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Inhibition of cortical spreading depression by L-701,324, a novel antagonist at the glycine site of the *N*-methyl-D-aspartate receptor complex

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1 Spreading depression (SD) is a propagating transient suppression of electrical activity, associated with cellular depolarization, which probably underlies the migraine aura and may contribute to neuronal damage in focal ischaemia. The purpose of this study was to examine whether L-701,324 (7-chloro-4-hydroxy-3-(3-phenoxy)phenyl-2-(1H)-quinolone), a high affinity antagonist at the glycine site of the *N*-methyl-D-aspartate (NMDA) receptor complex, inhibits the initiation and propagation of K^+-induced SD in the rat cerebral cortex *in vivo*.

2 Microdialysis probes incorporating a recording electrode were implanted in the cerebral cortex of anaesthetized rats and perfused with artificial cerebrospinal fluid (ACSF). Five episodes of repetitive SD were elicited by switching to a medium containing 130 mM K^+ for 20 min, each separated by 40 min of recovery (i.e. perfusion with normal ACSF). The brief negative shifts of the extracellular direct current (d.c.) potential, characteristic of SD elicitation, were recorded with the microdialysis electrode and a reference electrode placed under the scalp. Propagation of SD was examined using glass capillary electrodes inserted about 3 mm posterior to the microdialysis electrode. L-701,324 (5 or 10 mg kg^{-1}) or its vehicle were administered i.v. 10 min after the end of the second K^+-stimulus. The effects of L-701,324 were compared to those of dizocilpine (MK-801; 1 mg kg^{-1} i.v.), a NMDA-channel blocker known to potently block SD elicitation.

3 Potassium-induced SD initiation was inhibited by 10 mg kg^{-1} (but not by 5 mg kg^{-1}) of L-701,324. Thirty minutes after administration of 10 mg kg^{-1} L-701,324, the cumulative area of SD peaks elicited during 20 min was 15.3 ± 2.1 mV min, versus 23.2 ± 1.1 mV min in animals which received only the drug vehicle ($P < 0.02$; $n = 6$). The delay between application of 130 mM K^+ and occurrence of the first SD was also significantly increased. It was approximately doubled in animals treated with 10 mg kg^{-1} of L-701,324.

4 SD propagation was more sensitive than SD elicitation to L-701,324, as both 5 and 10 mg kg^{-1} produced an effective inhibition. Even at the lower dose of 5 mg kg^{-1}, L-701,324 completely blocked the propagation of SD elicited 30 min after drug administration. This differential sensitivity of SD elicitation and propagation is not specific to L-701,324 since it was previously observed with other drugs. At doses effective against SD, L-701,324 did not produce any marked alterations of the electroencephalogram.

5 L-701,324 (10 mg kg^{-1}) and MK-801 (1 mg kg^{-1}) had identical effects on the d.c. potential when administered during the recovery which followed the second K^+ stimulus. Both drugs produced a positive shift of around 4.5 mV within 10 min of i.v. drug administration, indicating rapid drug penetration into the CNS. Paradoxically, L-701,324 (10 mg kg^{-1}) was markedly less effective than MK-801 (1 mg kg^{-1}) in blocking SD, since this dose of MK-801 was sufficient virtually to abolish SD initiation and completely block its propagation. The higher potency of MK-801 against SD may reflect its use-dependency, i.e. binding of MK-801 and channel blockade are enhanced when the NMDA-receptor ionophore is open.

6 Taken together, these data demonstrate that L-701,324 has an inhibitory effect on both SD initiation and propagation. This action may be beneficial in focal ischaemia, and possibly also against migraine, especially as this drug was shown to be active when administered orally.

Keywords: L-701,324; spreading depression; focal ischaemia; migraine; microdialysis electrode; *N*-methyl-D-aspartate receptor glycine site; d.c. potential

Introduction

Spreading depression (SD) is a propagating transient suppression of electrical activity associated with cellular depolarization (Leão, 1944; Bureš *et al.*, 1974), and both experimental and clinical evidence point to cortical SD as the possible underlying mechanism of the migraine aura (i.e. neurological disturbances associated with the development of a migraine headache) (Lauritzen, 1994). Recurrent SD may also contribute to neuronal damage, subsequent to experimental focal ischaemia (Iijima *et al.*, 1992), very likely by producing marked disruption of ionic homeostasis, acidosis, enhanced energy demand and neurotransmitter effluxes in regions where residual blood supply can only sustain basal ionic homeostasis, such as the penumbra (Obrenovitch, 1995). Spontaneous cortical SD's were also reported in patients with severe head injury (Mayevsky *et al.*, 1995).

