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Pharmacological evidence for a correlation between hippocampal CA1 cell damage and hyperlocomotion following global cerebral ischemia in gerbils

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Abstract

Global ischemia, induced in Mongolian gerbils by bilateral occlusion of the carotid arteries for 5 min, produced a significant increase in locomotor activity at 1 day post-occlusion and a severe loss of hippocampal CA1 neurons at 4 days post-occlusion. To explore the pharmacological relationship between ischemia-induced hypermotility and CA1 cell death in the hippocampus, we evaluated the efficacy of diverse classes of putative neuroprotective agents for preventing hypermotility and delayed neuronal death. Administration of any drug 30 min before global ischemia dose-dependently, and with similar potency, ameliorated both hippocampal delayed neuronal death and locomotor hyperactivity, with a rank order: tacrolimus (FK506)>nizofenone>clonindine>dizocilpine (MK-801)>6-(1*H*-imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione hydrochloride (YM90K)>phencyclidine>pentobarbital>2-(4-(*p*-fluorobenzoyl)-piperidin-1-yl)-2′-acetonaphthone hydrochloride (E-2001)>*cis*-(±)-4-phosphonomethyl-2-piperidine carboxylic acid (CGS19755)>3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide (U-50,488H)>piroxicam>eliprodil>vinpocetine. Furthermore, potencies of the protective effect on delayed neuronal death and inhibitory effects on hypermotility were closely correlated ($r = 0.98$). These results suggest that post-ischemic CA1 injury and hypermotility share common mechanisms, and further imply that it is possible to predict the neuroprotective efficacy of drugs more easily by examining the inhibitory effects on post-ischemic hypermotility in global ischemia model in gerbils.
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Keywords: Delayed neuronal death; Hippocampus; Cerebral ischemia; Global ischemia; Locomotor activity; (Mongolian gerbil); Neuroprotective drug

1. Introduction

A brief, transient episode of global cerebral ischemia causes selective loss of CA1 pyramidal cells in the hippocampus. This phenomenon has been termed delayed neuronal death because neuronal damage is not morphologically evident until 2 or 3 days after ischemia (Kirino, 1982; Kirino and Sano, 1984). Delayed neuronal death in Mongolian gerbils is a useful animal model for exploring the mechanisms of neuronal cell death associated with cerebral ischemia, as well as a good screening method for evaluating neuroprotective drugs (Simon et al., 1984; Boast et al., 1988; Gill et al., 1988; Ide et al., 1996).

While global ischemia induces diverse physiological changes such as hyperthermia (Nakanishi et al., 1994; Ide et al., 1996) and amnesic behavior (Corbett et al., 1992; Karasawa et al., 1994; Matsuda et al., 1996), the most prominent behavioral change is a massive increase in locomotor activity (Chandler et al., 1985; Gerhardt and Boast, 1988; Babcock et al., 1993). This post-ischemic hypermotility is known to correlate specifically with the neuronal cell loss in the hippocampal CA1 region (Gerhardt and Boast, 1988; Kuroiwa et al., 1991). Mileson and Schwartz suggested that change of locomotor activity can be used as a predictor of CA1 damage, but not for the damage in other structures such as the striatum or cortex (Mileson and Schwartz, 1991). However, the relationship between CA1 neuronal death and hyperactivity is clearly more complex, since a second ischemic event rapidly induces hyperactivity despite prior degeneration of CA1 neurons (Karasawa et al., 1994).

