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In vivo and in vitro evaluation of AMPA receptor antagonists in rat hippocampal neurones and cultured mouse cortical neurones

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Abstract

The effects of four glutamate receptor antagonists on α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)- and *N*-methyl-D-aspartate (NMDA)-responses were evaluated using both in vitro and in vivo electrophysiological techniques: whole cell patch-clamp recordings from cultured mouse cortical neurones and microiontophoresis in the rat hippocampus. The compounds tested were NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline), GYKI 52466 (1-(4-amino-phenyl)-4-methyl-7,8-methyl-endioxyl-5*H*-2,3-benzodiazepine), PNQX (pyrido[3, 4-*f*]quinoxaline-2,3-dione, 1,4,7,8,9,10-hexahydro-9-methyl-6-nitro-, methanesulfonate), NS377 (7-ethyl-5-phenyl-1,6,7,8-tetrahydro-1,7-diaza-as-indacene-2,3-dione), and MK-801 ((+)-5-methyl-10,11-dihydro-5*H*-dibenz(*a*,*d*)cycloheptene-5,10-imine hydrogen maleate). In vitro, the IC_{50} values (in μM) for inhibition of AMPA-evoked inward currents were ~0.4 for NBQX, ~7.5 for GYKI 52466, ~1 for PNQX and ~15 for NS377. PNQX and NS377 also inhibited NMDA-induced currents with IC_{50} values at ~5 and ~18 μM, respectively, while NBQX at 60 μM and GYKI 52466 at 100 μM had only weak effects. The ED_{50} values in μmol/kg i.v. for inhibition of AMPA-evoked hippocampal neuronal spike activity in vivo were ~32 for NBQX, ~19 for GYKI 52466, ~17 for PNQX and ~11 for NS377 with efficacy values (maximal inhibition) between 71% and 81%. The ED_{50} values (in μmol/kg i.v.) and efficacy values for inhibition of NMDA-evoked hippocampal neuronal spike activity were ~28 with an efficacy of 61% for NBQX, ~16 with 35% for PNQX and ~6 with 61% for NS377. GYKI 52466 did not significantly affect NMDA responses, whereas MK-801 showed NMDA specificity in vivo.

Keywords: Excitatory amino acid; Patch-clamp, whole cell; GYKI 52466; NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline); PNQX; NS377; Iontophoretic; (Intravenous administration)

1. Introduction

Excitatory amino acids, especially glutamate, play a central role in neuronal activation. Glutamate interacts with three pharmacologically defined families of ionotropic receptors: α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), *N*-methyl-D-aspartate (NMDA) and kainate receptors. Evidence is accumulating that excessive stimulation of these receptors is related to a variety of disorders such as ischemia, epilepsy and neurodegenerative diseases (see Lipton and Rosenberg, 1994).

Brief transient cerebral ischemia causes death of pyramidal neurones in the CA1 part of the hippocampus, which has a high density of AMPA receptors (Nielsen et al., 1988), and it has been shown that this degeneration is due to excessive release of glutamate (Benveniste et al., 1989). In accordance with this, the delayed neuronal death can be avoided by administration of AMPA receptor antagonists (see Gill, 1994). Unfortunately, most currently available AMPA receptor antagonists have short half-lives, low systemic activity and limited blood–brain barrier penetration. By measuring evoked neuronal spike activity in hippocampal neurones after iontophoretic application of AMPA or NMDA, we evaluated the in vivo potency and selectivity (AMPA vs. NMDA) of known as well as novel AMPA receptor antagonists. Hippocampal neurones were selected because of their low spontaneous spike activity, prominent response to iontophoretic application of AMPA and high density of AMPA receptors in that specific area. Furthermore, this brain structure is the most vulnerable after transient, global ischemia.

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In order to evaluate in vitro potency and selectivity, the compounds were also tested on cultured cortical neurones, using the whole cell configuration of the patch-clamp technique. These neurones responded to repeated applications of AMPA or NMDA with inward currents which were stable for up to 30 min.

2. Material and methods

2.1. In vitro electrophysiology

All experiments were performed in voltage clamp using conventional whole cell patch-clamp methods (Hamill et al., 1981). The following salt solutions were used.

Extracellular solution for AMPA responses (mM): NaCl (140), KCl (4), $CaCl_2$ (2), $MgSO_4$ (4), HEPES (10, pH 7.4), tetrodotoxin (0.0003), bicuculline methiodide (0.005) and sucrose (30). For NMDA responses, the extracellular solution contained 10 μM glycine and $MgSO_4$ was omitted.

Intracellular solution (mM): CsCl (120), CsF (20), $MgSO_4$ (4), EGTA (10), ATP (4), HEPES (10, pH 7.2).

Tetrodotoxin was purchased from Alomone Labs., Jerusalem, Israel and bicuculline methiodide from RBI, MA, USA. All other reagents were from Sigma, USA.