The potential clinical relevance of SD has triggered much interest in its pharmacology, and it is now established that both its elicitation and propagation require activation of the *N*-methyl-D-aspartate (NMDA)-receptor ionophore complex (Lauritzen & Hansen, 1992; Sheardown, 1993). NMDA channel blockers such as phencyclidine and dizocilpine (MK-

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801) potently block SD, but their undesirable psychotomimetic and pathomorphologic effects preclude their use in clinical practice (Willetts *et al.*, 1990; Fix *et al.*, 1994). As antagonists at the glycine site of the NMDA-receptor may have a better therapeutic ratio (Koek & Colpaert, 1990; Hargreaves *et al.*, 1993; see however Carter, 1994) the aim of this study was to examine whether one such compound, L-701,324 (7-chloro-4-hydroxy-3-(phenoxy)phenyl-2-(1H)-quinolone), inhibited the initiation and propagation of K^+-induced SD in the rat cerebral cortex *in vivo*. L-701,324 belongs to a new class of 3-substituted 4-hydroxyquinolinones which are high affinity antagonists of the NMDA-receptor glycine site, and showed potent *in vivo* activity against seizures (Kulagowski *et al.*, 1994; Leeson & Iversen, 1994; Priestley *et al.*, 1994). SD initiation was recorded with a microdialysis electrode (Obrenovitch *et al.*, 1994), and its propagation with a glass capillary, with both recording devices implanted in the cerebral cortex of anaesthetized rats. Our data demonstrate that L-701,324 inhibits SD, but its efficacy is moderate in comparison to that of the NMDA-channel blocker, MK-801.

Methods

Animal preparation and intracerebral microdialysis

Twenty four adult male Sprague-Dawley rats (weight, 307±4 g, mean±s.e.mean; Bantin & Kingman, Grimston, Hull UK) were used, with food and water available *ad libitum*. All animal procedures used were in strict accordance with the Home Office guidelines, and specifically licensed under the Animals (Scientific Procedures) Act 1986. Anaesthesia was induced and maintained during surgery with halothane (2.5% and 1.5–2.0%, respectively) in O_2 : N_2O (1 : 1), with the animal breathing spontaneously. Concentric microdialysis probes incorporating a recording electrode (i.e. microdialysis electrode ME-H2, Applied Neuroscience Ltd., London, U.K.) were prepared as previously described (Obrenovitch *et al.*, 1993; 1994) and implanted in the fronto-parietal cortex (coordinates: 1.3–1.5 mm anterior to bregma, 2 mm lateral, and 2 mm deep from the dural surface). Unless otherwise stated, microdialysis probes were perfused with artificial cerebrospinal fluid (ACSF) (composition in mmol l^{-1}: NaCl 125, KCl 2.5, $MgCl_2$ 1.18, $CaCl_2$ 1.26, NaH_2PO_4 0.2; pH 7.3 adjusted with 1 M NaOH) at 1 μl min^{-1} with a syringe pump (CMA/100; CMA/Microdialysis, Stockholm, Sweden). The microdialysis probe was used to trigger repetitive SD, by switching from normal ACSF to a medium containing 130 mM K^+ (see below). Its electrode was used for the recording of SD initiation. A conventional glass capillary electrode (10–20 μm tip), inserted around 3 mm posterior to the microdialysis probe and 1 mm deep into the cortex, was used to record propagating SD.

A femoral artery was catheterized for continuous monitoring of arterial blood pressure, and a vein for drug administration and induction of cardiac arrest. To minimize any possible interference of halothane anaesthesia with the processes under study (Piper *et al.*, 1991; Verhaegen *et al.*, 1992; Saito *et al.*, 1993), once the surgical procedure had been completed, the depth of anaesthesia was carefully controlled by monitoring electroencephalogram (EEG) and arterial blood pressure, and the concentration of halothane in the breathing mixture kept to a minimum (0.8 to 1.2%). Body temperature was maintained at 37°C throughout the experiment. The halothane concentration was not altered after administration of the NMDA-receptor antagonist.

Recording of extracellular direct current (d.c.) potential and EEG

The d.c. potential and EEG were derived from the potential between the electrode built into the probe and a Ag/AgCl reference electrode placed under the scalp (Obrenovitch *et al.*, 1994), and that between the glass capillary electrode and the same reference. These two potentials were first amplified (×10) with a multichannel, high-impedance input pre-amplifier (NL834, Neurolog System, Digitimer Ltd., Welwyn Garden City, U.K.). With each channel, the alternating current component in the 1–30 Hz window, amplified 6,000–8,000 times, provided EEG, and the d.c. component, the d.c. potential. A dedicated application programme allowed all parameters to be continuously acquired, displayed and stored (Obrenovitch *et al.*, 1989). The d.c. potentials were converted to absolute values (mV) from prior calibration.

