Br. J. Pharmacol. (1991), **103**, 2030–2036

The neuroprotective action of dizocilpine (MK-801) in the rat middle cerebral artery occlusion model of focal ischaemia

[1]R. Gill, C. Brazell, G.N. Woodruff & [2]J.A. Kemp

Merck, Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex

1 An acute model of focal ischaemia, which involves permanent occlusion of the middle cerebral artery of the rat with 4 h survival, was used to find the minimum effective plasma concentration of dizocilpine (MK-801) and to determine its dose-effect relationship.

2 MK-801 was administered at the time of occlusion and was given as an i.v. bolus followed by an infusion for 4 h to maintain a steady state plasma concentration of the drug throughout the study. MK-801 was given at 3 dose levels; 0.04 mg kg^{-1} i.v. bolus + 0.6 μg kg^{-1} min^{-1} infusion; 0.12 mg kg^{-1} i.v. bolus + 1.8 μg kg^{-1} min^{-1} infusion; 0.4 mg kg^{-1} i.v. bolus + 6 μg kg^{-1} min^{-1} infusion, which gave mean plasma levels over the 4 h of 8.0 ng ml^{-1}, 18.9 ng ml^{-1} and 113.2 ng ml^{-1} respectively.

3 MK-801 at 8.0 ng ml^{-1} gave 10% reduction in the volume of ischaemic brain damage in the cerebral cortex which just reached significance. The middle dose of MK-801 (18.9 ng ml^{-1}) gave a highly significant reduction in the volume of ischaemic brain damage in the cerebral cortex and hemisphere, volumes of ischaemic tissue being reduced by 60% and 50% compared to saline-treated animals, respectively. The highest plasma concentration of MK-801 (113.2 ng ml^{-1}) resulted in a 35% reduction in the volume of hemispheric damage and a 40% reduction in the volume of cortical damage, which were significant.

4 The reduction in the amount of protection afforded by the highest dose of MK-801 may be due to the hypotensive effect of this dose. There was no protection against the volume of damage in the caudate nucleus for any of the doses of MK-801 tested.

5 Therefore the minimum effective plasma concentration of MK-801 was 8.0 ng ml^{-1}, although the greatest protection was seen with a plasma level of 18.9 ng ml^{-1}. This correlates well with the concentration of MK-801 required to block N-methyl-D-aspartate (NMDA) receptors and prevent NMDA receptor mediated neurotoxicity *in vitro*.

Keywords: Excitatory amino acids; glutamate; cerebrovascular disease; neurotoxicity; MK-801; NMDA antagonists; ischaemia; neuroprotection; focal ischaemia

Introduction

The neuronal degeneration which results from a period of cerebral ischaemia is thought to be due, at least in part, to an overexcitation at synapses using L-glutamate or L-aspartate as their neurotransmitter (Rothman & Olney, 1986; Choi, 1988). The initial evidence for this came mainly from transient forebrain ischaemia studies in which a massive increase in the extracellular concentrations of glutamate and aspartate was seen following ischaemia (Benveniste *et al.*, 1984; Hagberg *et al.*, 1985), and lesions of glutamatergic pathways projecting to the hippocampus ameliorated neuronal damage to this region (Wieloch *et al.*, 1985; Benveniste *et al.*, 1989).

The N-methyl-D-aspartate (NMDA) subtype of excitatory amino acid receptors appears to be the key receptor involved in ischaemia-induced neuronal degeneration for a number of reasons. Firstly, an important property of the NMDA receptor is that its associated ion channel is highly permeable to Ca^{2+} (MacDermott *et al.*, 1986). Thus, under ischaemic conditions, NMDA receptor activation, due to the excessive glutamate release, results in large increases in intracellular [Ca^{2+}], which are thought to be involved in pathways resulting in cell death (Siesjö, 1981). These increases in intracellular [Ca^{2+}] are mirrored by a large decrease in extracellular Ca^{2+} levels during the ischaemic episode (Benveniste *et al.*, 1988; Andiné *et al.*, 1988). Secondly, the neurones which are selectively vulnerable to brief periods of ischaemia (Pulsinelli & Brierley, 1979; Kirino, 1982) possess the highest density of NMDA receptors in the brain (Monaghan & Cotman, 1985). Finally, studies with selective NMDA antagonists (Simon *et al.*, 1984; Gill *et al.*, 1987; 1988; Boast *et al.*, 1988) have shown that these are neuroprotective in animal models of transient forebrain ischaemia, although there have been some negative results reported as well (Block & Pullsinelli, 1987; Jensen & Auer, 1988; Wieloch *et al.*, 1989).