Stock solutions of test compounds were prepared as follows: GYKI 52466 (1-(amino-phenyl)-4-methyl-7,8-methylendioxy-5*H*-2,3-benzodiazepine, RBI) and NS377 (7-ethyl-5-phenyl-1,6,7,8-tetrahydro-1,7-diaza-as-indacene-2,3-dione, NeuroSearch, Denmark), 50 mM in 50% dimethylsulfoxide (DMSO). PNQX (pyrido[3, 4-*f*]quinoxaline-2,3-dione, 1,4,7,8,9,10-hexahydro-9-methyl-6-nitro-, methanesulfonate, Parke Davis, USA), and NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline, NeuroSearch), 30 mM in 1 M of NaOH.

2.1.1. Cell cultures

Mouse neocortical neurones were cultured essentially as described by Drejer et al. (1987). Briefly, the forebrains from 17-day old embryonic NMRI (Naval Marine Research Institute) mice (M&B, Denmark) were removed under sterile conditions. The tissue was chopped into 0.4-mm cubes and then triturated with trypsin (12.5 μg/ml) and DNAse (2.5 μg/ml), 15 min, 37°C. The cells were suspended at a concentration of 3×10^6 cells/ml in a slightly modified Dulbecco's Eagle medium (Life Technologies, Roskilde, Denmark) which contained horse serum (10% v/v), penicillin (333 U/ml), paraaminobenzoic acid (1 mg/l), L-glutamine (0.5 mM), insulin (0.08 U/ml) and KCl (23.8 mM). The cell suspension was subsequently inoculated into poly-L-lysine-coated 35-mm Petri dishes (2 ml/dish). Glass coverslips (3.5 mm) were placed in the dishes before coating. After 24 h in culture, the medium was replaced by freshly made medium containing no serum but with added 1% N2 supplement (Life Technologies); final concentrations (mg/l): insulin (5); human transferrin (100); progesterone (0.0063); putrascine (16.11); and selenite (0.0052). Every second day, half the culture medium was refreshed.

The cells were kept in culture for 7–13 days at 37°C (5% CO_2/95% O_2) before experiments were carried out.

2.1.2. Electronics, programs and data acquisition

The amplifier used was the EPC-9 (HEKA-electronics, Lambrect, Germany) run by a Macintosh 7600/120 computer via an ITC-16 interface. Experimental conditions were set with the Pulse software accompanying the amplifier. Data were low-pass filtered and sampled directly to hard-disk at a rate of three times the cut-off frequency.

2.1.3. Pipettes and electrodes

Pipettes were pulled from borosilicate glass (Modulohm, Copenhagen, Denmark) using a horizontal electrode puller (Zeitz-Instrumente, Augsburg, Germany). The pipette resistances were 2–3 MΩ in the salt solutions used in these experiments. The pipette electrode was a chloridized silver wire, and the reference was a silver chloride pellet electrode (In Vivo Metric, Healdsburg, USA) fixed to the experimental chamber. The electrodes were zeroed with the open pipette in the bath just prior to sealing.

2.1.4. Experimental procedure

Coverslips were transferred to a 15-μl experimental chamber mounted on the stage of an inverted microscope (IMT-2, Olympus) supplied with Nomarski optics. Cells were continuously superfused with extracellular saline at a rate of 2.5 ml/min. After giga-seal formation (1–5 GΩ, success-rate ≈ 90%) the whole cell configuration was obtained by suction.

The holding potential was −60 mV and at the start of each experiment the current was measured continuously for 30 s to ensure a stable baseline. Agonist-containing solutions were delivered to the chamber through a custom-made gravity-driven flowpipe, the tip of which was placed approximately 50 μm from the cell. Application was triggered when the tubing connected to the flowpipe was compressed by a valve controlled by the Pulse software. In general, agonists were applied for 1.5 or 3 s every 45 s. The sample interval during application was 600 μs. After stable responses were obtained, the extracellular saline as well as the agonist-containing solution were replaced by solutions containing the compound to be tested. The compound was present until responses of a repeatable amplitude were achieved. Currents were measured at the plateau phase of the responses just before deactivation. The effect of compounds was calculated as the current at compound equilibrium divided by the current evoked by the pulse immediately before the compound was included.

The patch-clamp experiments were performed at room temperature (20–22°C).

2.2. In vivo electrophysiology

2.2.1. Preparation

Experiments were performed on 42 male Wistar rats (M&B) weighing 280–380 g and housed two per cage with free access to food and water. The rats were anaesthetised with urethane (1.1 g/kg i.p.) and the femoral artery and vein were catheterised for monitoring the blood pressure and for intravenous injection of drugs, respectively. When necessary, additional urethane (220 mg/kg i.v.) was given in order to maintain anaesthesia. The rats were placed in a stereotaxic frame and ventilated with a rodent ventilator (Ugo Basile, Italy). Core body temperature was maintained at 37°C with a DC heating pad. The left and dorsal part of the parietal bone was removed by craniotomy and the dura was withdrawn, exposing the pia mater and underlying brain, which was superfused with a standard Krebs solution (37°C) (Paxinos and Watson, 1986).