Experimental procedure

Experiments were performed at least 2 h after electrode implantation. Five episodes of repetitive SD were produced by switching the perfusion medium from normal ACSF to a solution containing 130 mmol l^{-1} K^+ and 2.5 mmol l^{-1} Na^+ for 20 min, using a liquid switch (CMA/110, CMA/Microdialysis). Preliminary experiments showed that such a high concentration of K^+ is required to evoke consistently periodic SD under our experimental conditions. This agrees with previous work from other laboratories (Szerb, 1991; Herreras & Somjen, 1993; Saito *et al.*, 1993). Each K^+ stimulus was followed by 40 min of recovery, i.e. perfusion with normal ACSF. The drug or vehicle was injected i.v. 10 min into the recovery period of the 2nd K^+ stimulus. Four separate groups of 6 animals were studied: (i) Controls, which received the vehicle for L-701,324 (10% PEG 300); (ii) 5 mg kg^{-1} of L-701,324; (iii) 10 mg kg^{-1} of L-701,324; and (iv) 1 mg kg^{-1} of MK-801.

Drugs

L-701,324 was a generous gift from Dr R.G. Hill (Merck Sharp & Dohme, Harlow, U.K.). Dizocilpine ((+)-MK-801 hydrogen maleate) was purchased from Research Biochemicals Inc. (Natick, MA, U.S.A.). Polyethylene glycol (PEG 300) was supplied by Sigma Chemicals (Pool, U.K.). L-701,324 was dissolved in 10% PEG 300 with pH adjusted to 10 with 1 M NaOH. MK-801 was dissolved in saline. All drug solutions were prepared on the day of experiment.

Data presentation and analysis

In order to conform to previous papers, in Figures 1, 2, 3 and 5 (i.e. representative recordings of SD initiation and propagation), and in Figure 6, the polarity of the d.c. potential was reversed so that depolarization produces an upward deflection. To facilitate comparison of the data, the d.c. potential sequences presented in Figures 2, 3 and 5 were aligned by setting the 3 min period preceding changes in perfusion medium to 0 mV. As inhibition of the elicitation of recurrent K^+-induced SD is characterized by a reduction of both the number and magnitude of SD, for each 20 min 130 mmol l^{-1} K^+-stimulus, SD elicitation and propagation were quantified by calculating the cumulative area (mV min) of the corresponding peaks, as shown in Figure 1. All values in Results are mean±s.e.mean. Statistical analysis was by Student's t test (paired or unpaired), or overall significance determined by analysis of variance or covariance.

Results

Effect of L-701,324 on mean arterial pressure (MAP)

Intravenous administration of 5 mg kg^{-1} of L-701,324 produced a rapid, but transient and moderate reduction of the MAP (from 82.4±3.2 to 70.5±2.5 mmHg; $n=5$, $P<0.001$ by Student's paired t test). Return to control levels was biphasic, with around 50% of recovery occurring within 2–3 min, followed by gradual normalization within 1 h (81.2±4.2 mmHg). Doubling the dose exacerbated this effect (i.e. rapid drop from 82.2±1.9 to 59.4±3.7 mmHg; $n=5$, $P<0.001$) but return to

normal still occurred within 1 h (82.6 ± 1.9 mmHg). The drug vehicle (10% PEG 300, pH 10) had no effect on the MAP.

K^+-evoked SD initiation and propagation in controls

Application of 130 mmol l^{-1} K^+ for 20 min through the cortical microdialysis electrode consistently produced a sustained negative shift of the d.c. potential, onto which were superimposed between 5 to 8 peaks of further depolarization (Figure 1). Each of these peaks corresponded to the initiation of a wave of SD (Obrenovitch *et al.*, 1993). The magnitude of SD elicitation, expressed as the cumulative peak area, decreased progressively with repeated K^+ applications ($P<0.001$; analysis of variance within the control group) especially with the first 3 stimuli (Figure 4) ($P<0.02$; comparison of the 3rd stimulus to the 1st in the control group by Student's paired t test).

Not all elicited SDs propagated up to the glass capillary electrode located 3 mm posteriorily (Figures 1 and 2): Only the first SD consistently and fully propagated, and with most K^+ stimuli in control experiments, another wave triggered 10 to 15 min into the K^+ challenge. The delay between triggering of the first SD and the recording of its propagation with the capillary electrode was 50.2 ± 2.3 s ($n=18$), i.e. a rate of propagation of around 3.5 mm min^{-1}.