MK-801 is one of the most potent and selective NMDA antagonists available (Wong *et al.*, 1986). We have previously demonstrated it to be neuroprotective in a gerbil model of transient forebrain ischaemia when given prior to or up to 24 h post-ischaemia. The neuronal damage seen in the gerbil is of the delayed type. That is, neuronal damage does not occur immediately but occurs over a 24–48 h period (Kirino, 1982; Gill *et al.*, 1987). This phenomenon is also seen in human post mortem tissue from patients who have suffered a cardiac arrest (Petito *et al.*, 1987).

Following this work in transient forebrain ischaemia studies, NMDA antagonists have subsequently been shown to be potent neuroprotective agents in animal models of focal cerebral ischaemia (Duverger *et al.*, 1987; Ozyurt *et al.*, 1988; Park *et al.*, 1988a,b; Bielenburg, 1989) which are more akin to stroke in man. The protective effects of NMDA antagonists in these models appear to be more reproducible than in transient forebrain ischaemia models. MK-801, as a single dose, was found to be neuroprotective against cortical infarction when given prior to or post-ischaemia following permanent middle cerebral artery (MCA) occlusion in the rat or cat (Ozyurt *et al.*, 1988; Park *et al.*, 1988a,b). The aim of the present series of experiments was to determine the minimum effective plasma concentration of MK-801 in a focal ischaemia model in the rat and to investigate its dose-effect relationship. Therefore, we used an infusion regimen to maintain a steady state plasma level of MK-801 throughout the ischaemic period. For these experiments we used the acute rat MCA occlusion model of Tamura *et al.* (1981) because it enabled continuous monitor-

[1] Present address: Department of Veterinary Basic Sciences, Royal Veterinary College, Royal College Street, London NW1 0TU.
[2] Author for correspondence.

ing of all physiological parameters, and because it allowed us to monitor closely the plasma levels of MK-801 throughout the study.

Methods

Male Sprague Dawley rats weighing 300–350 g were used for these studies; the animals were maintained on a 12 h light:dark cycle and fed *ad libitum* prior to experimentation.

The method used for permanent MCA occlusion was essentially as described by Tamura *et al.* (1981) and Shigeno *et al.* (1985). Briefly rats were anaesthetized with a mixture of 4% isoflurane, 30% oxygen and 70% nitrous oxide, a trachaeostomy was performed and the animals ventilated with a mixture of 2–3% isoflurane, 30% oxygen and 70% nitrous oxide.

The left femoral artery and vein were cannulated to enable continuous blood pressure recordings, blood sampling, injections and infusions. All animals then underwent a subtemporal craniectomy and exposure of the left MCA (Tamura *et al.*, 1981), the zygomatic arch was left intact. The stem of the artery and its lenticulostriate branches were occluded with microbipolar coagulation. Ventilation was subsequently maintained with a mixture of 1% isoflurane, 30% oxygen and 70% nitrous oxide. The blood gases were monitored prior to MCA occlusion then every hour following the occlusion for the duration of the experiment. They were maintained at $Paco_2$ of >30 mmHg and Pao_2 of >100 mmHg, the animals were also kept normothermic at 37 ± 0.5°C, throughout the experiment, by use of a rectal probe and heating blanket (Harvard apparatus, U.K.). The blood pressure was continuously monitored during the experiment; it was maintained at 80 mmHg for the surgical procedures and following MCA occlusion it was allowed to return to normal.

The animals were maintained under these conditions for 4 h after which they were perfusion fixed with 40% formaldehyde, glacial acetic acid and methanol (FAM; 1:1:8, v/v/v). The brains were processed and embedded in paraffin wax, 8 μm sections were stained with haematoxylin-eosin or with a combination of cresyl violet and luxol fast blue. The sections were examined by light microscopy and areas of early infarction were delineated at 8 preselected coronal levels from anterior 10.5 mm to anterior 1.0 mm (Konig & Klippel, 1963). This was done blind to the drug treatment of the animals. The areas of brain damage were drawn on scale diagrams ($\times 4$ actual size) of forebrain and measured in terms of hemisphere, cortex and caudate on an image analyzer (Quantimet 760, Cambridge Instruments). The areas of ischaemic damage were used to determine the total volume of ischaemic tissue in each brain (Osborne *et al.*, 1987), which was done by integration of areas with the distance between each level. The end points for integration for the cortex and hemisphere were anterior 12.5 and posterior 0.05. The amount of ischaemic damage was expressed in absolute terms (mm^3) for hemisphere, cortex and caudate.

