYKI 53655 and NBQX reduced AMPA responses to a comparable degree ($23 \pm 6\%$ and $27 \pm 6\%$ control, respectively) whereas for NMDA the values were $77 \pm 7\%$ vs $59 \pm 7\%$ control respectively. Similar data that are more directly comparable were obtained by selecting those cells on which both antagonists were tested under directly comparable conditions (Figure 2c). On these cells the highest doses of each antagonist tested (NBQX 16 mg kg⁻¹ and GYKI 53655 8 mg kg⁻¹) again reduced AMPA responses to a very similar degree, but NBQX reduced NMDA responses significantly more than did GYKI 53655 ($P < 0.05$, based on percentage data).

When administered by iontophoresis as opposed to intravenously, NBQX was a highly selective antagonist at the top currents tested (5–10 nA), reducing AMPA responses to $14 \pm 4\%$ control ($P < 0.001$ vs control) whilst NMDA responses remained at $85 \pm 4\%$ control (nonetheless significantly less than control, $P < 0.01$).

Spontaneous firing rates were reduced by all antagonists, but significantly more by systemic GYKI 53655 and NBQX (respectively to $50 \pm 13\%$ and $36 \pm 10\%$ control at the highest doses tested on 10 cells having spontaneous activity) than by microiontophoretic NBQX ($70 \pm 7\%$ control; 9 cells with spontaneous activity).

Discussion

Both the competitive AMPA antagonist NBQX, and a weaker analogue of the non-competitive antagonist, GYKI 53655, have been reported to be therapeutically effective in different animal models of epilepsy and global brain ischaemia (Rogawski, 1993). However, most of the data on the receptor profile of these compounds come from *in vitro* studies (e.g. Zeman & Lodge, 1992; Zorumski *et al.*, 1993). A variety of factors may change this profile of the drugs *in vivo*, particularly after systemic administration, making the interpretation of the mechanism of their effects difficult. Thus, GYKI 52466, which selectively blocked AMPA-activated currents in cultured hippocampal neurones (Donevan & Rogawski, 1993), has been reported to be a non-selective antagonist of both AMPA and NMDA-induced seizures when tested

Figure 1 (a) Effects of a bolus intravenous injection of GYKI 53655 on neuronal responses to iontophoretic AMPA (8 nA). (b) Selectivity of GYKI 53655 between responses to iontophoretic NMDA (N, 25 nA) and AMPA (A, 7 nA), and to noxious heat stimulation applied to the plantar surface of the hindpaw (H, 49°C for 15 s); a bolus injection was supplemented by infusion of half the bolus dose over the half-recovery time estimated from experiments such as that in (a). Data from two spinal dorsal horn neurones in α -chloralose anaesthetized, spinalized rats.

Figure 2 The effects of intravenous GYKI 53655 and NBQX (including tests with iontophoretically administered NBQX) on dorsal horn neurone responses to iontophoretic NMDA (solid columns) and AMPA (cross-hatched columns). (a) Pooled data for GYKI 53655; (b) pooled data for NBQX; (c) direct comparison of the selectivity of GYKI 53655 and NBQX when tested on the same cells at lower (left) and higher i.v. doses (right). Asterisks show significant difference from control: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. #Significant difference between effects on NMDA responses of NBQX (16 mg kg⁻¹) and GYKI 53655 (8 mg kg⁻¹), $P < 0.05$.

systemically (Bisaga *et al.*, 1993). When tested on a small number of spinal motoneurons by either iontophoretic or intravenous administration *in vivo*, this compound did, however, appear selective for AMPA-evoked responses (Engberg *et al.*, 1993). NBQX has also been shown to antagonize AMPA-induced responses in the spinal cord *in vitro* (Zeman & Lodge, 1992) and *in vivo* after localized iontophoretic administration (Lodge *et al.*, 1991) without marked effects on NMDA responses.

In this study GYKI 53655, a more potent derivative of GYKI 52466 (Tarnawa *et al.*, 1993) was more selective than NBQX in reducing responses to AMPA vs NMDA. Even GYKI 53655, however, did reduce NMDA responses to some extent. The reduction of background activity (which presumably results from antagonism of AMPA receptor-mediated spontaneous inputs onto the cells) reflects a decreased excitability that would in turn reduce responses to NMDA as well as those to AMPA; this effect is therefore likely to contribute to this minor degree of non-selectivity. Such a mechanism cannot, however, explain the difference in the degree of selectivity between systemic NBQX and either iontophoretic NBQX or systemic GYKI 53655 (see data in Figure 2), particularly when the two agents were compared on the same cells.

Since NBQX is evidently metabolized rapidly (the half-

recovery time under these conditions was 15 min), it seems likely that a metabolic product may underlie the observed low selectivity of NBQX after systemic administration. GYKI 53655, whilst more selective, was even shorter-acting (half recovery time 7 min), which would limit the possible therapeutic use of this compound. Disubstituted reduced analogues of GYKI 52466 are longer lasting (Tarnawa *et al.*, 1993); development in this direction may yield more useful compounds.

In conclusion, the present work has demonstrated that both NBQX and GYKI 53655 can be effective AMPA antagonists following systemic administration. Care should, however, be taken in the interpretation of results obtained with systemic NBQX since its antagonist profile appears to be less selective than that observed either *in vitro* or following localized administration *in vivo*. This seems likely to be due to conversion of NBQX to a less selective metabolite. GYKI 53655 therefore seems to be a more valuable tool than NBQX for the investigation of AMPA receptor-mediated processes *in vivo*.

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