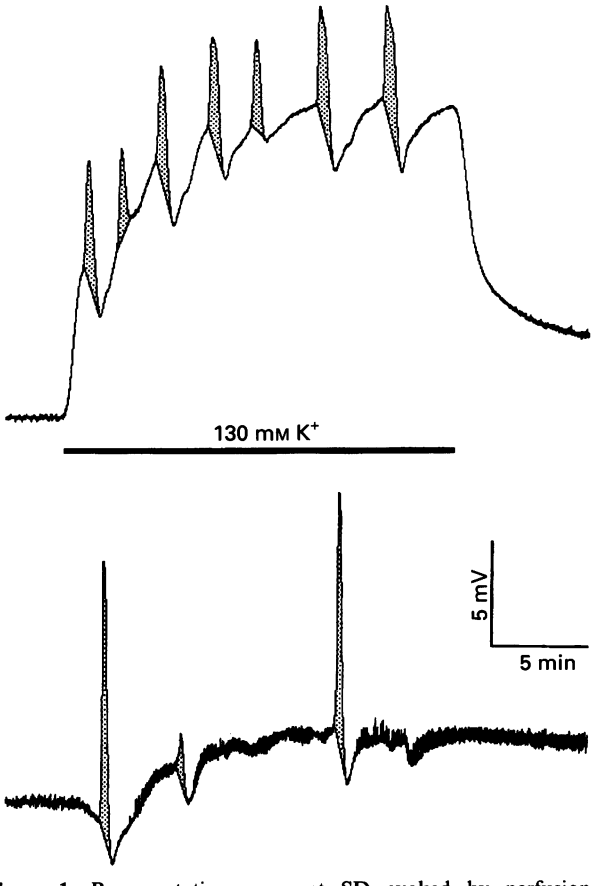

Figure 1 Representative recurrent SD evoked by perfusion of 130 mmol l^{-1} K^+ for 20 min at 1.0 μl min^{-1} through a microdialysis electrode implanted in the rat fronto-parietal cortex (top trace), and corresponding propagating waves recorded around 3 mm posteriorly (bottom trace). In the top trace, the sustained negative shift of the d.c. potential corresponds to persistent local depolarization produced by the applied K^+, whereas each further transient depolarization indicates the elicitation of an SD wave. Note in the bottom trace that not all elicited SDs propagated to the posterior recording electrode. Horizontal bar: 20 min K^+-stimulus. Dotted areas illustrate how the magnitude of SD elicitation and propagation was quantified by integration, for each 20 min K^+ stimulus. In this case, values for the cumulative areas of SD initiation and propagation were 31.6 and 13.9 mV min, respectively.

Effects of L-701,324 (5 and 10 mg kg^{-1}) on SD initiation and propagation

Potassium-elicitation of SD was moderately inhibited by 10 mg kg^{-1} (but not by 5 mg kg^{-1}) of L-701,324 (Figure 3;

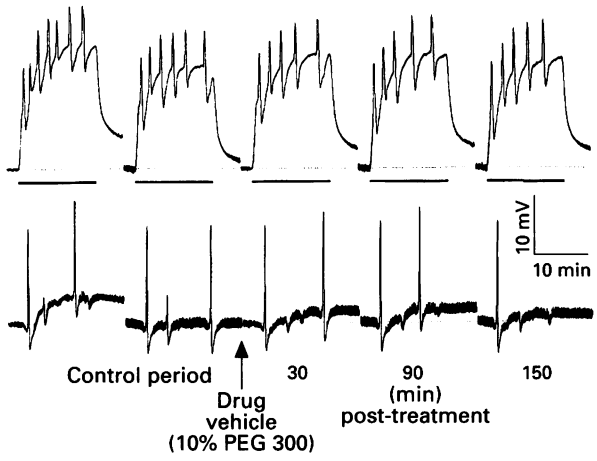

Figure 2 Initiation and propagation of recurrent SDs, evoked by 5 separate 20 min perfusions of 130 mM K^+ through the microdialysis electrode (solid horizontal bars). Each stimulus was followed by 40 min of recovery (largely truncated in this figure). This experiment is typical of the control group which only received the vehicle of L-701,324 ($n=6$).