MK-801 was tested at 3 doses, 0.04 mg kg^{-1} as an i.v. bolus + 0.6 μg kg^{-1} min^{-1} infusion; 0.12 mg kg^{-1} i.v. bolus + 1.8 μg kg^{-1} min^{-1} infusion and 0.4 mg kg^{-1} i.v. bolus + 6 μg kg^{-1} min^{-1} infusion. Initial dosing was started immediately following MCA occlusion, the bolus dose was administered over a 30 s period and the infusion was continued for the duration of the experiment (4 h). Plasma samples (100 μl) were taken prior to MCA occlusion and then every hour following occlusion. The plasma samples were assayed in the MK-801 radioimmunoassay described below. The control groups of animals received 1 ml kg^{-1} i.v. bolus of saline followed by an infusion of saline at a rate of 0.0298 ml min^{-1} for 4 h. There were 10–12 animals in each of the groups.

MK-801 was measured in plasma samples by radioimmunoassay (RIA) (Hichens *et al.*, 1991). Briefly, antisera were raised to N-glutaryl-MK-801 coupled to bovine serum albumin, and the radioligand was an iodotyramine conjugate of the same derivative. Specificity was for the N-acyl derivatives and the drug was extracted from plasma and N-acetylated prior to using a double antibody RIA procedure. Metabolites were eliminated by the extraction method. Recovery (51 ± 3.8%) was independent of concentration. Inter- and intra-assay coefficients of variation were 12% and 3.8%, respectively.

Statistical analysis

Differences between the volume of ischaemic damage in the control and MK-801-treated animals were tested by analysis of variance (ANOVA) with a Bonferroni correction; this was done for each of the brain regions (hemisphere, cortex and caudate). The curves for the area of ischaemic damage in the cortex were analysed by BMDP 2v (BMDP Statistical Software, Ireland), which is an analysis of variance and covariance test with repeated measures. The area of ischaemic damage at each coronal plane was compared between control and MK-801-treated animals by the BMDP 2v analysis of variance test with repeated measures. All data are presented as the mean $\pm$ s.e.mean for n animals.

Results

The lowest dose of MK-801 (0.04 mg kg^{-1} i.v. bolus + 0.6 μg kg^{-1} min^{-1}) gave a plasma concentration in the range of 8–12 ng ml^{-1} with a mean plasma concentration of 8.0 ng ml^{-1} over the 4 h period (Figure 1*a*). MK-801 at a dose of 0.12 mg kg^{-1} i.v. bolus + 1.8 μg kg^{-1} min^{-1} as an infusion gave a range of 10–52 ng ml^{-1} with a mean plasma concentration of 18.9 ng ml^{-1} over the 4 h period (Figure 1b). The highest dose of MK-801 of 0.4 mg kg^{-1} i.v. bolus + 6 μg kg^{-1} min^{-1} gave plasma levels in the range of 100–140 ng ml^{-1} with a mean of 113.2 ng ml^{-1} over the 4 h period (Figure 1c). The early peak plasma concentration of MK-801 was not monitored in this study, however, a study using the same dosing regime as this (Willis *et al.*, 1991) has shown that the initial peak levels of MK-801 in both plasma and CSF which occur following the bolus dose reach equilibrium within 10 min. Therefore with these dosing regimes a steady state plasma level of MK-801 was achieved during the 4 h period of ischaemia.

There were no significant differences observed for any of the MK-801-treated groups and their saline-treated controls with respect to respiratory blood gas levels or blood glucose levels (Table 1). The rectal temperature of all animals was maintained at 37 ± 0.5°C throughout the experimental period using a thermostatically controlled blanket. Ischaemic damage was observed only within the territory of the occluded MCA, that is, in the dorsolateral cortex and in the neostriatum. These areas showed the morphological characteristics of early ischaemic cell change with microvaculation, shrinkage and triangulation of the nucleus and cytoplasm. The nucleus also became very darkly stained and pyknotic. The histological appearance of ischaemic brain tissue in rats treated with MK-801 was similar to that in the saline-treated group in areas where ischaemic damage was present but in areas showing protection the cells appeared normal.