2.2.2. Drugs

AMPA (Sigma) was dissolved at 10 mM in 0.2 M of NaCl. NMDA (Sigma) was dissolved at 100 mM in 100 mM of NaCl. Both solutions were adjusted to pH 7.5–8.0 with NaOH. NBQX (NeuroSearch) was dissolved in distilled water and a small amount of 1 M of NaOH to a final concentration of 30 mM, pH was adjusted to 8 with 1 M of HCl. PNQX (Parke Davis, USA) was dissolved in a minimum amount of 85% lactic acid, diluted to 5 mM with distilled water and adjusted to pH 4 with sodium hydroxide. The solution was filtered (22 μm). NS377 (NeuroSearch) was dissolved at 12 mM in distilled water and adjusted to pH 6 with NaOH. GYKI 52466 (RBI) was dissolved at 15 mM in 0.9% NaCl (pH 5). MK-801.HCl ((+)-5-methyl-10,11-dihydro-5*H*-dibenz(*a*,*d*)cycloheptene-5,10-imine hydrogen maleate) (RBI) was dissolved in distilled water (0.3–3.0 mM, pH 5).

2.2.3. Test procedure

Extracellular recordings of single CA1 neurone spikes (action potentials) were made with five-barrel glass microelectrodes (5B120F-6, World Precision Instruments, USA) with a tip diameter of 10–12 μm. The individual barrels were filled with 5 M of NaCl and 2% pontamine sky blue (recording), 400 mM of NaCl (current balancing), 200 mM of NaCl (control current), and the last two barrels were filled with solutions of AMPA or NMDA.

Experiments were performed on 70 hippocampal neurones ($A = 5.5$–6.5 mm, $L = 1.5$–2.0 mm, $H = 2.0$–3.0 mm, according to Paxinos and Watson, 1986). Neuronal spike activity was evoked by iontophoretic application of AMPA or NMDA for 10–15 s with 1.5 min between each agonist stimulation. Neuronal signals were amplified 5000 times by a FET input amplifier, filtered at 0.3–3 kHz (4th-order Bessel) and recorded by a computer, using the Spike2 programme. The amplifier was connected to the computer via a 1401*plus* interface (Cambridge Electronic Design, UK). The computer programme also recorded mean arterial blood pressure and both monitored and controlled the iontophoretic application.

AMPA and NMDA were ejected into the hippocampus in regular cycles of 3 min. During each cycle a saline (200-mM NaCl) ejection was also performed in order to rule out artefacts. When neuronal responses were stable (less then 10% deviation between groups of three responses) for at least 1/2 h and the responses to AMPA or NMDA did not differ by more than a factor of three, a single dose of either PNQX (9.0–36 μmol/kg, corresponding to 2.5–10 mg/kg i.v.), NS377 (6.0–25 μmol/kg, corresponding to 2.5–10 mg/kg i.v.), NBQX (30–60 μmol/kg, corresponding to 10–20 mg/kg i.v.), GYKI 52466 (15–60 μmol/kg, corresponding to 5.0–20 mg/kg i.v.), or MK-801(1.5–3.0 μmol/kg, corresponding to 0.5–1.5 mg/kg i.v.) was injected into the femoral vein. Normally, only a single dose was used per rat, but when the antagonist activity was brief (less than 30 min) or not effective at that specific dose, an additional injection was performed 90 min after first injection and after 30 min of stable responses to AMPA and NMDA. Recording of neuronal spike activity was continued for at least 45 min after intravenous injection and only experiments where AMPA and NMDA responses were reversed to at least 50% of pre-drug level were accepted for analysis. Five or six experiments were performed with each dose.

2.2.4. Statistics

Evoked neuronal spike activity was analysed on-line by a computer, saving single spikes and time of event. Neuronal spike activity (spikes/s) was monitored on a pulse rate histogram together with indicators for AMPA and NMDA application. Drug effects were calculated as percent decreases in spike activity during AMPA or NMDA stimulation from pre-drug to post-drug level, using the average of three responses for each agonist. The Wilcoxon matched pairs sign test ($\alpha = 0.05$) was used for statistical analysis of original spike counts in order to test for significant differences between pre- and post-drug levels of spike activity for each dose group. The dose needed to obtain 50% of the maximal inhibition (ED_{50} value, potency) and the efficacy values were estimated by dose–response curve fitting (Microcal, Origin 5.0) using the following equation: $Y = A_2 + (A_1 - A_2)/(1 + 10\ \hat{}((\log(x_0) - x) * P))$, where Y corresponds to the inhibition obtained, x to dose in μmol/kg, x_0 to ED_{50} value, A_1 to the initial inhibition obtained, A_2 to maximal inhibition (efficacy value) and P to the Hill slope.