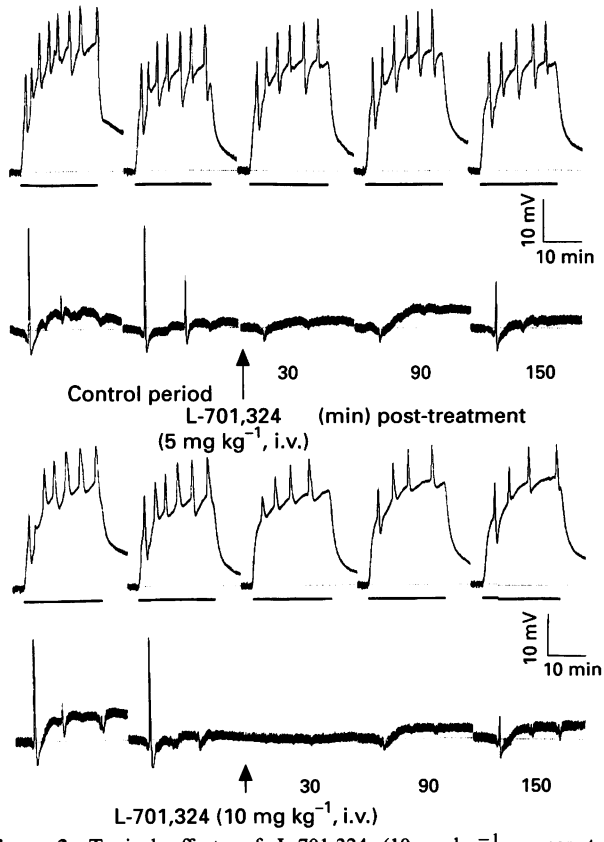

Figure 3 Typical effects of L-701,324 (10 mg kg^{-1}, upper two traces; 10 mg kg^{-1}, lower traces) on the initiation and propagation of K^+-induced SD. Note that only the higher dose reduced moderately SD elicitation, whereas both doses markedly inhibited SD propagation.

and Figure 4a). Thirty minutes after administration of 10 mg kg^{-1} L-701,324, the cumulative area of SD peaks elicited during 20 min was 15.3 ± 2.1 mV min, versus 23.2 ± 1.1 mV min in animals who had received only the drug vehicle ($P < 0.02$, Student's t test; $n = 6$), and this inhibition persisted throughout the recording period (i.e. at least 150 min post-treatment) (Figure 3; and Figure 4a). The delay between application of 130 mM K^+ and occurrence of the first SD was also significantly increased. It was 110 ± 4 s 30 min post-'treatment' in controls, 127 ± 9 s with 5 mg kg^{-1} L-701,324, and 202 ± 41 s with 10 mg kg^{-1} ($P < 0.02$; comparison of L-701,324 groups with control by Student's t test).

The propagation of SD was more sensitive to L-701,324 than its elicitation, since both 5 and 10 mg kg^{-1} produced a marked inhibition (Figure 3; and Figure 4b). With both doses, SD propagation was completely abolished 30 min after drug injection, but the inhibition did not appear as marked 90 and 150 min post-treatment.

At the doses tested, L-701,324 did not produce any obvious changes in EEG amplitude (data not shown).

Comparison with the blockade of SD by MK-801 (1 mg kg^{-1})

Even at the low dose of 1 mg kg^{-1}, MK-801 virtually abolished SD initiation (Figure 5) and completely blocked its propagation (data not shown), thus indicating that this NMDA channel blocker is much more potent against SD than L-701,324.

Effect of NMDA-receptor blockade on the d.c. potential

Our experimental design also allowed us to examine the effect of L-701,324 and MK-801 on the d.c. potential, 10 min into the recovery period following the 2nd K^+ challenge. L-701,324 produced a dose-dependent positive shift of the d.c. potential (Figure 6). The amplitude of this shift was already maximal 10 min after drug injection, with either L-701,324 or MK-801, and the effect of 10 mg kg^{-1} L-701,324 was very similar to that produced by 1 mg kg^{-1} MK-801.

