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Lack of Protection by the N-Methyl-D-aspartate Receptor Blocker Dizocilpine (MK-801) after Transient Severe Cerebral Ischemia in the Rat

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Glutamate is an important factor in the mechanisms of neuronal damage following cerebral ischemia. Blockade of one type of glutamate receptor, the N-methyl-D-aspartate (NMDA) receptor, decreases brain infarct size in experimental models of permanent focal ischemia, but protection in models of transient reversible ischemia is ambiguous. We investigated the effect of the noncompetitive NMDA receptor antagonist dizocipiline (MK-801) on neuronal damage in the CA1 region of the rat hippocampus, using two models of reversible cerebral ischemia: 10 or 15 min of bilateral common carotid occlusion combined with hypotension, or 6–8.5 min of cardiac arrest. Histopathologic evaluation of neuronal damage was performed 7 days after the ischemic insults. Thirteen groups of rats (a total of 129 animals) were treated with saline or dizocilpine in single or multiple doses ranging from 0.1 to 5 mg · kg^{-1}, given intravenously or intraperitoneally prior to and/or after the ischemic insult. In none of the dizocilpine-treated groups could neuronal protection be demonstrated in the CA1 region of the septal as well as dorsotemporal hippocampus, compared to a corresponding saline-treated group. We conclude that systemically administered noncompetitive NMDA receptor antagonists do not provide a marked protection against neuronal damage after a transient period of severe forebrain ischemia. (Key words: Antagonists, glutamate: dizocilpine. Brain: hippocampus. Brain, ischemia: cell death; protection. Receptors: glutamate.)

DURING RECENT YEARS, attention has focused on the transmitter glutamate as a prime factor in the initiation and development of neuronal damage,[1,2] and several lines of evidence support an involvement of glutamatergic neurotransmission in the mechanism of ischemic brain damage.[3,4] First, distribution of neuronal damage coincides to some extent with the distribution of excitatory afferent nerve terminals and glutamate receptors in the brain.[3] Second, the extracellular concentration of glutamate increases during ischemia.[5] Third, lesions of the glutamate pathways to the hippocampus can reduce neuronal damage.[6] Finally, during the 6 yr since the first report on the protective effect of an N-methyl-D-aspartate (NMDA) receptor antagonist against ischemic damage,[7] a vast literature has accumulated confirming this finding.[8]

However, more recently, the general importance of glutamate in the mechanism of ischemic brain damage has been disputed.[9–12] For example, in experimental models of transient cerebral ischemia in rats,[9] cats,[10] dogs,[11,12] and monkeys,[13] protection by glutamate receptor antagonists could not be demonstrated. It was hypothesized that the cerebroprotective properties of the NMDA receptor antagonists depend on residual tissue energy stores during the ischemic insult, and that after severe ischemic insults leading to extensive energy depletion, the NMDA receptor antagonists are less effective cerebroprotectants.[14]

This investigation describes the effects of dizocilpine (MK-801) ([+]-5-methyl-10,11-dihydro-5H-dibenzo[a,d]-cyclohepten-5,10-imine maleate), a noncompetitive NMDA receptor antagonist, on neuronal damage in the rat hippocampus in two models of transient cerebral ischemia. These models, the two-vessel occlusion (2-VO) model of ischemia (common carotid occlusion combined with hypotension)[15] and a cardiac arrest model of complete ischemia,[16] were developed in our laboratory and are extensively used in experimental ischemia research. Dizocilpine was chosen since it readily crosses the blood–brain barrier and frequently has been used in research on glutamate cytotoxicity.[17] Some of the results have been presented previously, in abstract form.‡

Materials and Methods

All operational procedures were approved by the Ethical Committee of Lund University Hospital. Male Wistar rats (Møllegaard A/S, Denmark) weighing 290–370 g were anesthetized in a plastic jar by inhalation of 3% isoflurane in a mixture of oxygen/nitrous oxide (30/70%). After tracheal intubation, the animals' lungs were mechanically ventilated with a rodent respirator (7025 Rodent Ventilator, Ugo Basile Biological Research Apparatus, Comeno, Italy), while anesthesia was maintained with 1–2% isoflurane in the oxygen/nitrous oxide (30/70%) gas mixture.

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An external jugular vein catheter (602-175, Silastic, Dow Corning) was inserted with its tip placed in the superior caval vein. A tail artery catheter and one tail vein catheter (models 800/110/200/100 polythene tubing, Portex, England) were inserted to allow blood sampling, arterial blood pressure recording, and infusions of drugs. The blood pressure was recorded (blood pressure recorder SE 120, ABB Goerz, Germany) continuously until the tracheas were extubated. In animals subjected to 2-VO ischemia, the common carotid arteries were exposed and encircled by loose ligatures. Blood samples (200 μl) were collected 15 min prior to ischemia and 15 min postischemia to measure blood gases (AcidBase analyzer ABL 30, Radiometer, Copenhagen, Denmark) and blood glucose (Glucometer II, Miles Laboratories, Inc.). After each sample, the catheters were flushed with Krebs' solution to maintain the circulating blood volume. If blood gases could not be corrected to normal values (arterial oxygen tension > 90 mmHg, arterial carbon dioxide tension 35–45 mmHg, and *p*H 7.35–7.45) by adjustments of tidal volume or frequency (normally, frequency = 85 breaths per min) of the respirator or if the blood glucose was < 4 mM, the rats were excluded from the study.