3. Results

3.1. In vitro electrophysiology

Brief applications of AMPA (30 μM) to voltage-clamped neurones resulted in fast activating inward cur-

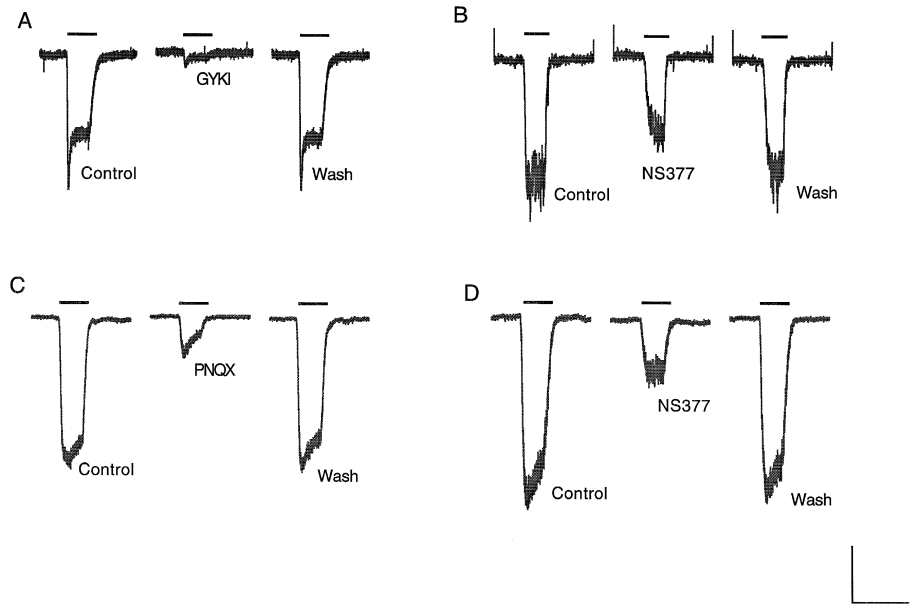

Fig. 1. Cortical neurones were voltage-clamped at −60 mV in the whole cell configuration. Every 45 s, each cell was exposed to a pulse of 30 μM AMPA (A and B) or 100 μM NMDA (C and D) as indicated by the black horizontal bars. After obtaining responses of a reproducible amplitude ('Control') the compound to be tested was included in the solutions (A: GYKI 52466, 30 μM; B: NS377, 30 μM; C: PNQX, 60 μM; D: NS377, 60 μM). After attainment of a new stable level (for example 'GYKI' in panel A), solutions were changed back to control conditions ('Wash'). The experiments with NMDA were performed using Mg^{2+}-free solutions with 10 μM of glycine added. The traces in panel C are low-pass filtered with a cut-off frequency of 100 Hz. Scale bars: (A) 100 pA/3 s, (B) 25 pA/2 s, (C) 600 pA/3 s, (D) 400 pA/3 s.

rents, which displayed very different desensitisation kinetics from cell to cell. Some, as shown in Fig. 1A, desensitised rapidly to reach a plateau level, others desensitised almost completely, while others again did not desensitise at all.

Two examples are shown in Fig. 1. It is seen that 30 μM GYKI 52466 reversibly and almost completely inhibited AMPA-evoked responses (Fig. 1A). NS377 also at 30 μM, inhibited AMPA-evoked responses, although less effectively (Fig. 1B).

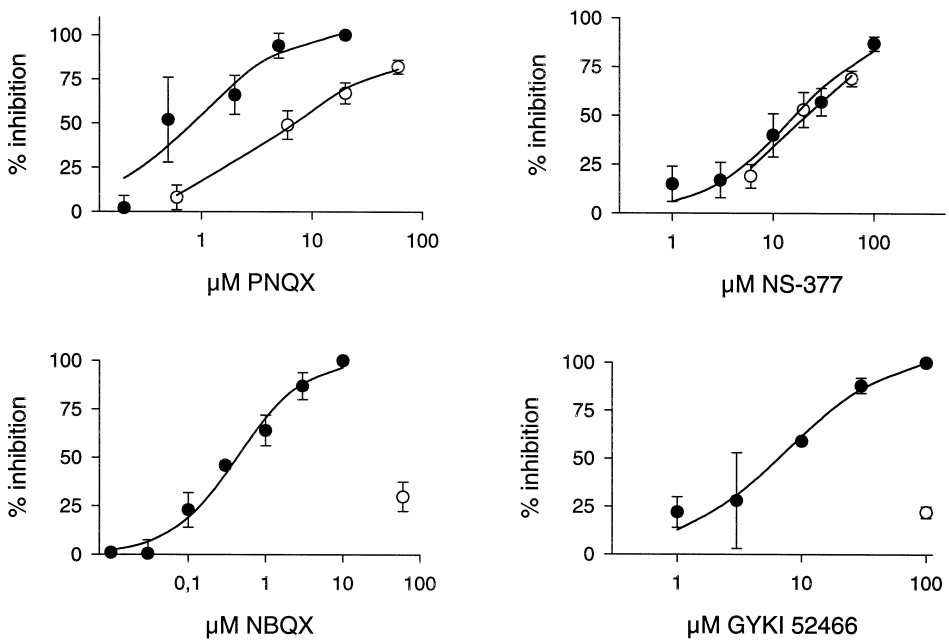

Fig. 2. The four panels summarise the experiments from Fig. 1. Effect (percent inhibition) of the compounds was calculated as described in Section 2. Dose–effect relationships of the four compounds tested on NMDA-evoked responses (○) and on AMPA-evoked responses (●) are shown. Error bars represent standard deviation ($n = 3$–6). Data points are fitted (solid curves) to the equation: $y = (A \times C)/(B + C)$, where A is the maximal inhibition ('100') and B is the IC_{50}.