Abilities of neuroprotective compounds to ameliorate delayed neuronal death in this model are well established; however, the mechanisms underlying the subsequent hyper-

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motility and their potential pharmacological targets are poorly understood. Better understanding of these mechanisms could lead to development of newer and better methods for discovering novel neuroprotective agents. Therefore, the aim of this study was to identify common pharmacological mechanisms for ameliorating delayed neuronal death and hyperactivity and thus to clarify the relationship between the changes in these two endpoints. We investigated the neuroprotective agents: non-competitive antagonists of *N*-methyl-D-aspartate (NMDA) receptors (dizocilpine ((+)-MK-801), phencyclidine), competitive NMDA receptor antagonists (*cis*-(±)-4-phosphonomethyl-2-piperidine carboxylic acid, CGS19755), an NMDA receptor NR2B-subunit selective antagonist (eliprodil), an antagonist of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor (6-(1*H*-imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione hydrochloride, YM90K), a glutamate release inhibitor (2-(4-(*p*-fluorobenzoyl)-piperidin-1-yl)-2′-acetonaphthone hydrochloride, E-2001), an anti-platelet agent (ticolopidine), anti-inflammatory agents (piroxicam, aspirin), an opioid κ-receptor agonist (3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide, U-50488H), an α_2-adrenoceptor agonist (clonidine), an immunosuppressant (tacrolimus, FK506), an anesthetic (pentobarbital), a radical scavenger (nizofenone) and a phospodiesterase inhibitor (vinpocetine).

2. Materials and methods

2.1. Animals

Male Mongolian gerbils (*Meriones unguiculatus*) weighing 60–80 g (Japan SLC, Shizuoka, Japan) were used. The animals were housed for 7 days before the experiment in a cage in an air-conditioned room with controlled temperature (23 ± 2.0 °C), humidity (55 ± 5.0%) and light (07:00 on–19:00 off). They were allowed free access to food and water. All animal procedures were performed under the Guidelines of the Experimental Animal Ethical Committee of Fujisawa Pharmaceuticals.

2.2. Transient global ischemia in gerbils

Transient forebrain ischemia was induced by bilateral occlusion of the common carotid arteries, as described previously (Nakanishi et al., 1994). Briefly, gerbils were anesthetized with 3% halothane and anesthesia was maintained during the operation with 1% halothane in a gas mixture of 70% nitrogen and 30% oxygen. The bilateral common carotid arteries were exposed carefully to avoid damage to the neighboring vagus nerve fibers. Common carotid arteries were occluded bilaterally for 5 min with Sugita aneurysm microclips. Five minutes later, the clips were removed and reperfusion was verified. The duration of the initial halothane anesthesia was kept constant at 10 min including the period of ischemia. Rectal temperature of animals was carefully monitored and maintained at 38.0 °C throughout the surgery using a feedback-controlled heating pad (TR-100, PS-100, Fine Science Tool, North Vancouver, Canada), as changes in body temperatures are known to have impact on consequences of global ischemia (Dietrich et al., 1996). Animals were returned to their homecage after the surgery and they were maintained subsequently for 1–2 h under Xenon heating lamp. Sham-operated non-ischemic animals underwent the identical procedures except clips were not applied.

2.3. Locomotor activity

Motor activity of the animals was assessed by measuring spontaneous locomotor activity for 30 min using an ANIMEX apparatus (L35 × W30 × H20 cm; Muromachi Kikai, Tokyo, Japan). For the time-course study, locomotor activity of the ischemia-treated and sham operated animals were measured for 30 min at 1, 4, 7 and 14 days after ischemia. Repeated testing of locomotor activity is known to produce habituation to the apparatus and greatly interferes with the measurement of activity (Babcock et al., 1993), so different animals were prepared for groups of each time-point. For drug interaction studies, locomotor activity of animals was measured for 30 min 24 h after the reperfusion.