At the end of surgery, bipolar electroencephalography (EEG) electrodes were inserted into the temporal muscles, and the EEG activity was recorded (Mingograf 34, Elema-Schönander, Sweden) every 5–to 10 min prior to ischemia, continuously during the ischemic insult, and intermittently in the postischemic period. The EEG electrodes were removed once EEG activity had returned. At the beginning of a 30-min steady-state period prior to induction of ischemia, the inspired isoflurane concentration was decreased to 0.5%, and 150 IU · kg^{-1} heparin was administered intravenously (iv). Vecuronium (Organon, Teknika, Boxtel, Holland) was given either as an iv infusion of 3 mg · h^{-1} or as incremental bolus doses of 0.4 mg. One minute prior to induction of ischemia, isoflurane was discontinued, and a bolus dose of 0.4 mg vecuronium was administered.

The animals exposed to 2-VO ischemia were fasted overnight but had free access to tap water. The ischemia was performed essentially as described earlier.[15] Briefly, blood was withdrawn *via* the jugular catheter to a mean arterial pressure (MAP) of 50 mmHg, and both carotid arteries were clamped. Blood pressure was maintained at 50 mmHg during the ischemic period by withdrawing or infusing blood through the jugular catheter. The ischemic insult was defined as the time from the onset of isoelectric EEG at an MAP of 50 mmHg. At the end of ischemia, the carotid clamps were removed. Then, after the blood was reinfused, 0.5–1 ml 0.6 M sodium bicarbonate was infused.

Complete cerebral ischemia[16] of 6–8.5-min duration was induced by iv administration of 0.3–0.4 ml 0.5 M potassium chloride into the jugular vein catheter to cause immediate cardiac arrest. Ventilation was interrupted, and 10 ml blood was withdrawn from the jugular catheter. Resuscitation was initiated after 5 min of cardiac arrest by resumption of ventilation with 100% oxygen and infusion of 10 ml oxygenated donor blood and 1 ml 0.6 M sodium bicarbonate concomitant with rapid manual chest compressions.

In all experiments, temperature was monitored before, during, and after ischemia (15 min of recovery) with thermistors placed in the rectum and on the scalp. The temperature was controlled with the aid of a heating lamp. Rectal as well as scalp temperature were maintained at approximately 37° C during the ischemic insult and until tracheal extubation (20–60 min postischemia).

After removal of the external jugular vein catheter, all wounds were sutured. Within 1 h after the ischemic insult, the animals resumed adequate spontaneous breathing; the oxygen/nitrous oxide mixture was discontinued; and the endotracheal tube could be removed. The animals were transferred to a cage and received supplementary oxygen. If they did not eat or drink spontaneously on the day after surgery, they received a suspension of nutritional fluid (Meritene, Wander AG, Bern Switzerland). This was particularly important for rats treated with 5 mg · kg^{-1} dizocilpine. In the postischemic phase, 1 or 2 animals in each group died. The mortality rate was similar in the saline-treated and dizocilpine-treated groups.

The investigation includes the following experimental groups:

Cardiac arrest ischemia: 0.3 mg · kg^{-1} dizocilpine intraperitoneally (ip) 30 min before ischemia (n = 6); 0.6 mg · kg^{-1} dizocilpine iv at reperfusion (n = 5).

2-VO ischemia (15 min): 5 mg · kg^{-1} dizocilpine ip 30 min prior to ischemia (n = 6); 5 mg · kg^{-1} dizocilpine ip at reperfusion after ischemia (n = 6).

2-VO ischemia (10 min): 0.3 mg · kg^{-1} ip (n = 6), 1 mg · kg^{-1} ip (with (n = 4) or without (n = 6) 0.4 mg · kg^{-1} diazepam iv) or 3 mg · kg^{-1} dizocilpine ip (n = 6) 0.5–1 h prior to ischemia; 3 mg · kg^{-1} dizocilpine ip followed by an infusion of 1 mg · kg^{-1} · h^{-1} for 6 h starting at reperfusion (n = 6); 0.1 mg · kg^{-1} dizocilpine iv at reperfusion with (n = 6) or without 0.4 mg · kg^{-1} diazepam iv (n = 6); dizocilpine 1 mg · kg^{-1} ip at 15 min and 5 h postischemia + diazepam 0.4 mg · kg^{-1} iv (n = 7) within 20 min postischemia; diazepam 0.4 mg · kg^{-1} iv immediately at reperfusion (n = 6).

When administered iv as bolus or as a continuous infusion, the drugs where given *via* the tail vein catheter.

After 1 week of survival the animals were reanesthetized in a jar by inhalation of 2% halothane in a mixture of oxygen/nitrous oxide (30/70%). After tracheostomy, the lungs were ventilated mechanically by a rodent ven-

tilator, and the brains were perfusion-fixed *via* the ascending aorta with an infusion of prewarmed (37° C) 4% formaldehyde solution, buffered to *p*H 7.35, preceded by a short saline rinse.[18] The brains were removed, embedded in paraffin, and sectioned at 6 μm. The sections were stained with celestine blue and acid fuchsin. Irreversibly injured neurons[18] stained bright red; surviving neurons stained violet.