The lowest dosing regime of MK-801, which resulted in a mean plasma concentration of 8.0 ng ml^{-1}, gave only just significant reduction of the volume of ischaemic brain damage in the cerebral cortex (ANOVA; $F = 4.51$, $P < 0.05$), the volume of ischaemic tissue was reduced by 10% compared with saline treated controls (Figure 2a), but not in the cerebral hemisphere (ANOVA; $F = 4.13$, $P > 0.05$) or caudate nucleus (ANOVA; $F = 0.06$; $P > 0.8$; Figure 2a). The middle dose of MK-801 resulted in a mean plasma concentration of 18.9 ng ml^{-1} over the 4 h period, this gave a highly significant reduction in the volume of ischaemic brain damage in the cerebral cortex (ANOVA; $F = 111.63$, $P < 0.0001$) and hemisphere (ANOVA; $F = 115.58$, $P < 0.0001$) volumes of isch-

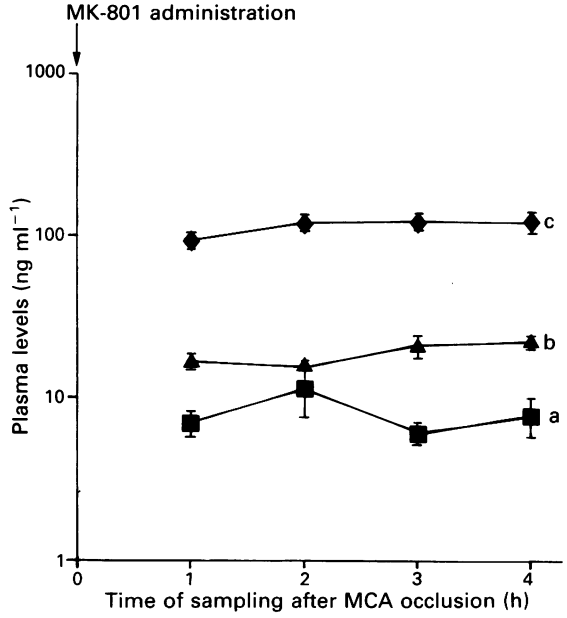

Figure 1 Plasma levels of MK-801 at different times of sampling following middle cerebral artery (MCA) occlusion. The different dosing regimes of MK-801 were all initiated immediately after MCA occlusion. (a) Plasma levels of MK-801 following a dose of $0.04\,mg\,kg^{-1}$ i.v. bolus + $0.6\,\mu g\,kg^{-1}\,min^{-1}$ infusion for 4 h. The mean plasma concentration over this period was $8.0\,ng\,ml^{-1}$. (b) The plasma concentration of MK-801 achieved by a dose of $0.12\,mg\,kg^{-1}$ i.v. bolus + $1.8\,\mu g\,kg^{-1}\,min^{-1}$ infusion for 4 h. The mean plasma concentration was $18.9\,ng\,ml^{-1}$ over the 4 h period. (c) Plasma levels of MK-801 following a dose of $0.4\,mg\,kg^{-1}$ i.v. bolus + $6\,\mu g\,kg^{-1}\,min^{-1}$ infusion for 4 h. The mean plasma level was $113.2\,ng\,ml^{-1}$. Each point on the curves represents the mean of 9–10 animals, vertical bars show s.e.mean.

aemic tissue were reduced by 60% and 50% compared to saline-treated animals, respectively (Figure 2b). There was no significant reduction in the volume of damage in the caudate nucleus (ANOVA; $F = 0.82$, $P > 0.4$) of the MK-801-treated group (Figure 2b). The highest plasma concentration of $113.2\,ng\,ml^{-1}$ was achieved with a dose of $0.4\,mg\,kg^{-1}$ i.v. bolus + $6\,\mu g\,kg^{-1}\,min^{-1}$ infusion, this resulted in a significant reduction of the volume of ischaemic brain damage in the cerebral cortex (ANOVA; $F = 8.26$, $P < 0.01$) and hemisphere (ANOVA; $F = 8.78$, $P < 0.01$); the volumes of ischaemic tissue were reduced by 40% and 35% compared with controls, respectively (Figure 2c). There was no change in the volume of caudate damage (ANOVA; $F = 1.19$, $P > 0.3$) between the control and MK-801-treated animals at this dose either.

The area of damage at different stereotactic levels was then studied for the 3 plasma concentrations of MK-801. At the plasma concentration of $8.0\,ng\,ml^{-1}$ there was no overall significant (BMDP 2v; $P = 0.053$) effect for the area of cortical damage in the control and MK-801 treated groups (Figure 3a); however, when individual stereotactic levels were compared there was a significant (BMDP 2v; $P < 0.05$) difference at 2 stereotactic levels only (Figure 3a). For the medium dose of MK-801 (mean plasma level $18.9\,ng\,ml^{-1}$) the area of ischaemic damage in the cerebral cortex was significantly (BMDP 2v; $P < 0.0001$) smaller in the MK-801 treated group than those in the saline treated group (Figure 3b). There was a significant (BMDP 2v; $P < 0.01$) reduction in the ischaemic area at each stereotactic coronal plane (Figure 3b), the greatest reductions were observed in the caudal coronal plane. However, there was no significant difference in the area of ischaemic damage for the caudate nucleus between the two groups of animals at this dose (data not shown). For the highest dose of MK-801 (mean plasma level of $113.2\,ng\,ml^{-1}$) tested, there was a significant (BMDP 2v; $P < 0.01$) decrease in the overall area of ischaemic damage in the cerebral cortex for the MK-801-treated group compared to the saline-treated controls (Figure 3c). There were also significant ($P < 0.05$) decreases in the area of damage at coronal points between anterior 7.19 and anterior 2.18 mm from bregma. There was no significant change in the area of ischaemic damage at the different stereotactic levels for the caudate nucleus ($P > 0.2$; data not shown).