2.4. Histopathology

Gerbils were anesthetized deeply with pentobarbital-Na (50 mg/kg, i.p.) and transcardiac perfusion was performed with heparin (10 U/ml) in 0.9% saline, followed by 3.5% formaldehyde in 0.1 M phosphate buffer (pH 7.4) at 130 cm H_2O. Brains were removed and kept in the same fixative for more than 3 days. Coronal blocks were embedded in paraffin and 3-μm-thick coronal sections through the dorsal hippocampus (between 1.5 and 2.0 mm posterior to the bregma) were cut and stained with Luxol fast blue, Cresyl violet, and with hematoxylin and eosin. The CA1 field of each brain slice was analyzed in graphic analyzer (Adobe Photoshop 4.0J) using images taken at × 400 magnification with a CCD camera (FUJIX HC-2000, Fujifilm, Tokyo, Japan). The number of intact neurons in the hippocampal CA1 subfield per mm of the pyramidal cell layer was counted by a blinded investigator according to the method of Kirino (1982). The values of the left and right hippocampus were averaged for each animal and subjected to further statistical analysis.

2.5. Drugs

All drugs were synthesized at the Fujisawa Pharmaceuticals (Osaka, Japan). Nizofenone, phencyclidine, CGS19755 and clonidine were dissolved in physiological saline. Dizocilpine ((+)-MK-801) was dissolved in aqueous HCl and adjusted to pH 7.4 with 0.1 N NaOH. YM90K was dissolved

in aqueous NaOH and adjusted to pH 7.4 with 0.1 N HCl. E-2001, eliprodil, piroxicam, aspirin, ticlopidine, vinpocetine and U50488H were suspended in 0.5% methylcellulose. The injection formulation of tacrolimus (FK506) was diluted with physiological saline and, for the control group, placebo ampoule synthesized at Fujisawa Pharmaceuticals was similarly diluted with physiological saline. All drugs were administered intraperitoneally 30 min before the occlusion in a volume of 1 ml/100 g body weight. The doses used were selected based on preliminary experiments to identify the maximum tolerated dose by observing general animal behavior.

2.6. Data analysis

All values are expressed as the mean ± S.E.M. Statistical analysis was carried out by using Student's *t*-test between the sham-operated or normal and ischemia-treated groups and by using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test for ischemia-treated groups against drug-treated groups. ED_{50} values of the neuroprotective effects and inhibitory effects of drugs on hypermotility were calculated by the Probit method. The relationship between these two parameters was analyzed by linear correlation analysis.

3. Results

We first examined the time-course of hippocampal CA1 pyramidal cell damage and locomotor activity after global cerebral ischemia and the data were shown in Fig. 1. While the number of neurons per millimeter of the CA1 region of the hippocampus in ischemic animals was comparable with that in normal animals at 1 and 2 days after ischemic insult, it was markedly reduced after 3–14 days of ischemia, as compared with normal control group (Fig. 1A; $P<0.01$ by one-way ANOVA followed by Dunnett's multiple comparison test). Loss of CA1 neurons demonstrated a delayed onset, plateauing 4 days after ischemia. As shown in Fig. 1B, locomotor activity of ischemic animals increased markedly at 1–4 days compared with the sham-operated animals measured at each corresponding time-point ($P<0.01$ by Student's *t*-test). This hyperactivity gradually returned to control levels, but the tendency for the increment was still observed at 7 and 14 days after cerebral ischemia.

We evaluated the efficacy of various classes of neuroprotective compounds with diverse mechanisms of action in preventing hyperlocomotion and delayed neuronal death in hippocampal CA1 sector measured at 1 and 4 days after the ischemia, respectively (Table 1). Most of agents investigated showed dose-dependent protection to greater or lesser extent on CA1 cell death. Tacrolimus, nizofenone and clonidine were the most potent drugs. Tacrolimus (0.1, 0.32, 1 and 3.2 mg/kg, i.p.) administered 30 min before occlusion, dose-dependently inhibited the loss of CA1 pyramidal neurons