Neuronal damage was assessed independently by two individuals blinded to treatment. Neurons were counted in the light microscope (400×) in three areas of the hippocampal CA1 region at the same rostrocaudal level, each 400 μm long. In each animal the mean of neuronal counts from both hemispheres was calculated. Two rostrocaudal levels of the hippocampus were investigated; the septal hippocampus at a level approximately 3.8–4.0 mm caudal to the bregma and the dorsal temporal hippocampus at a level 5.8 mm caudal to the bregma.[19] Neuronal necrosis in the CA1 sector of the hippocampus was expressed as the percentage of damaged neurons within the total neuronal population.[18]

Data are presented as means ± standard deviations (SD). When assessing differences in neurologic damage, by comparing one treated group with the corresponding control group, a two-tailed unpaired Student's *t* test was used with significance at $P < 0.05$. An analysis of variance with a *post hoc* Dunnett's test was used when several drug treated groups were compared with a control group.

Results

Changes in Physiologic Variables

The values of blood glucose, temperature and MAP, measured 15 min prior to and 15 min after the ischemic insults, are shown in tables 1–3. The MAP increased significantly in five groups treated with dizocilpine compared with saline-injected animals (tables 1 and 3). Blood glucose in fasted animals subjected to 2-VO ischemia was between 5.8–8.8 mM prior to ischemia and 5.0–10.0 mM after ischemia (tables 1 and 2). In these experiments, a slightly but significantly lower blood glucose was noted in two dizocilpine-treated groups compared to the corresponding saline-treated groups. In fed animals subjected to cardiac arrest ischemia, blood glucose levels prior to ischemia were 11.5–13.3 mM and after ischemia 15.6–16.9 mM (table 3). There was no difference in blood glucose levels between dizocilpine- and saline-treated animals in these experiments. Small differences in rectal temperature after dizocilpine treatment were noted in three experimental groups. In one group, temperature was 0.7° C higher prior to ischemia than in the corresponding saline-treated group (table 3). In two other dizocilpine-treated groups, rectal temperature was 0.1° C higher and 1.1° C lower, respectively, than in the corresponding saline-treated group after ischemia (tables 1,2).

TABLE 1. Data before and after 10 min of Forebrain Ischemia in Rats Treated with Dizocilpine (MK-801), Diazepam, or Saline

Drug	Dose (mg·kg⁻¹)	n	MABP (mmHg)		Temperature (°C)		Blood Glucose (mM)	
			Pre	Post	Pre	Post	Pre	Post
Dizocilpine	3[a]	6	131 ± 12	181 ± 10*	37.0 ± 0.4	36.3 ± 0.4	7.5 ± 1.1	7.4 ± 1.1
Saline		5	118 ± 21	130 ± 22	36.6 ± 0.6	36.2 ± 0.3	8.8 ± 1.8	9.3 ± 1.8
Dizocilpine	3[b]	6	147 ± 14‡	177 ± 14*	36.6 ± 0.4	36.5 ± 0.5	7.8 ± 0.4	8.4 ± 1.0
Saline		6	123 ± 14	141 ± 13	36.8 ± 0.5	36.6 ± 0.8	8.4 ± 1.7	8.5 ± 1.8
Dizocilpine	1[c]	6	152 ± 7	152 ± 8	37.1 ± 0.3	37.1 ± 0.1‡	5.9 ± 1.8	6.4 ± 1.3
Saline		6	145 ± 10	149 ± 6	36.0 ± 0.2	37.0 ± 0.0	6.8 ± 1.2	5.0 ± 1.5
Dizocilpine	0.3[d]	6	138 ± 24	165 ± 14‡	37.2 ± 0.4	36.8 ± 0.4	6.9 ± 0.9‡	7.0 ± 1.0‡
Saline		6	118 ± 12	137 ± 20	36.9 ± 0.3	36.7 ± 0.3	8.3 ± 1.0	8.6 ± 1.0
Dizocilpine	1 + diazepam[e]	4	162 ± 9‡	164 ± 5	37.3 ± 0.1	37.3 ± 0.2	6.3 ± 1.2	5.0 ± 1.0
Dizocilpine	0.1 post[f]	6	127 ± 15	148 ± 7	37.0 ± 0.2	37.0 ± 0.4	5.8 ± 1.0	5.4 ± 1.3
Dizocilpine	0.1 + diazepam[g]	6	142 ± 8	148 ± 15	37.0 ± 0.2	37.0 ± 0.2	6.1 ± 1.0	5.7 ± 0.7
Diazepam	0.4[h]	6	136 ± 12	137 ± 9	37.0 ± 0.2	36.9 ± 0.1	6.5 ± 2.1	6.4 ± 2.1
Saline		10	138 ± 11	148 ± 13	37.1 ± 0.3	37.0 ± 0.2	6.9 ± 1.4	5.6 ± 0.7
Dizocilpine	1 + diazepam[i]	7	119 ± 17	123 ± 13	36.7 ± 0.5	36.7 ± 0.6	7.5 ± 1.2	6.8 ± 1.1
Saline		6	112 ± 11	127 ± 9	37.0 ± 0.4	36.8 ± 0.6	7.7 ± 1.6	7.7 ± 1.6

Data are presented as means ± SD. Statistics used were two-tailed, unpaired Student's *t* test or ANOVA with a *post hoc* Dunnett's test, when multiple groups are compared with the saline group.