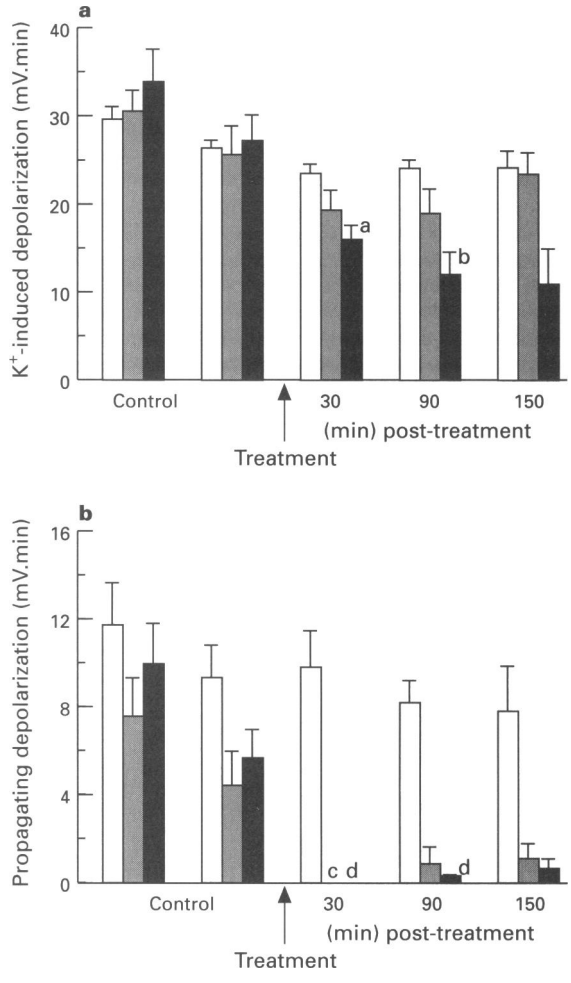

Figure 4 Comparison of the magnitude of SD elicitation (a) and propagation (b) between L-701,324 treated rats (5 and 10 mg kg^{-1}; stippled and solid columns, respectively) and control (open columns). Columns represent mean ± s.e.mean, with $n = 6$ throughout, except at time 150 min after 5 mg kg^{-1} L-701,324 (two missing values). $^aP < 0.02$, $^bP < 0.005$; comparison to control group by Student's unpaired t test; $^cP < 0.05$, $^dP < 0.01$; comparison to pretreatment values by paired t test.

Discussion

Methodological considerations

Simultaneous investigation of SD initiation and propagation in the living brain with microdialysis electrodes is a powerful

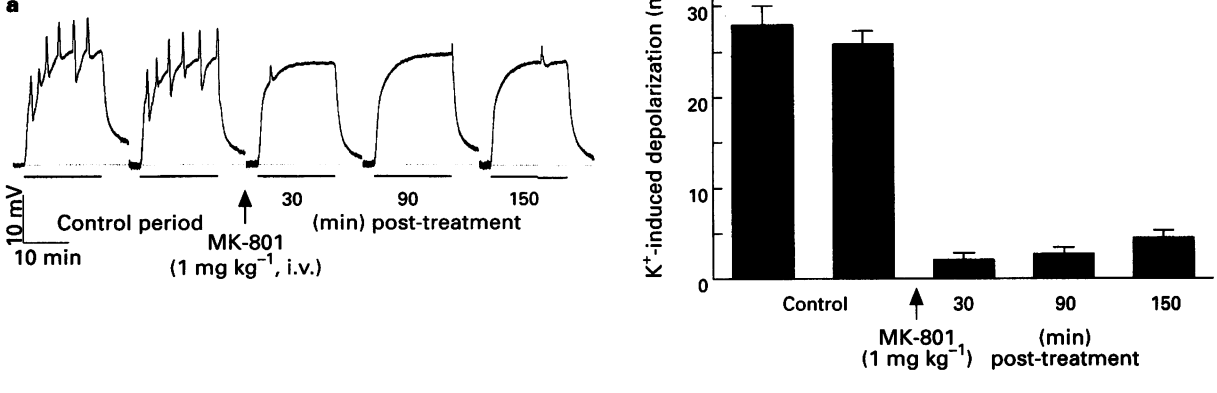

Figure 5 Effects of MK-801 (1 mg kg^{-1}) on the initiation of K^+-induced SD. Panel (a) shows a typical, strong inhibition of SD by MK-801. Columns in (b) are mean ± s.e.mean ($n = 6$); $P < 0.001$ for all post-treatment values (comparison to SD initiation before treatment by Student's paired t test).