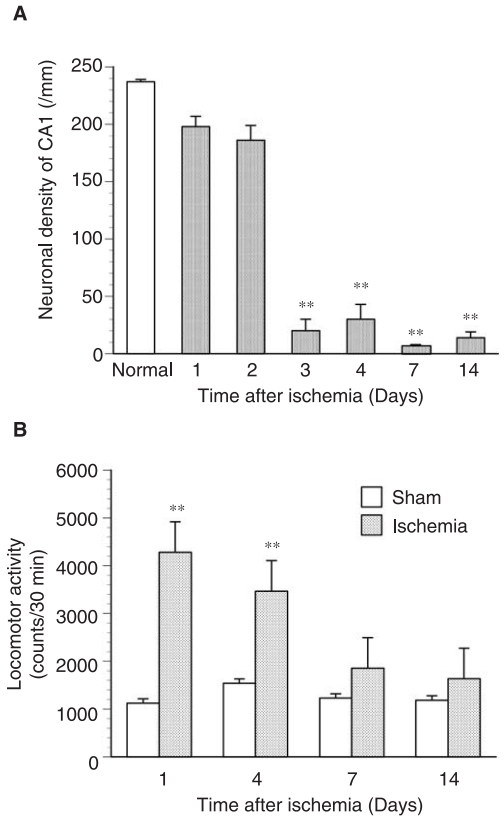

Fig. 1. Time-course of changes in locomotor activity and CA1 neuronal cell numbers in the hippocampus in sham-operated and ischemia–animals following 5-min global ischemia. (A) The neuronal density in the CA1 region of the hippocampus of normal animals and ischemic animals at 1, 2, 3, 4, 7 and 14 days after recirculation. Histological results are expressed as mean ± S.E.M. of neuronal density per millimeter of CA1 region ($n=4–7$ animals/group). $^{**}P<0.01$, significantly different from normal group (one-way ANOVA followed by Dunnett's multiple comparison test). (B) Locomotor activity was measured for 30 min and is illustrated as total counts (mean ± S.E.M., $n=3–6$ animals/group). Significant increases of locomotor activity were observed in post-ischemic animals at 1–4 days after ischemia. $^{**}P<0.01$, statistically significant compared to corresponding sham animals (by Student's *t*-test).

with statistical significance at higher dosages than 1 mg/kg ($P<0.01$ by one-way ANOVA followed by Dunnett's multiple comparison test). Tacrolimus significantly ameliorated not only CA1 damage but also the locomotor hyperactivity with similar potency (ED_{50} 0.85 mg/kg for CA1 damage, 0.54 mg/kg for hypermotility). Similarly, clonidine exerted potent protection on both parameters (ED_{50} 0.40 and 0.26 mg/kg for CA1 damage and hypermotility, respectively). Nizofenone showed potent protection on both parameters (ED_{50} 0.32 and 0.21 mg/kg for CA1 damage and hypermotility, respectively). The second group of compounds included dizocilpine (ED_{50} for CA1 damage was 1.08 mg/kg), YM90K (2.81 mg/kg) and phencyclidine (7.60 mg/kg). These compounds all showed almost complete blockade of delayed neuronal death at the highest doses and similar potencies for hyperactivity. E-2001 (ED_{50} for CA1 damage was 30.9 mg/kg), CGS19755 (37.9 mg/kg),

Table 1
Effect of neuroprotective agents on locomotor hyperactivity and hippocampal CA1 pyramidal cell damage