MABP = mean arterial blood pressure. Temperature is rectal temperature. Pre = 15 min before and post = 15 min after ischemia.

* $P < 0.001$.

† $P < 0.01$.

‡ $P < 0.05$.

[a] 0.5 h preischemia + at reperfusion1 mg·kg⁻¹·h⁻¹ for 6 h.

[b] 0.5 h preischemia

[c] 1 h preischemia

[d] 1 h preischemia

[e] 0.5 h preischemia + 0.4 mg·kg⁻¹ diazepam at reperfusion.

[f] At reperfusion.

[g] At reperfusion + 0.4 mg·kg⁻¹ diazepam 0.5 h postischemia.

[h] At reperfusion.

[i] 0.25 h + 5 h postischemia + 0.4 mg·kg⁻¹ diazepam within 20 min postischemia.

TABLE 2. Data before and after 15 min of Forebrain Ischemia in Rats Treated with Dizocilpine (MK-801) or Saline

Drug	Dose (mg·kg^{-1})	n	MABP (mmHg)		Temperature (°C)		Blood Glucose (mM)	
			Pre	Post	Pre	Post	Pre	Post
Dizocilpine	5[a]	6	102 ± 25	143 ± 24	36.7 ± 0.6	37.0 ± 0.8	6.6 ± 1.1	6.2 ± 1.1*
Dizocilpine	5[b]	6	111 ± 24	113 ± 18	37.1 ± 0.5	36.7 ± 0.6*	7.4 ± 1.2	7.3 ± 2.7
Saline		8	101 ± 12	121 ± 13	36.9 ± 0.6	37.8 ± 0.4	7.5 ± 1.4	10.0 ± 1.8

Data are presented as means ± SD. ANOVA with a *post hoc* Dunnett's test is used for comparison between groups.
MABP = mean arterial blood pressure. Temperature is rectal temperature. Pre = 15 min before and post = 15 min after ischemia.
* $P < 0.01$.
[a] 0.5 h preischemia.
[b] At reperfusion.

Effect of Dizocilipine on Hippocampal Damage after 2-VO Ischemia

Figure 1 shows neuronal necrosis in the septal CA1 region in saline-treated animals and animals given dizocilipine 5 mg·kg^{-1} ip either 30 min prior to 15 min of 2-VO ischemia or at reperfusion. The neuronal damage (mean ± SD) amounted to 91.4 ± 3.8% in saline-injected animals, 85.8 ± 8.0% in animals pretreated with dizocilipine, and 71.7 ± 27.3% in animals given dizocilipine immediately after ischemia. In the dorsotemporal hippocampus (fig. 2), damage in the saline-treated group was 67.3 ± 23.8% and in the dizocilipine-pretreated animals or in animals treated in the postischemic phase was 50.2 ± 24.6 and 45.6 ± 19.3%, respectively. The effects of dizocilipine 1 mg·kg^{-1} given ip at 15 min and 5 h after 10 min of 2-VO ischemia in combination with diazepam 0.4 mg·kg^{-1} injected iv within 20 min postischemia, are shown in figures 3 and 4. The neuronal necrosis in the septal part of the CA1 region in saline-treated ischemic animals was 65.4 ± 28.3% and in those subjected to dizocilipine and diazepam treatment 89.4 ± 7.6%. In the dorsal temporal part of CA1, 30.9 ± 26.7% neuronal damage was noted in the saline group, compared with 55.4 ± 28.8% in the dizocilipine-treated groups.

Table 4 summarizes the effects of different doses of dizocilipine and diazepam on neuronal damage in the septal hippocampus after 10 min of 2-VO ischemia. Damage in saline-treated animals ranged from 65 to 85% and in the dizocilipine-treated animals from 53 to 92%. Table 5 demonstrates the neuronal damage of the dorsotemporal hippocampus of rats exposed to 10 min of 2-VO ischemia. Damage in the saline-treated groups ranged from 18 to 40% and in the dizocilipine-treated groups from 12 to 45%.

Effects of Dizocilipine on Hippocampal Damage after Cardiac Arrest Ischemia

In the transient cardiac arrest model, 55.0 ± 26.5% neuronal damage was found in the septal hippocampus (CA1 sector) of saline-treated animals. In animals treated with 0.3 mg·kg^{-1} dizocilipine ip 1 h preischemia or with 0.6 mg·kg^{-1} dizocilipine iv at reperfusion, 55.4 ± 36.3% and 65 ± 23.2% neuronal damage was observed, respectively (fig. 5). In the dorsotemporal CA1 sector of hippocampus, 20.0 ± 23.6% neuronal damage was observed in the saline-treated group, and neuronal necrosis in the dizocilipine-treated groups was 29.2 ± 30.6 and 35.7 ± 35.3%, respectively (fig. 6).

Statistical Data Analysis

In five of seven saline-treated groups and in five of nine dizocilipine-treated groups, neuronal damage in the septal hippocampus was significantly denser than in its dorsotemporal part ($P < 0.05$, Student's t test).