In general, responses evoked by NMDA (100 μM) were considerably greater than the AMPA responses and the desensitisation kinetics varied from cell to cell. As seen in Fig. 1C, PNQX (60 μM) significantly and reversibly delayed the activation time and decreased the amplitude. NS377 (60 μM) also inhibited NMDA-evoked responses, as shown in Fig. 1D.

In Fig. 2, the effects of PNQX, NS377, NBQX and GYKI 52466 on AMPA- and NMDA-evoked currents are shown as dose–response curves.

3.2. In vivo electrophysiology

Preliminary experiments in our laboratory showed that some of the AMPA receptor antagonists, especially GYKI 52466, induced an inhibitory effect on respiration and had secondary effects on blood pressure. Therefore, all experiments were performed on artificially ventilated rats. The average mean arterial blood pressure was 93 mmHg with a range of 72–120 mmHg (Table 1), and there was no significant difference between the groups. The mean arterial blood pressure was below 70 mmHg in two rats and they were excluded from the calculations. PNQX reduced mean arterial blood pressure dose dependently for 2–3 min, followed by a minor increase, and returned to the baseline level 10–15 min after administration (Fig. 3A). Administration of NS377 resulted in an initial short-lasting (30–60 s) reduction of mean arterial blood pressure followed by a longer-lasting (2–3 min) increase (Fig. 3B). Intravenous injection of NBQX only resulted in an initial short-lasting (30–60 s) reduction of mean arterial blood pressure (Fig. 3C). Administration of GYKI 52466 resulted in a long-lasting decrease of mean arterial blood pressure, with return to baseline 10–15 min after i.v. injection (Fig. 3D). None of the effects on AMPA- and NMDA-evoked spike activity seemed to be correlated to changes in arterial blood pressure.

Application of AMPA or NMDA evoked CA1 neuronal spike activity in a dose-dependent manner (data not shown). Population spikes could be observed for a period, 0.5–1 h after surgery, especially when minor damage to the pia mater and cortex had occurred during removal of bone and dura mater. Occasionally, population spikes were also evoked by AMPA or NMDA, but these experiments were excluded and only single neuronal spikes were used to evaluate the effects of the AMPA receptor antagonists. Iontophoretic application of sodium chloride did not induce CA1 neuronal spike activity (data not shown).

The two lowest doses of PNQX (9.0 and 18 μmol/kg i.v.) affected neither AMPA- nor NMDA-evoked spike activity, whereas the highest dose (36 μmol/kg i.v.) significantly inhibited AMPA responses for 15–30 min (Fig. 3AFig. 4A).

The lowest dose (6.0 μmol/kg i.v.) of NS377 had no effect on AMPA- or NMDA-evoked spike activity in the

Table 1
Summary of in vivo data

	PNQX			NS377			NBQX			GYKI 52466			MK-801	
Dose (μmol/kg i.v.)	9	18	36	6	12	25	30	45	60	15	30	60	1.5	3.0
AMPA-specific	2		2	1							3	3		
AMPA-selective			1	1		1	1	2	2			2		
Non-selective	1	3	2	1	5	4		3	3		2			
No effect		1		2			3			3				
Enhancing effects	2	1					1			2				
NMDA selective													3	2
Potency (ED_{50}, AMPA)	17 ($n = 5$)			11 ($n = 5$–6)			32 ($n = 5$–6)			19 ($n = 5$–6)				
Efficacy (AMPA)	74%			78%			81%			71%				
Potency (ED_{50}, NMDA)	16 ($n = 5$)			9 ($n = 5$)			28 ($n = 5$)			n.d. ($n = 5$–6)				
Efficacy (NMDA)	35%			61%			61%			n.d.				
Spontaneous activity														
Total number	4	1	2	1	2	4	3	2	4	3	3	3	2	
Inhibited	2	1	2		1	3		2	4		1	1		
Enhanced	1									2		1		
MABP (mmHg)	75–120			87–113			80–107			75–95			72–97	

First row shows the number of experiments in which specific, selective or non-selective inhibition of AMPA-evoked spike activity was observed.
Second row shows the number of experiments where either no effects, enhancing effects on NMDA-evoked spike activity, or specific inhibition of NMDA-evoked spike activity was observed.
Third row shows the potency (ED_{50} values, in μmol/kg i.v.) and efficacy values for each of the four AMPA receptor antagonists. The effects on NMDA-evoked spike activity were not determined for GYKI 52466 as indicated by 'n.d.'.
Fourth row shows the number of experiments in which spontaneous spike activity was observed and lists the number of experiments where the activity was either inhibited or enhanced by drug administration.
Fifth row shows the range of mean arterial blood pressure (MABP).