The lowest plasma concentration of MK-801 of $8.0\,ng\,ml^{-1}$ produced no significant effect on the mean arterial blood pressure (MABP) of the animals (Figure 4a). However, a plasma concentration of $18.9\,ng\,ml^{-1}$ of MK-801 gave a decrease in the MABP straight after the bolus dose had been administered. The MABP was reduced significantly ($P < 0.05$) by 28 mmHg from the preinjection level although it returned to control levels within 1 h (Figure 4b). The highest dose of

Table 1 The physiological parameters for the animals measured throughout the experiment

	Pre-MCA	*1 h post*	*2 h post*	*3 h post*	*4 h post*
A					
Pao_2 Control	126 ± 7	133 ± 6	126 ± 4	129 ± 3	123 ± 3
Pao_2 MK-801	118 ± 5	122 ± 4	118 ± 4	124 ± 4	119 ± 3
$Paco_2$ Control	35 ± 1	36 ± 1	36 ± 1	34 ± 1	36 ± 1
$Paco_2$ MK-801	37 ± 1	36 ± 1	37 ± 1	35 ± 1	36 ± 1
Gluc Control	14 ± 1	8 ± 1	7 ± 1	8 ± 0	7 ± 0
Gluc MK-801	17 ± 1	9 ± 1	8 ± 0	7 ± 0	7 ± 0
B					
Pao_2 Control	141 ± 6	135 ± 7	145 ± 7	143 ± 5	136 ± 6
Pao_2 MK-801	128 ± 5	128 ± 7	144 ± 5	129 ± 4	126 ± 2
$Paco_2$ Control	35 ± 1	36 ± 1	36 ± 1	34 ± 2	37 ± 2
$Paco_2$ MK-801	38 ± 1	37 ± 1	35 ± 1	35 ± 1	35 ± 1
Gluc Control	18 ± 2	13 ± 2	12 ± 3	12 ± 2	11 ± 2
Gluc MK-801	19 ± 2	16 ± 3	14 ± 3	10 ± 2	8 ± 2
C					
$Paco_2$ Control	127 ± 6	148 ± 5	145 ± 5	144 ± 4	147 ± 5
Pao_2 MK-801	120 ± 9	135 ± 8	130 ± 4	136 ± 4	140 ± 4
$Paco_2$ Control	36 ± 2	38 ± 1	37 ± 1	36 ± 1	36 ± 1
$Paco_2$ MK-801	38 ± 2	37 ± 1	36 ± 1	36 ± 1	35 ± 1
Gluc Control	20 ± 1	11 ± 1	8 ± 1	8 ± 1	8 ± 1
Gluc MK-801	20 ± 1	8 ± 1	7 ± 1	7 ± 1	7 ± 1

The physiological parameters of animals used in these studies were measured prior to middle cerebral artery (MCA) occlusion then at 1, 2, 3 and 4 h post occlusion. The Pao_2 and $Paco_2$ values are expressed in mmHg. Plasma glucose (Gluc) concentration is given in $mmol\,l^{-1}$. (A) These are the values from the experiment in which plasma MK-801 levels were maintained at $8.0\,ng\,ml^{-1}$. (B) This group of animals had plasma MK-801 levels of $18.9\,ng\,ml^{-1}$. (C) The final group had plasma MK-801 levels of $113.2\,ng\,ml^{-1}$. Each value represents the mean ± s.e.mean from 10–12 animals.