A statistically significant difference could not be discerned between any dizocilipine-treated group and the corresponding saline-treated group. A power analysis[20]

TABLE 3. Data before and after Transient Cardiac Arrest of 6–8.5 Min in Rats Treated Either with Dizocilipine (MK-801) or Saline

Drug	Dose (mg·kg^{-1})	n	MABP (mmHg)		Temperature (°C)		Blood Glucose (mM)	
			Pre	Post	Pre	Post	Pre	Post
Dizocilipine	0.3[a]	6	141 ± 23	130 ± 10	37.6 ± 0.4*	37.2 ± 0.4	12.7 ± 1.5	16.3 ± 1.5
Dizocilipine	0.6[b]	5	127 ± 12	139 ± 4†	37.2 ± 0.2	37.5 ± 0.3	11.5 ± 1.5	15.6 ± 2.1
Saline		6	127 ± 15	128 ± 4	36.9 ± 0.2	36.9 ± 0.6	13.3 ± 2.0	16.9 ± 1.4

Data are presented as means ± SD. ANOVA; a *post hoc* Dunnett's test is used for comparison between groups.
MABP = mean arterial blood pressure. Temperature is rectal temperature. Pre = 15 min before and post = 15 min after cardiac arrest.
* $P < 0.01$.
† $P < 0.05$.
[a] 1 hr prior to cardiac arrest.
[b] At reperfusion.

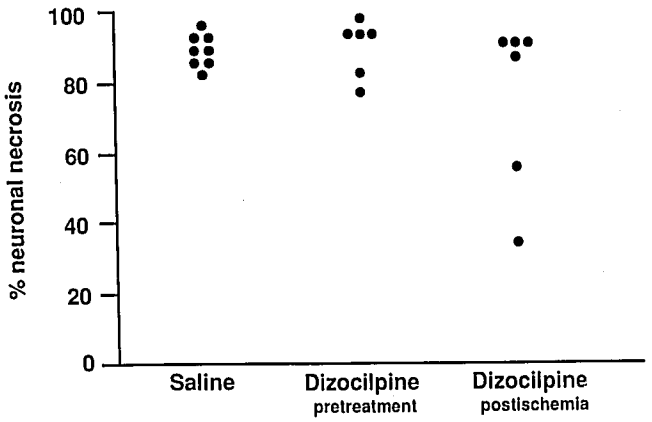

FIG. 1. Hippocampal CA1 damage (mean percentage neuronal damage ± SD) in septal hippocampus after 15 min of forebrain ischemia using a two-vessel occlusion model in rats. Animals were treated with 5 $mg \cdot kg^{-1}$ dizocilipine either 30 min prior to ischemia (85.8 ± 8.0%) or directly postischemia (71.7 ± 27.3%). The neuronal necrosis is compared to saline-treated rats (91.6 ± 3.5%). Each dot is the mean damage of the two hemispheres. Using ANOVA with a *post hoc* Dunnett's test, no significant difference between groups was found.

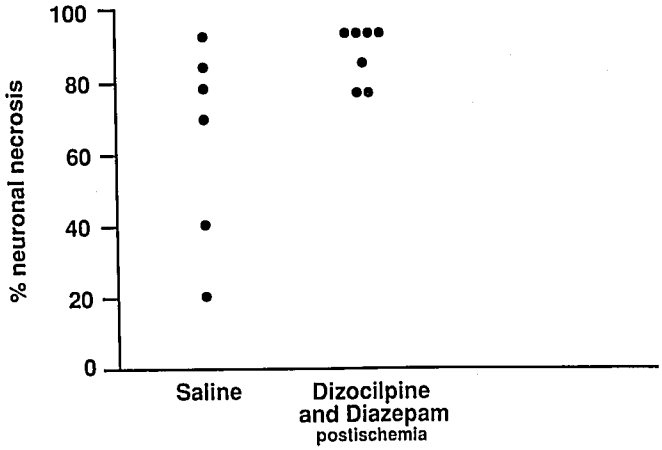

FIG. 3. Neuronal damage in the septal hippocampal CA1 region (mean percentage ± SD) of rats exposed to 10 min of forebrain ischemia using a two-vessel occlusion model. Dizocilpine was given as doses of 1 $mg \cdot kg^{-1}$ ip 15 min and 5 h postischemia, combined with 0.4 $mg \cdot kg^{-1}$ of diazepam iv postischemia within 20 min (89.4 ± 7.6%) and was compared with saline-treated rats (65.4 ± 28.3%) using a two-tailed unpaired Student's *t* test. Each dot represents the mean damage of the two hemispheres, and no significant difference between groups could be detected.

showed that a minimal decrease in neuronal damage, by 32 and 35%, can be detected in the septal and dorsotemporal hippocampus, respectively, after 10 min of ischemia, with a power of 0.8 when the significance level is 0.05. The corresponding values for the damage in the septal and dorsotemporal hippocampus in the groups undergoing 15 min of ischemia are 20 and 40% respectively, and in the cardiac arrest model 44 and 45%.