Drugs	Dose (mg/kg)	*N*	Locomotor activity		Hippocampal CA1 damage	
			(Counts/30 min)	ED_{50} (mg/kg)	(Number/mm)	ED_{50} (mg/kg)
Tacrolimus	sham	7	1730.0 ± 173.2	0.54	223.9 ± 10.4	0.85
	vehicle	7	3889.0 ± 239.6		20.1 ± 13.4	
	0.1	7	3877.0 ± 150.2		8.3 ± 1.6	
	0.32	7	3463.0 ± 201.8		45.6 ± 21.5	
	1	7	2482.0 ± 469.0[a]		127.0 ± 34.0[b]	
	3.2	7	1275.0 ± 144.6[b]		209.1 ± 7.5[b]	
Nizofenone	sham	5	1565.8 ± 222.8	0.21	214.7 ± 10.7	0.32
	vehicle	5	3417.2 ± 262.7		36.4 ± 22.6	
	0.1	5	2560.0 ± 349.0		64.2 ± 34.9	
	1	5	2322.0 ± 279.1[a]		206.8 ± 7.2[a]	
	10	6	1541.0 ± 169.0[b]		218.5 ± 7.7[a]	
Clonidine	sham	5	1306.8 ± 136.8	0.26	223.6 ± 13.1	0.40
	vehicle	4	3249.0 ± 500.9		28.0 ± 26.3	
	0.1	4	2610.5 ± 348.0		27.0 ± 14.5	
	0.32	4	2516.0 ± 400.7[a]		121.0 ± 25.6	
	1	5	1430.2 ± 215.6[b]		182.2 ± 31.9[a]	
Dizocilpine	sham	5	1760.3 ± 28.8	0.55	180.6 ± 2.1	1.08
	vehicle	6	4755.7 ± 247.2		4.5 ± 0.6	
	0.32	6	4061.0 ± 700.7		4.3 ± 1.2	
	1	5	2397.6 ± 549.4[a]		87.0 ± 46.7	
	3.2	7	NA		106.9 ± 35.7[b]	
	10	5	NA		179.0 ± 5.7[b]	
YM90K	sham	3	1382.0 ± 156.0	3.46	190.8 ± 14.8	2.81
	vehicle	7	3572.0 ± 337.0		4.6 ± 1.4	
	3.2	6	2424.0 ± 466.0		104.5 ± 30.7[a]	
	10	5	2157.0 ± 629.0		109.7 ± 40.8[a]	
	32	6	1374.0 ± 305.0[b]		144.7 ± 10.6[a]	
Phencyclidine	sham	5	1655.8 ± 89.9	4.64	234.4 ± 8.4	7.60
	vehicle	5	3325.0 ± 317.8		13.7 ± 8.6	
	3.2	5	2730.8 ± 454.4		31.6 ± 20.4	
	10	5	2010.0 ± 80.5		188.3 ± 28.5[a]	
	32	5	1181.4 ± 33.8[a]		224.0 ± 9.0[b]	
E-2001	sham	5	1568.9 ± 136.5	18.9	194.6 ± 5.6	30.9
	vehicle	5	3077.2 ± 219.7		57.4 ± 23.1	
	3.2	5	4185.2 ± 532.6		65.4 ± 41.1	
	10	5	2868.8 ± 413.5		50.8 ± 23.3	
	32	5	1838.0 ± 296.4[b]		145.8 ± 34.4[a]	
Piroxicam	sham	5	1098.3 ± 93.9	33.0	253.6 ± 7.2	48.3
	vehicle	4	3519.3 ± 379.4		64.8 ± 5.5	
	1	5	3117.4 ± 271.6		37.0 ± 23.3	
	3.2	5	2974.0 ± 323.7		35.8 ± 15.9	
	10	5	2544.2 ± 303.5[a]		118.4 ± 31.2[a]	
Pentobarbital	sham	3	1970.3 ± 220.8	22.8	249.6 ± 6.1	26.4
	vehicle	5	3609.2 ± 163.2		4.8 ± 1.2	
	20	4	3164.3 ± 344.7		48.5 ± 24.3	
	40	5	1175.0 ± 257.9[b]		245.2 ± 10.0[b]	
CGS19755	sham	5	1527.7 ± 91.2	22.1	228.2 ± 5.8	37.9
	vehicle	5	2932.9 ± 442.2		60.2 ± 35.8	
	10	5	2813.0 ± 393.9		34.0 ± 17.3	
	32	5	1951.1 ± 241.2		151.6 ± 35.3	
	100	4	1155.7 ± 246.0[a]		208.0 ± 2.3[a]	
U50488H	sham	5	1425.2 ± 123.8	42.3	200.0 ± 7.2	47.5
	vehicle	5	3164.5 ± 159.1		29.8 ± 13.0	
	10	5	3519.3 ± 161.5		10.2 ± 3.3	
	32	5	2800.5 ± 264.2		74.8 ± 32.2	
	100	5	1380.0 ± 242.0[a]		175.0 ± 8.9[a]	
Eliprodil	sham	5	1156.6 ± 170.5	51.6	202.8 ± 8.6	75.5
	vehicle	5	2493.0 ± 573.6		70.2 ± 35.1	
	10	4	3087.8 ± 194.9		23.0 ± 14.0	
	32	5	2139.4 ± 179.0		102.4 ± 24.4	
	100	5	1345.2 ± 306.5		145.6 ± 27.3	