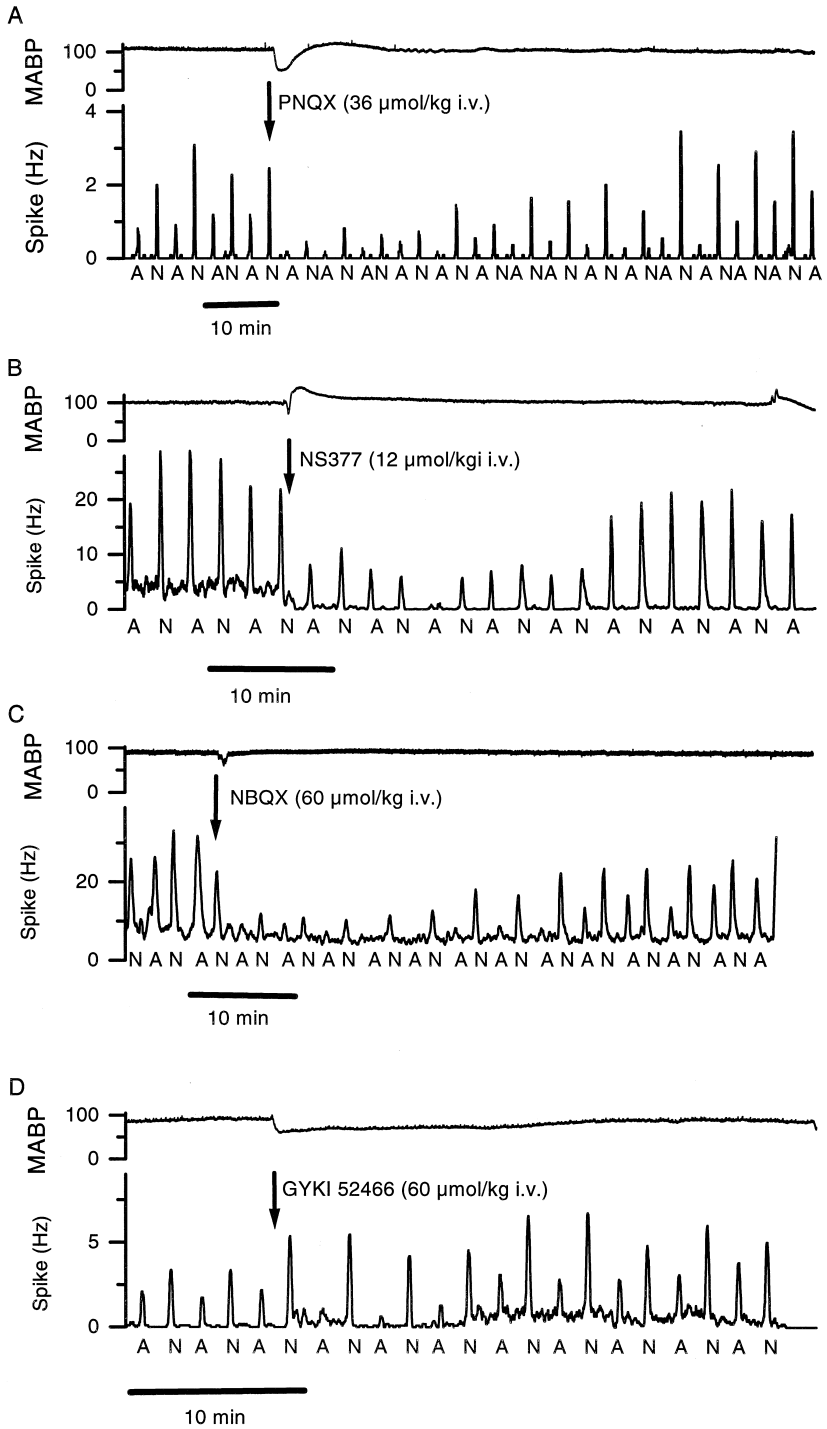

Fig. 3. Original experiments showing effects on AMPA (indicated by 'A') or NMDA- (indicated by 'N') evoked spike activity and mean arterial blood pressure (MABP, mmHg) after single dose i.v. of either PNQX, NS377, NBQX or GYKI 52466. (A) PNQX (36 μmol/kg i.v.) inhibited AMPA- (22 nA) and NMDA- (26 nA) evoked spike activity for 20–30 min. (B) NS377 (12 μmol/kg i.v.) inhibited AMPA- (20 nA) and NMDA- (16 nA) evoked spike activity for 15–20 min. (C) Intravenous injection of NBQX (60 μmol/kg) inhibited both NMDA- (15 nA) and AMPA- (14 nA) evoked spike activity for 25 and 40 min, respectively. (D) GYKI 52466 (30 μmol/kg i.v.) selectively inhibited AMPA (28 nA) for 6–9 min and showed some enhancing effect on NMDA- (18 nA) evoked spike activity. The spike activity traces were adjacent averaged over 10 s. Vertical arrows indicate time of i.v. injection.

CA1 region of the hippocampus, but at higher doses (12 and 24 μmol/kg i.v.) AMPA and NMDA responses were inhibited for 15–30 and 20–40 min, respectively (Fig. 4B). Fig. 3B shows an example of non-selective inhibition of evoked spike activity by an i.v. injection of NS377 (12 μmol/kg i.v.). The effect lasted 20 min.