Discussion

In the following discussion we first compare the cerebroprotective efficiencies of NMDA receptor antagonists in experimental models of *permanent focal ischemia* (accomplished by occlusion of a major cerebral artery) with

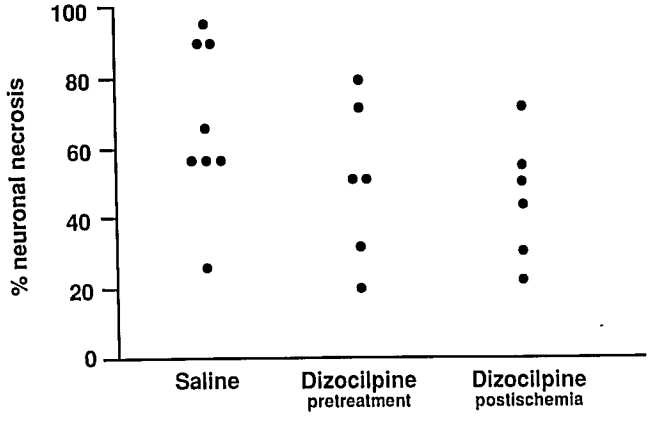

FIG. 2. Hippocampal CA1 damage (mean percentage neuronal damage ± SD) in dorsotemporal hippocampus after 15 min of forebrain ischemia using a two-vessel occlusion model in rats. Animals were treated with 5 $mg \cdot kg^{-1}$ dizocilipine either 30 min prior to ischemia (50.2 ± 24.6%) or directly postischemia (45.6 ± 19.3%). The neuronal necrosis is compared to saline-treated rats (67.3 ± 23.8%). Each dot is the mean damage of the two hemispheres. Using ANOVA with a *post hoc* Dunnett's test, no significant difference between groups was found.

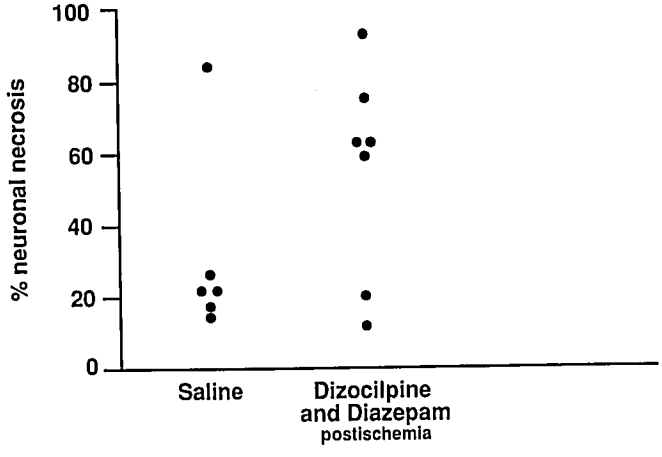

FIG. 4. Neuronal damage in the dorso temporal hippocampal CA1 region (mean percentage ± SD) of rats exposed to 10 min of forebrain ischemia using a two-vessel occlusion model. Dizocilpine was given as doses of 1 $mg \cdot kg^{-1}$ ip 15 min and 5 h postischemia, combined with 0.4 $mg \cdot kg^{-1}$ diazepam iv postischemia within 20 min (55.4 ± 28.8%) and was compared with saline-treated rats (30.9 ± 26.7%) using a two-tailed unpaired Student's *t* test. Each dot represents the mean damage of the two hemispheres, and no significant difference between groups could be detected.

TABLE 4. Neuronal Damage in Septal Hippocampal CA1 Sector in Rats in a Two-vessel Occlusion Model of 10-min of Forebrain Ischemia

Drug	n	Dose (mg·kg^{-1})	Neuronal Damage in CA1 (Septal) (% of Total Neurons)
Dizocilpine	6	3[a]	77.9 ± 21.1
Saline	5		65.4 ± 35.6
Dizocilpine	6	3[b]	85.8 ± 16.5
Saline	6		75.0 ± 31.5
Dizocilpine	6	1[c]	52.7 ± 25.5
Saline	6		65.0 ± 24.3
Dizocilpine	6	0.3[d]	92.5 ± 2.7
Saline	6		81.2 ± 15.1
Dizocilpine	4	1 + diazepam[e]	91.1 ± 4.7
Dizocilpine	6	0.1[f]	86.1 ± 15.3
Dizocilpine	6	0.1 + diazepam[g]	91.9 ± 5.1
Diazepam	6	0.4[h]	78.0 ± 35.8
Saline	10		84.8 ± 15.3

Data are presented as means ± SD. Statistics used are two-tailed, unpaired Student's *t* test or ANOVA with a *post hoc* Dunnett's test, when multiple groups were compared.

No significant difference of neuronal damage between drug-treated animals and saline-treated animals were found.

[a] 0.5 h preischemia + at reperfusion 1 mg·kg^{-1}·h^{-1} for 6 h.
[b] 0.5 h preischemia.
[c] 1 h preischemia.
[d] 1 h preischemia.
[e] 0.5 h preischemia + 0.4 mg·kg^{-1} diazepam at reperfusion.
[f] At reperfusion.
[g] At reperfusion + 0.4 mg·kg^{-1} diazepam 0.5 h postischemia.
[h] At reperfusion.

that in models of *transient ischemia* (performed by reversible occlusion of the neck arteries supplying the brain). Second, we discuss the apparently contradictory results described in the literature on the effects of NMDA antagonists in different experimental paradigms of transient cerebral ischemia.