Table 1 (*continued*)

Drugs	Dose (mg/kg)	*N*	Locomotor activity		Hippocampal CA1 damage	
			(Counts/30 min)	ED_{50} (mg/kg)	(Number/mm)	ED_{50} (mg/kg)
Vinpocetine	sham	5	1165.8 ± 175.1	80.2	195.8 ± 6.5	>100
	vehicle	5	4139.8 ± 56.5		3.0 ± 0.8	
	10	6	3812.8 ± 185.9		3.8 ± 0.8	
	32	6	3535.0 ± 154.0[b]		19.0 ± 14.2	
	100	5	2361.6 ± 441.0[a]		94.0 ± 40.7[a]	
Aspirin	sham	5	1713.6 ± 164.2	>32	242.6 ± 11.0	>32
	vehicle	5	2967.4 ± 335.2		26.2 ± 19.2	
	3.2	4	2946.2 ± 293.8		55.0 ± 51.8	
	10	4	2751.8 ± 403.5		71.3 ± 59.9	
	32	5	3027.8 ± 223.0		22.6 ± 14.9	
Ticlopidine	sham	6	1637.5 ± 201.9	>32	230.3 ± 3.9	>32
	vehicle	6	3519.8 ± 248.7		9.2 ± 4.3	
	3.2	6	3129.0 ± 251.3		30.2 ± 20.3	
	10	6	3144.3 ± 161.4		52.5 ± 15.7	
	32	6	3485.3 ± 160.5		3.8 ± 1.3	

Gerbils were treated with drugs (i.p.) 30 min before ischemia. The locomotor activity was measured for 30 min using an Animex at 1 day after the ischemia and hippocampal delayed neuronal death was histologically measured at 4 days after the ischemia. NA; the locomotor activity was not monitored due to severe ataxia of animals treated with higher doses of dizocilpine. Values are mean ± S.E.M. [a] $P<0.05$. [b] $P<0.01$, statistically significant compared with each vehicle-treated control group (by one-way ANOVA followed by Dunnett's multiple comparison test).

piroxicam (48.3 mg/kg), pentobarbital (26.4 mg/kg), U-50,488H (47.5 mg/kg) and eliprodil (75.5 mg/kg) were less potent but the highest dosage of any agents provided more than 50% protection both for hippocampal delayed neuronal death as well as hypermotility. Vinpocetipine, aspirin and ticolopidine did not show neuroprotection at the dosages employed. As shown in Fig. 2, correlation analysis revealed that the ED_{50} values of these agents on the two parameters were perfectly correlated and the correlation factor ($r=0.98$) was statistically significant ($P<0.0001$).