The lowest dose of NBQX (30 μmol/kg i.v.) had no effect on either AMPA- or NMDA-evoked spike activity,

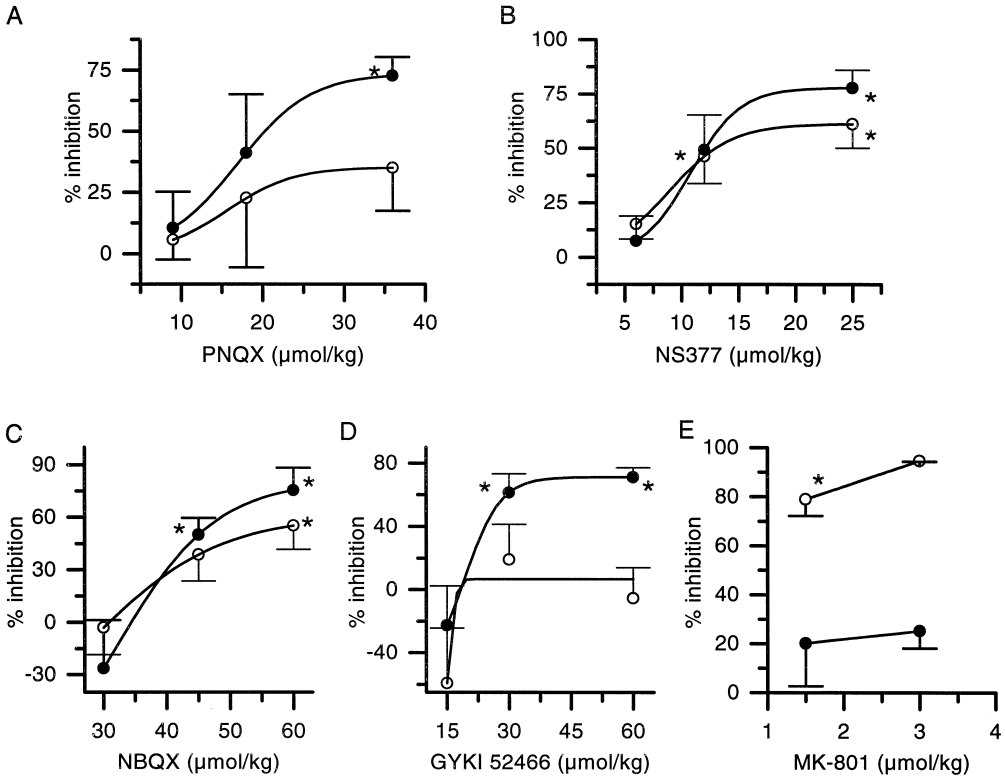

Fig. 4. Mean (±S.E.) percent inhibition of AMPA (•)- or NMDA (o)- evoked spike activity in the CA1 region of hippocampus by five glutamate receptor antagonists: PNQX (9.0–36 μmol/kg i.v., $n = 5–6$) (A), NS377 (6.0–24 μmol/kg i.v., $n = 5–6$) (B), NBQX (30–60 μmol/kg i.v., $n = 5$) (C), GYKI 52466 (15–60 μmol/kg i.v., $n = 5–6$) (D), and MK-801 (1.5, $n = 3$ and 3.0 μmol/kg, $n = 2$) (E). Positive values indicate inhibition of neuronal spike activity from predrug level, whereas negative values indicate stimulation. The solid curves represent dose–response curve fitting. Asterisks indicate significant difference between predrug and drug level (Wilcoxon, $\alpha = 0.05$).

but at 45 and 60 μmol/kg i.v. the AMPA-evoked activity was significantly inhibited. NMDA-evoked spike activity was significantly inhibited only at the highest dose (60 μmol/kg i.v.) (Fig. 3C; Fig. 4C). In some experiments, NBQX elicited a non-selective inhibition for 20–30 min followed by recovery of the NMDA-evoked spike activity, and 15–30 min later by recovery of the AMPA responses (e.g., Fig. 3C).

The lowest dose of GYKI 52466 (15 μmol/kg i.v.) induced no significant effects, whereas the two highest doses (30 and 60 μmol/kg i.v.) induced a significant inhibition of AMPA-evoked spike activity (Fig. 3D; Fig. 4D) which lasted 10–20 min and 15–40 min, respectively. In two experiments, GYKI 52466 enhanced the NMDA-evoked spike activity (Table 1, Fig. 3D).

MK-801 specifically inhibited NMDA-evoked spike activity (1.5 and 3.0 μmol/kg i.v., $n = 3$ and 2, respectively, Fig. 4E).

Estimated potency (ED_{50} values) and efficacy values for the AMPA receptor antagonists are shown in Table 1. The effects induced by NS377, PNQX and NBQX were all found to be non-selective, as these compounds inhibited AMPA and NMDA responses with equal potency. However, in contrast to NBQX and NS377, PNQX displayed significantly higher efficacy towards AMPA-evoked spike activity. The non-competitive AMPA receptor antagonist, GYKI 52466, induced a specific effect. The ranking of in vivo AMPA receptor potency was NS377 ≥ PNQX = GYKI 52466 > NBQX.

Spontaneous spike activity (average spike frequency, 2.5 Hz) was observed in some experiments (Table 1). Spontaneous spikes were especially inhibited by NS377, NBQX and PNQX, whereas GYKI 52466 increased activity in three out of nine experiments in which spontaneous spikes were observed.