There seems to be agreement that NMDA receptor antagonists decrease infarct size and improve neurologic outcome in models of permanent focal ischemia in which one or several major arteries in the brain are permanently occluded.[8] In these models, mimicking the clinical situation of stroke, an area of mixed density of ischemia is formed by collateral vessels to the territory normally supplied by the occluded arteries. In the core of the infarct, *i.e.*, the area of severe or complete ischemia where energy depletion is severe enough to cause membrane depolarization, total tissue necrosis develops rapidly. In the tissue surrounding the ischemic focus, called the "penumbra" (a condition of incomplete ischemia), the residual blood flow is sufficient for some remaining energy production,[21] support of Na^+–K^+ adenosine triphosphatase activity, and preservation of ion gradients across the plasma membrane.[22] In this area, the glutamate levels are significantly elevated[23] and may aggravate neuronal damage and expand the infarcted area by imposing continuous stress on the neurons. It has been proposed that NMDA receptor antagonists preclude the recruitment of irreversibly damaged tissues *during* ischemia by inhibiting ion cycling across the plasma membrane through the NMDA-operated ion channel in the penumbra area, and thereby preserve energy, enhance glutamate uptake, and terminate intracellular second messenger formation.[24]

In models of transient cerebral ischemia, the situation is in some respects different from that found in permanent focal ischemia. Here neurons experience a transient period of energy depletion followed by recovery and degeneration of vulnerable neurons. Transection of glutamatergic afferent nerves[25] or administration of glutamate receptor antagonists in the reperfusion phase protect hippocampal neurons after transient cerebral ischemia. This strongly suggests that glutamate-receptor-mediated events leading to neuronal damage take place *after* ischemia and that ischemia inflicts an insult on vulnerable neurons and so makes them prone to glutamatergic excitatory neurotransmission in the reperfusion period. The severity of the ischemic insult may establish the degree of sensitization of vulnerable neurons to postischemic glutamate

TABLE 5. Neuronal Damage in Dorsal Temporal Hippocampal CA1 Sector in Rats in a Two-vessel Occlusion Model of 10-min Forebrain Ischemia

Drug	n	Dose (mg·kg^{-1})	Neuronal Damage in CA1 Sector (Temporal) (% of Total Neurons)
Dizocilipine	6	3[a]	45.0 ± 35.2
Saline	5		26.5 ± 22.1
Dizocilipine	6	3[b]	44.2 ± 22.5
Saline	6		18.2 ± 17.9
Dizocilipine	6	1[c]	12.4 ± 8.4
Saline	6		31.2 ± 19.7
Dizocilipine	6	0.3[d]	38.4 ± 16.3
Saline	6		39.6 ± 35.7

Data are presented as means ± SD. Statistics: two-tailed, unpaired Student's *t* test. No significant differences in neuronal damage between dizocilipine- or saline-treated animals were found.

[a] 0.5 h preischemia + at reperfusion 1 mg·kg^{-1}·h^{-1} for 6 h.
[b] 0.5 h preischemia.
[c] 1 h preischemia.
[d] 1 h preischemia.

FIG. 5. Neuronal damage (mean percentage ± SD) in the septal hippocampal CA1 region of rats exposed to transient cardiac arrest for 6–8.5 min. Dizocilpine was administered either 1 h prior to ischemia in a dose of 0.3 mg · kg^{-1} ip (55.4 ± 36.3%) or at reperfusion in a dose of 0.6 mg · kg^{-1} (65.0 ± 23.2%). The neuronal damage is compared with saline-treated animals (55.0 ± 26.5%) using ANOVA with a *post hoc* Dunnett's test. Each dot is the mean damage of the two hemispheres. No significant difference between groups was found.

stimulation, which in turn may determine the contribution of glutamate receptors to the degenerative processes in the reperfusion phase.

In the current study it was demonstrated that dizocilpine, given to the rat systemically in doses of 0.1–5 mg · kg^{-1}, either prior to or after a transient ischemic insult, does not decrease ischemic neuronal damage in the hippocampus. These results agree with several recent reports[9–13] demonstrating lack of cerebral protection by competitive or noncompetitive NMDA receptor antagonists, but they conflict with some data[26,27] obtained in rat and gerbil models of forebrain ischemia.

In the four-vessel occlusion model of cerebral ischemia, neither competitive nor noncompetitive NMDA receptor antagonists decreased neuronal necrosis in the hippocampal CA1 region.[9] In a cardiac arrest model in cats, no mitigation of ischemic damage could be obtained with dizocilpine 0.33 mg · kg^{-1} given postischemia combined with 10-h iv infusion of 0.073 mg · kg^{-1}.[10] Likewise, after 11 min of aortic occlusion in dogs, no improvement of neurologic outcome could be demonstrated by 0.15 mg · kg^{-1} dizocilpine given 5 min postischemia followed by an infusion of 1.25 μg · kg^{-1} · h^{-1} for 8 h.[11] In another study conducted in dogs, dizocilpine 0.3 mg · kg^{-1} given after 17 min of cardiac arrest and followed by a 12-h infusion of 0.075 mg · kg^{-1} · h^{-1} was unable to improve neurologic outcome.[12] Also, in a monkey model of 17-min global cerebral ischemia, no improvement in neurologic outcome could be demonstrated with dizocilpine 0.3 mg · kg^{-1} over 5 min followed by an infusion of 0.15 mg · kg^{-1} · h^{-1} for 10 h.[13] In these experiments, in which protection by the NMDA receptor antagonist was not achieved, ischemia can be characterized as severe or complete (cardiac arrest).