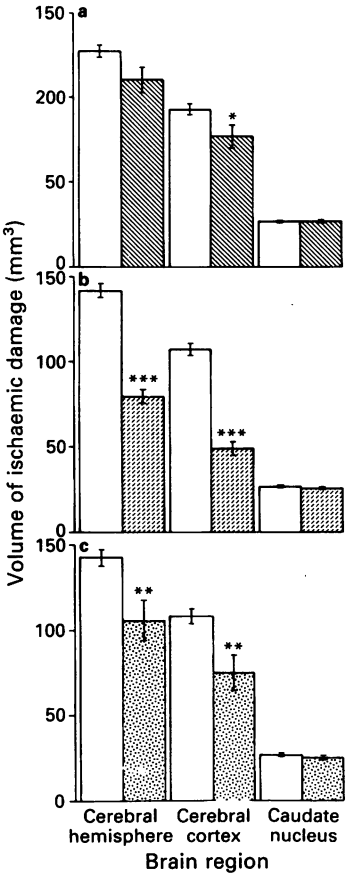

Figure 2 Volume of ischaemic damage in the cerebral hemisphere, cerebral cortex and caudate nucleus for the different doses of MK-801 and saline-treated animals (open columns). (a) Data for the 8.0 ng ml^{-1} plasma level of MK-801 (hatched columns). There was only significant protection against cortical damage, ANOVA $F = 4.5$ (* $P < 0.05$). There was no significant difference in the volume of ischaemic damage for the hemisphere (ANOVA $F = 4.13$, $P > 0.06$) or the caudate nucleus (ANOVA $F = 0.06$, $P > 0.8$). (b) Histogram for dose of MK-801 which gave plasma levels of 18.9 ng ml^{-1} (hatched columns). This gave significant protection against cortical (ANOVA $F = 111.63$, *** $P < 0.0001$) and hemispheric (ANOVA $F = 115.58$, *** $P < 0.0001$) ischaemic damage. However no protection against caudate damage was seen (ANOVA $F = 0.82$, $P > 0.4$). (c) The highest dose of MK-801 tested gave a plasma level of 113.2 ng ml^{-1} (stippled columns). This gave significant protection against cortical (ANOVA $F = 8.26$, ** $P > 0.01$) and hemispheric (ANOVA $F = 8.78$, ** $P < 0.01$) ischaemic damage. There was no protection against caudate (ANOVA $F = 1.19$, $P > 0.3$). Each column is mean for 9–10 animals, vertical bars show s.e.mean.

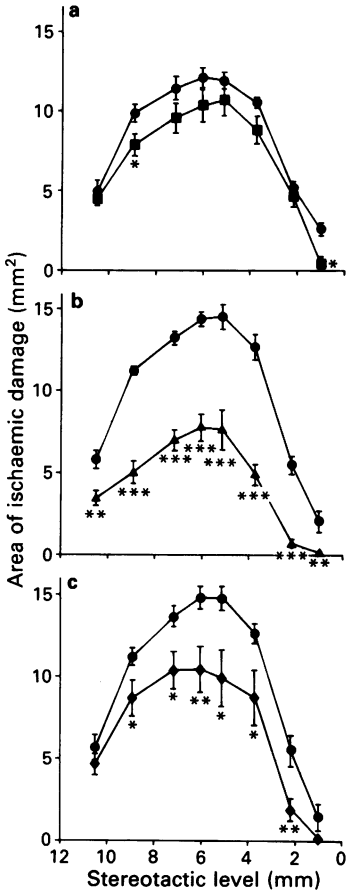

Figure 3 The area of ischaemic damage in the cerebral cortex at different stereotactic levels for the 3 doses of MK-801. (a) This illustrates data from the MK-801 group which had a plasma level of 8.0 ng ml^{-1} (■). The area of damage for the treated group was less than that for the saline-treated group (●) but there was no overall ($P > 0.05$) significant effect. Although statistical significance (* $P < 0.05$) was seen at 2 stereotactic levels. (b) The area of ischaemic damage for the 18.9 ng ml^{-1} dose of MK-801 (▲) was significantly ($P < 0.0001$) less than that for the saline-treated controls (●). There was a significant (*** $P < 0.0001$, ** $P < 0.01$) reduction in the ischaemic area at each stereotactic level. (c) The highest plasma concentration of MK-801 (113.2 ng ml^{-1}) (◆) also produced a signficant ($P < 0.01$) decrease in the overall area of ischaemic damage in the cerebral cortex. There was a significant (* $P < 0.05$, ** $P < 0.01$) decrease in the area of ischaemic damage for the MK-801-treated animals compared to the saline-treated controls (●) at coronal points between anterior 7.19 mm and anterior 2.18 mm from bregma. Each point represents the mean for 9–10 animals; vertical bars show s.e.mean.

MK-801 produced a marked hypotension in the rats. Immediately after administration of MK-801, MABP was reduced significantly ($P < 0.001$) to 60% of the preinjection level (MABP reduced from 99 ± 6 to 60 ± 2 mmHg; Figure 4c).