4. Discussion

In this study, we examined four AMPA receptor antagonists using both in vitro and in vivo electrophysiological techniques. NBQX and GYKI 52446 have previously been shown to have little effect on NMDA responses recorded from cultured hippocampal neurones (Donevan and Rogawski, 1993; Parsons et al., 1994), while GYKI 52466 has been reported to inhibit AMPA-evoked responses recorded from hippocampal neurones or from superior colliculus neurones with IC_{50} values of 11 and 4.4 μM, respectively (Donevan and Rogawski, 1993; Parsons et al.,

1994). In superior colliculus neurones NBQX has been shown to inhibit responses evoked by 50 μM AMPA with an IC_{50} value of 0.7 μM (Parsons et al., 1994).

In the present study, IC_{50} values were ~0.4 μM for NBQX and ~7.5 μM for GYKI 52466. With respect to NBQX, which is a competitive antagonist (Donevan and Rogawski, 1993), the difference can be explained by the higher concentration of AMPA used in the study by Parsons et al. (1994).

In the present study, PNQX was found to be a potent inhibitor of AMPA-evoked currents (IC_{50} ~1 μM). The compound also inhibited NMDA responses with an IC_{50} value of ~5 μM. NS-377 was found to be a rather weak antagonist with virtually no selectivity for NMDA or AMPA receptors. The IC_{50} values for NMDA and AMPA responses were ~18 and ~15 μM, respectively.

In vivo, NBQX inhibited AMPA-evoked as well as NMDA-evoked spike activity. Similar effects of NBQX after i.v. injection were reported by Headley's group (Chizh et al., 1994; Cumberbatch et al., 1994). Since the compound was highly selective in vitro, the inhibition of NMDA-evoked spike activity must have involved mechanisms other than direct interaction with the NMDA receptor. It may be speculated that inhibition of AMPA receptors on presynaptic glutamatergic neurones reduces the overall excitatory load on the neurone, leading to a decreased ability of NMDA to overcome the Mg^{2+} blockade of the receptor channel. This hypothesis is supported by the finding that NMDA-evoked spike activity often recovered earlier than the AMPA-evoked spike activity (Fig. 3C). Furthermore, Chizh et al. (1994) found that NBQX did not inhibit NMDA-evoked spike activity when applied iontophoretically together with NMDA. According to this, one might expect a similar effect of GYKI 52466 on NMDA-evoked spike activity. This was not found, and the apparent discrepancy might be explained by the very different blockage kinetics of these two compounds (Parsons et al., 1994).

We observed that GYKI 52466 specifically inhibited AMPA responses in vivo, which was consistent with our in vitro results. The observed specificity and short-lasting effect of GYKI 52466 in vivo are consistent with the findings of Ouardouz and Durand (1991).

PNQX had fairly high potency, in the same range as GYKI 52466, and was a partly selective AMPA receptor antagonist in the in vivo test. Similar data were obtained in vitro. The low effectiveness in vivo compared to in vitro might have been due to difficulties with penetration of the blood–brain barrier and/or to metabolism of the compound.

NS377 was slightly more potent in vivo than the other compounds tested, and the lack of specificity agreed with our in vitro data. However, NS377 was fairly weak in the in vitro experiments, indicating that NS377 either penetrated the blood–brain barrier more easily than the other compounds and/or is metabolised more slowly. Another possibility is that, in vivo, the compound is converted to a more potent metabolite.

The level of spontaneous spike activity might influence the degree of evoked spike activity. Chizh et al. (1997) have shown that the spontaneous activity in the spinal dorsal horn, which is partly mediated by NMDA receptor activation, influences the responsiveness to nociceptive stimuli. The same relationship between spontaneous and evoked spike activity might qualitatively explain the differences between in vitro and in vivo selectivity. We observed that the compounds which were non-selective (i.e., NS377 and NBQX) also to some extent inhibited spontaneous spike activity, whereas GYKI 52466 actually enhanced spontaneous activity in some cases. Thus, it is possible that NS377 and NBQX inhibited overall excitability, as seen for spontaneous activity, and thereby decreased the responsiveness to NMDA receptor activation, contradictory to the findings by Chizh et al. (1997). The depolarisation associated with the spontaneous spike activity might enhance the ability to overcome the voltage-dependent magnesium block of the NMDA receptor channel during continuous stimulation by iontophoretically applied NMDA, and thereby indirectly influence the observed inhibition of AMPA- and NMDA-evoked spike activity obtained after an intravenous injection of an AMPA receptor antagonist. Thus, the lack of correlation between in vitro and in vivo data is probably related to: (1) the fact that compounds with different chemical structures have different abilities to penetrate the blood–brain barrier, (2) differences in effects on AMPA receptor kinetics between the compounds tested, and (3) changes in responsiveness influenced by spontaneous spike activity.

5. Conclusion

In vivo data showed that AMPA responses were specifically inhibited by GYKI 52466. PNQX preferentially inhibited AMPA responses, whereas NBQX and NS377 induced non-selective effects compared to the effects on NMDA responses. In vitro data supported these findings for GYKI 52466, NS377 and PNQX. NBQX was highly selective in vitro, but inhibited both AMPA- and NMDA-evoked spike activity in vivo. MK-801 specifically inhibited NMDA-evoked spike activity.

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