In contrast, in some studies using rat and gerbil models of transient cerebral ischemia, dizocilpine was found to decrease neuronal damage. For example, in a gerbil model of transient cerebral ischemia, dizocilpine decreased neuronal damage when administered in doses ranging from 0.1 to 10 mg · kg^{-1} after ischemia.[26] Furthermore, in the rat 2-VO model, several different competitive or noncompetitive NMDA receptor antagonists protected the hippocampal CA1 area against ischemic damage.[27] Dizocilpine 1 mg · kg^{-1} given twice postischemia in combination with a single dose of diazepam, 0.4 mg · kg^{-1}, decreased neuronal damage in the CA1 region of the hippocampus from approximately 73% damage in saline-treated animals to approximately 30% damage in dizocilpine-treated animals.[27] Our data are unable to confirm these latter findings, even though some of our experiments used similar treatment paradigms (figs. 3 and 4).

The conflicting results may be due to differences in some of the factors influencing neuronal damage, such as brain temperature,[28] blood glucose concentration, or the density and duration of ischemia.[29] In the current and one conflicting study,[27] skull and rectal temperatures were carefully controlled during and after ischemia, preventing a change in body temperature induced by dizocilpine, as described in gerbil experiments.[30] Moreover, the blood glucose concentrations in these investigations did not increase because of dizocilpine treatment. Residual cerebral blood flow during the ischemic insult, and thus the density

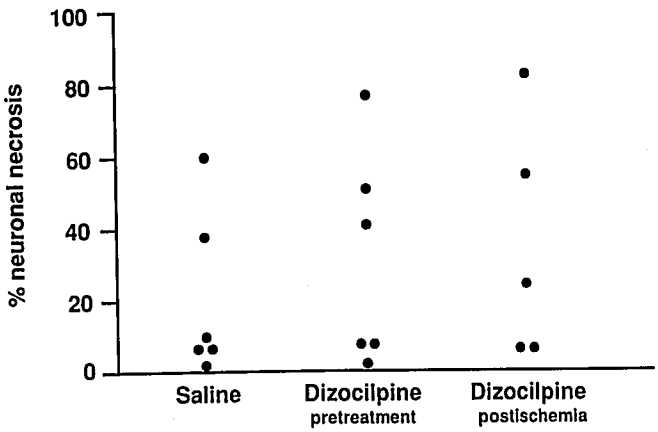

FIG. 6. Neuronal damage (mean percentage ± SD) in the dorsal temporal hippocampal CA1 region of rats exposed to transient cardiac arrest for 6–8.5 min. Dizocilpine was administered either 1 h prior to ischemia in a dose of 0.3 mg · kg^{-1} ip (29.2 ± 30.6%) or at reperfusion in a dose of 0.6 mg · kg^{-1} (35.7 ± 35.3%). The neuronal damage is compared with saline-treated animals (20.0 ± 23.6%) using ANOVA with a *post hoc* Dunnett's test. Each dot is the mean damage of the two hemispheres. No significant difference between groups was found.

of ischemia, may be decisive factors influencing the efficiency of NMDA receptor antagonists. During ischemia, cerebral blood flow may vary among animal species and strains and among the diverse ischemia models used. In hypoglycemia, during which adenosine triphosphate levels are reduced by approximately 50% and lactate levels are low, neuronal damage in the striatum decreases by *posthypoglycemic* administration of dizocilpine.§

It therefore is possible that the density of an ischemic insult, and thus the degree of lactic acidosis and the severity of energy depletion, determine the importance of NMDA receptors in the postischemic development of neuronal damage. If some energy formation remains during the insult, such as after moderate or incomplete ischemia, the activation of NMDA receptors in the postinsult period could be detrimental. Conversely, in situations in which the ischemic insult is severe or complete, such as in the current investigation and in some of the investigations mentioned earlier,[9–13] postischemic treatment with NMDA receptor antagonists is not effective. The reason why NMDA receptor blockade does not affect neuronal damage after transient severe ischemia may be inhibition of the receptor at low *p*H values[31] or receptor down-regulation *via* covalent modification.[32]

Since lesions of the glutamatergic input to the hippocampus decrease neuronal damage also in models of severe or complete ischemia, nonNMDA glutamate receptors may be important for the development of neuronal damage after a more severe ischemic insult. Recently, blockade of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor, an ionotropic nonNMDA receptor, provided significant neuronal protection after transient bilateral common carotid occlusion in the gerbil.[33] Therefore, we propose that the efficiency of the glutamate receptor blockade in preventing neuronal damage after transient severe ischemia depends on the severity of the ischemic insult. After moderate (incomplete) ischemia, the NMDA receptor antagonists are effective, whereas after transient severe (complete) ischemia, AMPA receptor antagonists provide effective protection of neurons.

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