Discussion

The aim of the present experiments was to determine the minimum effective plasma level of MK-801 which was neuroprotective and to examine the dose-effect relationship of MK-801 in a focal ischaemia model. The acute model was used for these studies because it enabled critical physiological variables to be assessed repeatedly, such as MABP, $Paco_2$, Pao_2, and importantly the temperature of the animals which was maintained at 37 ± 0.5°C throughout the study. Furthermore, repeated blood samples were available enabling close monitoring of MK-801 plasma levels over the 4 h duration of the experiment. The infusion regimes were chosen to give a constant plasma level of MK-801 over the 4 h period, based on the assumption that the plasma half life of MK-801 in the rat is 1 h (Hucker *et al.*, 1982). The results of these studies indicate that the lowest effective plasma concentration of MK-801 is 8.0 ng ml^{-1} in this model because it produced a just significant reduction in the volume of cortical ischaemic cell damage. Maximum reduction (60%) in infarct size was seen with the plasma concentration of 18.9 ng ml^{-1}, this degree of infarct reduction correlates well with that observed by other investigators (Park *et al.*, 1988a,b; Oyzurt *et al.*, 1988). The plasma concentration of MK-801 of 113.2 ng ml^{-1} produced a 40% decrease in the infarct volume, which was less than that seen with a plasma concentration of 18.9 ng ml^{-1}. The reason for this may be due to the hypotensive effect of this dose of MK-801 as it has previously been shown that hypotension can increase infarct size in this model (Osborne *et al.*, 1987). Thus, the higher dose of MK-801 may result in a reduced protective effect as a result of its hypotensive action. However, the hypotensive effect of MK-801 is seen only in anaesthetized animals. In conscious, lightly restrained animals, MK-801 is hypertensive at these doses (Hargreaves, unpublished data) and this has also been reported in conscious rats following i.v. bolus doses (Lewis *et al.*,

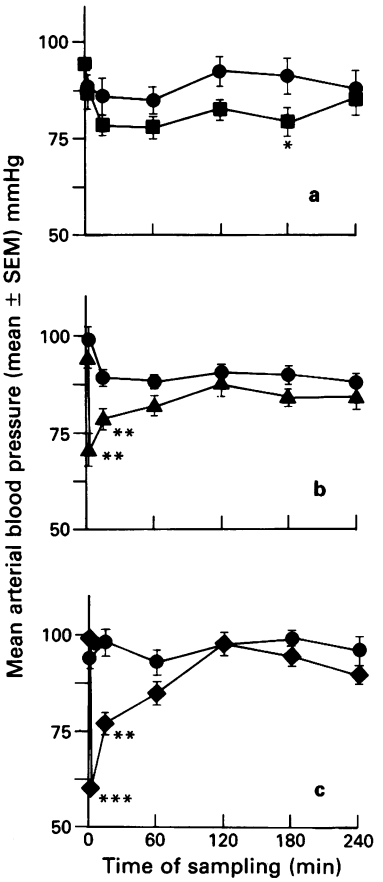

Figure 4 Changes in mean arterial blood pressure (MABP) produced by the 3 doses of MK-801 compared to their control groups. (a) Blood pressure effects produced by 8.0 ng ml^{-1} of MK-801 (■). There was no overall significant difference between the MK-801 and control group ($P = 0.2$) (●), although at the 2 h time point the MABP of the MK-801 group was significantly ($P < 0.05$) less than that of the control group. (b) MABP changes produced by a plasma concentration of 18.9 ng ml^{-1} of MK-801 (▲). The MABP was reduced significantly ($P = 0.006$) by 28 mmHg from the pre-injection level although it returned to level of controls (●) within 1 h. There was a significant (** $P < 0.01$) difference at 2 min and 15 min post MCA occlusion. (c) The highest dose of MK-801 (113.2 ng ml^{-1}) (◆) produced a marked hypotension in the rats. Following administration of MK-801, MABP was reduced significantly ($P = 0.004$) to 60% of the pre-injection level (MABP reduced from 99 to 60 mmHg). There was significant difference at 2 min (*** $P < 0.0001$) and 15 min (** $P < 0.001$) after MCA occlusion although the blood pressure had returned to same level as control animals (●) by 1 h. Values are mean with s.e.mean shown by vertical bars.

1989). There was no protection seen against caudate nucleus damage with any of the doses of MK-801, the reason for this being that the lenticulostriate branches are occluded in this model and these are the main blood supply to the caudate nucleus. Thus, with a much reduced blood flow no protection is seen in the caudate nucleus although the cell death is still through excitotoxic mechanisms.