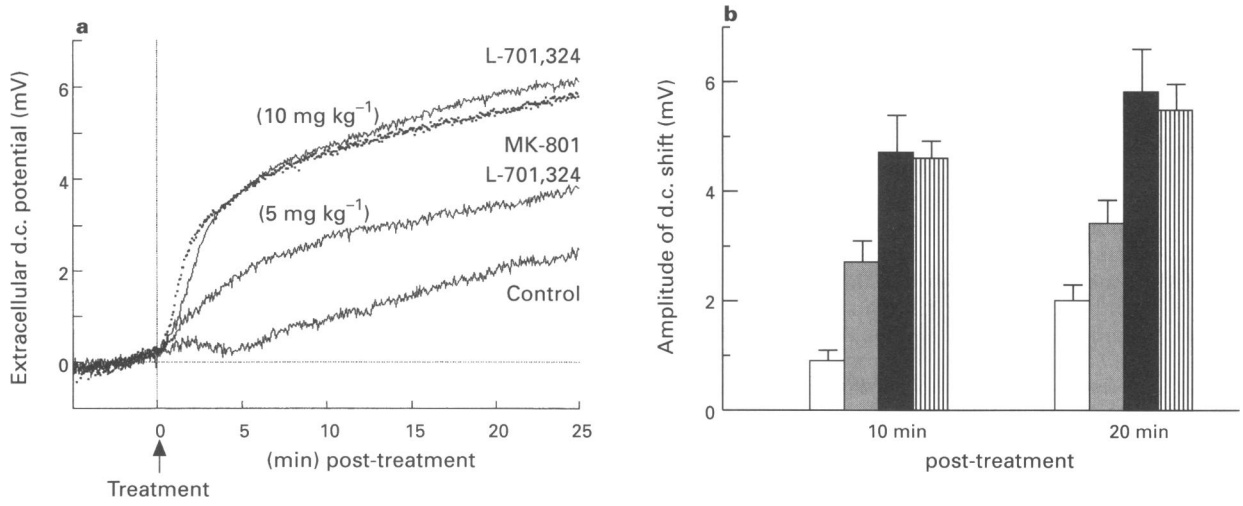

Figure 6 Effect of NMDA-receptor block by L-701,324 (5 and 10 mg kg^{-1}) and MK-801 (1 mg kg^{-1}) on the d.c. potential. Each trace in (a) is the average of 6 experiments (positive d.c. shift are upwards in this figure). The small, underlying rise in the d.c. potentials (see control trace) reflects recovery from the previous K^+ challenge. Columns in (b) are mean ± s.e.mean ($n=6$): Open columns are controls; stippled and solid columns, 5 and 10 mg kg^{-1} L-701,324, respectively; and hatched columns, MK-801.

approach, but the cost of the methodology precluded us from determining the full dose-response curves for L-701,324 and MK-801. Five and 10 mg kg^{-1} L,701,324 were selected from a separate study which demonstrated that these doses inhibited NMDA activation (Obrenovitch *et al.*, 1996). As MK-801 was known to block potently SD evoked by either high K^+ (Lauritzen & Hansen, 1992) or focal ischaemia (Iijima *et al.*, 1992), our strategy was to use a single dose (1 mg kg^{-1}) of this drug to compare, in our model, the effects of L-701,324 with those of a reference compound. Our data on SD initiation and elicitation suggest that the effects observed were at the low end of the L-701,324 dose-response curve, and at the top end of the MK-801 curve.

Halothane reduced the susceptibility of the cat cortex to SD evoked by K^+ or local injury (Piper *et al.*, 1991; Saito *et al.*, 1993), but this effect was less pronounced in rats. Halothane and isoflurane had no dose-related effects on the electrical threshold for triggering cortical SD in rats, and high doses of these agents reduced only slightly the rate of SD propagation (Verhaegen *et al.*, 1992). In contrast, in rats, isoflurane increased the threshold at which NMDA induced SD as compared to pentobarbitone or N_2O-fentanyl anaesthesia (Patel *et al.*, 1995), and volatile anaesthetics reduced both glutamate-stimulated [^{3}H]-MK-801 binding to rat cortex membranes (Martin *et al.*, 1991; 1995) and responses induced by glutamate receptor agonists in cortical slices (Puil & El-Beheiry, 1990; Carlà & Moroni, 1992). On the basis of these findings, the depth of anaesthesia was carefully controlled and reduced to a minimum after surgical preparation, in order to minimize any possible interference of halothane in this study. Residual effects of halothane cannot be ruled out however, and they may have contributed to the fact that only a small proportion of the elicited SD propagated to the remote recording site (Figures 1 and 2).

In the control group, repeated perfusion of 130 mM K^+ through the microdialysis probe for 20 min consistently triggered SD, although SD elicitation decreased slightly with the first 3 challenges (Figures 2 and 4). Verhaegen and coworkers (1992) observed a similar phenomenon with electrically evoked SD, i.e. the triggering threshold increased with the second wave of SD as compared to the first. We concur with these authors in proposing that the reduced sensitivity to SD elicitation with repeated challenges is due to persistent effects of the first SD waves, possibly tissue acidosis (Mutch & Hansen, 1984; Taylor *et al.*, 1994) which inhibits SD (Gardner-Medwin, 1981; Marrannes *et al.*, 1985), and to which long-lasting reduction of cerebral blood flow after SD may contribute (Lauritzen, 1984; Duckrow, 1991). For this reason, two control K^+-stimuli preceded drug administration in this study.

Effect of L-701,324 on the mean arterial blood pressure (MAP)

Intravenous administration of L-701,324 reduced the MAP, but this action was short-lasting and, therefore, unlikely to have exacerbated the persistant hypoperfusion (Lauritzen, 1984) and tissue acidosis which follow SD (Mutch & Hansen, 1984; Taylor *et al.*, 1994). Consequently, this effect on MAP is unlikely to have contributed to the blockade of SD by the drug. This is supported by the fact that the inhibition of SD by L-701,324 persisted long after MAP had returned to normal (within <1 h) (Figures 3 and 4). ACEA-1021 (6,7-dichloro-5-nitro-1,4-dihydro-2,3-quinoxaline-dione), another potent antagonist at the glycine site of the NMDA receptor, was also reported to provoke hypotensive responses when administered i.v. (Martin *et al.*, 1994).

Effects of L-701,324 on SD initiation and propagation

At 5 and 10 mg kg^{-1}, L-701,324 virtually abolished the propagation of SD and the drug was effective within 30 min after administration and for up to 2.5 h (Figures 3 and 4). These doses are around 5–10 fold the ED_{50} for inhibition of audiogenic seizures in the DBA/2 mouse (Saywell *et al.*, 1995), a model extremely sensitive to NMDA-receptor blockade (Croucher *et al.*, 1982). In contrast, 10 mg kg^{-1} L-701,324 inhibited K^+ elicitation by around 50% only. This apparant 'differential' effect on propagation and elicitation of SD is not specific to L-701,324, as it was also observed with ketamine (Hernández-Cáceres *et al.*, 1987) and ACEA-1021 (Martin *et al.*, 1994). Elicitation of K^+-evoked SD is known to be less sensitive to pharmacological blockade than its propagation, and criteria for SD inhibition rank in the order: reduced velocity of SD propagation, blockade of SD propagation, and inhibition of SD elicitation (Hernández-Cáceres *et al.*, 1987).

It is interesting to note that SD inhibition by L-701,324 was not associated with major changes in the EEG amplitude, because this contrasts with the action of ACEA-1021. Increasing doses of this other glycine/NMDA receptor antagonist within the range 20–80 mg kg^{-1} markedly reduced EEG activity (Martin *et al.*, 1994), suggesting induction of deep anaesthesia (McFarlane *et al.*, 1995). Despite this suppression

of the EEG, even the maximal dose of ACEA-1021, around 4 fold greater than that required to reduce infarct volume in focal cerebral ischaemia (Warner *et al.*, 1995), only slowed SD propagation but did not block it (Martin *et al.*, 1994). Therefore, ACEA-1021 appears to have little inhibitory action against SD in comparison to its protective effects against ischaemia. This feature may result from the fact that a small degree of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor antagonism may be associated with ACEA-1021 (Woodward *et al.*, 1995), or from its reduction of electrical activity (Obrenovitch, 1995).

Comparison with the blockade of SD by MK-801

MK-801 (1 mg kg^{-1}) virtually abolished SD initiation (Figure 5) and completely blocked its propagation, confirming that this drug is an extremely potent inhibitor of SD (Marrannes *et al.*, 1988; Lauritzen & Hansen, 1992), and implying that it is at least 10 fold more effective than L-701,324 against SD. One possible explanation for the lower potency of L-701,324 in comparison to MK-801 may be its strong binding to plasma proteins (>99.9%) (Kari & Vyas, 1994), which allows only low levels of the drug to reach the brain. However, the effects of L-701,324 and MK-801 on the d.c. potential do not support this hypothesis. L-701,324 provoked a positive shift of the d.c. potential immediately after drug administration (Figure 6), indicating rapid penetration into the brain. Furthermore, the effect of 10 mg kg^{-1} L-701,324 on the d.c. potential, suggesting hyperpolarization of the cell population surrounding the recording electrode, was very similar to that produced by 1 mg kg^{-1} MK-801 (Figure 6).

The exclusive sensitivity of SD to MK-801 may rather be due to the fact that this drug is most effective when the receptor is activated (i.e. 'open channel block'). Exogenous glutamate agonists enhanced the binding of [^{3}H]-MK-801 to well-washed membranes by up to 700% (Foster & Wong, 1987) suggesting that MK-801 bound preferentially to the activated form of the NMDA receptor. Functional studies also showed that the blocking effect of MK-801 itself was markedly agonist-dependent (Wong *et al.*, 1986; Huettner & Bean, 1988). NMDA-receptors are likely to be activated with SD, because this event combines glutamate release with postsynaptic depolarization (Reid *et al.*, 1988; Obrenovitch & Zilkha, 1995; Obrenovitch *et al.*, 1995).

In conclusion, these data demonstrate that L-701,324 has an inhibitory effect on both SD initiation and propagation. This action may be beneficial in focal ischaemia, and possibly against migraine, especially as this drug has a number of encouraging features: high oral bioavailability (Saywell *et al.*, 1995), rapid onset of action, and absence of marked EEG abnormalities at doses effective against SD.

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