The plasma levels of MK-801 required to protect against ischaemia-induced neuronal degeneration in the present study are the same as those required to block NMDA receptor mediated neurotoxicity *in vivo* (Willis *et al.*, 1991). The minimum effective plasma level of MK-801 of 8.0 ng ml^{-1} equals a concentration of 36 nM. This corresponds to the estimated K_D of MK-801 for the NMDA ion-channel site (Wong *et al.*, 1986; Huettner & Bean, 1988) and the concentration required to produce significant protection of cortical neurones in culture from hypoxia-induced degeneration (Priestley *et al.*, 1990). Given that MK-801 equilibrates well between plasma and cerebrospinal fluid (Willis *et al.*, 1991) with this dosing regime this provides further evidence to support the hypothesis that MK-801 mediates its neuroprotective action *in vivo* as a result of NMDA receptor blockade.

In the present experiments we have attempted to simulate some of the conditions that may prevail in the clinical situation. Thus, the drug was given as an i.v. bolus dose followed by a slow infusion to maintain stable plasma concentrations for the duration of the study. This was done using an acute model of stroke and therefore it could be argued that MK-801 is merely delaying ischaemic cell death. However, a recent study by Park (1988c) has shown that following permanent MCA occlusion and treatment with MK-801 the outcome is the same at 3 h or 48 h of survival. This is in agreement with the results of Nedergaard (1988), who found no delayed neuronal degeneration following permanent MCA occlusion in the rat. This is an important difference between global ischaemia models and permanent MCA occlusion in the rat.

MK-801 is a non-competitive NMDA antagonist which antagonizes the activated state of the NMDA receptor (Kemp *et al.*, 1987) by an open-channel blocking action. Other NMDA antagonists that act at the same site as MK-801, such as phencyclidine (Bielenburg, 1989) and thienylcyclohexylpiperidine (Duverger *et al.*, 1987), have also been shown to be neuroprotective in rat permanent MCA occlusion models. Furthermore a recent report has shown that (E)-4-(3-phosphonoprop-2-enyl)piperazine-2-carboxylic acid (CPPene), a competitive NMDA antagonist acting at the transmitter recognition site on the NMDA receptor complex, is neuroprotective in cats when administered prior to permanent MCA occlusion (Bullock *et al.*, 1990). Kynurenic acid which is a broad spectrum excitatory amino acid receptor antagonist acting at NMDA and non-NMDA subtypes of receptors (Evans *et al.*, 1987) was also neuroprotective in a rat MCA occlusion model (Germano *et al.*, 1987) when administered prior to the occlusion but was without effect on post-ischaemic administration. Ifenprodil, which has been reported to act at the polyamine site on the NMDA receptor complex (Carter *et al.*, 1989), is also neuroprotective in focal ischaemia models in the cat and rat (Gotti *et al.*, 1988). Recently, additional evidence for the involvement of excitatory amino acid induced neuronal damage in the rat MCA model has come from *in vivo* dialysis studies which have reported a substantial increase in extracellular glutamate and aspartate levels both in the striatum and cortex (Hillered *et al.*, 1989; Butcher *et al.*, 1990).

A recent report (Buchan *et al.*, 1989), however, has suggested that the neuroprotective action of MK-801 in focal ischaemia is due not to NMDA antagonism but to an increase in cerebral blood flow produced by the drug. The effect of MK-801 on cerebral blood flow in the same rat model of focal ischaemia as used in the present study was investigated by Park *et al.* (1989). They reported a significant decrease in cerebral blood flow produced by MK-801 in the non-ischaemic hemisphere but no effect was seen in the ischaemic hemisphere. Therefore, it is unlikely that the effects of MK-801 on cerebral blood flow contribute to its neuroprotective effects in this model.

MK-801 appears to be one of the most powerful neuroprotective agents available at the present time because it is able to ameliorate ischaemic damage when administered after the ischaemic insult in rats, cats and gerbils (Park *et al.*, 1988a,b; Ozyurt *et al.*, 1988; Gill *et al.*, 1988). Phencyclidine has also been shown to be neuroprotective when administered up to 3 h after permanent MCA occlusion (Bielenburg, 1989). Thus, it appears that non-competitive NMDA antagonists acting at the level of the ion channel linked to the NMDA receptor complex are effective on post-ischaemic administration. This is more relevant to the clinical situation giving a time window of up to 3 h for therapeutic intervention.

We are most grateful to Prof. J. McCulloch and Dr C. Wallace for teaching us how to prepare this ischaemia model. We would also like to thank Kevin Bradford and Dave Smith for histological assistance, Roy Hammans and Andrew Butler for preparing the figures.

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(Received February 13, 1991
Revised April 23, 1991
Accepted April 25, 1991)