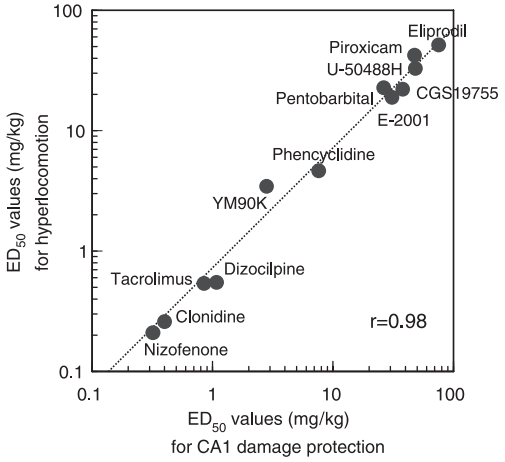

Fig. 2. Relationship between hypermotility and hippocampal CA1 pyramidal cell damage after global cerebral ischemia in gerbils. Protective potency for delayed neuronal death and reduced hypermotility was estimated by the Probit method, and ED_{50} values for each endpoint were plotted. The correlation coefficient between two parameters was 0.98 ($P<0.0001$). Vinpocetipine, aspirin and ticolopidine were excluded from the correlation analysis, as their efficacy was so modest at the dosages employed that ED_{50} values could not be calculated.

4. Discussion

The results presented here show: (1) gerbils subjected to 5-min bilateral forebrain ischemia develop significant locomotor hyperactivity 24 h after reperfusion, followed by delayed neuronal death in the hippocampal CA1 region; (2) a number of neuroprotective agents, via different mechanisms of action, dose-dependently ameliorated both delayed neuronal death in the hippocampus and locomotor hyperactivity; (3) inhibitory potencies for these two parameters were quite similar and showed statistically significant correlation.

Since delayed neuronal death in the hippocampus following global ischemia was first recognized (Kirino, 1982), extensive research using this animal model of global cerebral ischemia has been carried out in order to gain insights into the mechanisms of ischemic cell death. Although the precise mechanism underlying post-ischemic delayed neuronal death is not fully understood, the most widely supported explanation is the glutamate-Ca^{2+} theory, in which ischemia results in a loss of energy supply to the brain, leading to neuronal depolarization and massive release of excitatory amino acids such as glutamate (Choi, 1992). Glutamate activates ionotropic glutamate receptors (NMDA, AMPA and kainate) increasing Ca^{2+} entry and resulting in the activation of Ca^{2+}-dependent enzymes, ultimately leading to cell death (Choi, 1992). Based on this hypothesis, a number of glutamate receptor antagonists have been developed as anti-stroke agents, although none are currently used clinically. In the present study, supporting this hypothesis, most of the glutamate-related inhibitors such as NMDA open channel blockers (dizocilpine and phencyclidine), the competitive NMDA receptor antagonist (CGS19755), the NR2B-subunit selective NMDA receptor antagonist (eli-

prodil) and the competitive AMPA receptor antagonist (YM90K) had potent neuroprotective effects, in accordance with earlier investigations by others (Boast et al., 1988; Gill et al., 1988; Judge et al., 1991; Bath et al., 1996; Kawasaki-Yatsugi et al., 1997). E-2001, an inhibitor of glutamate release, also showed neuroprotective actions in the present study, in accordance with previous report (Kaneko et al., 1989). Excitotoxicity directly and/or indirectly generates a variety of radical species that are known to contribute to ischemic brain injury. The present study demonstrates the efficacy of nizofenone, a potent radical scavenger, in accordance with previous findings (Matsumoto et al., 1994).

In addition to glutamate, other neurotransmitters such as noradrenaline, serotonin, γ-amino butyric acid (GABA), dopamine, adenosine and opioid peptides are also known to be modulated during an ischemic episode and contribute to the complex pathophysiological cascade of ischemia. The α_2-adrenoceptor agonist clonidine is a potent neuroprotectant, indicating for the first time that α_2-adrenoceptors play an important role in ischemic brain damage, perhaps by modulating hypoxia-induced Ca^{2+} mobility (Bickler and Hansen, 1996). The κ-opioid receptor agonist, U-50,488H, was also neuroprotective in the present study, supporting the previously proposed beneficial actions of κ-opioid receptors agonists (Contreras et al., 1991).

The potent immunosuppressant tacrolimus was shown to be neuroprotective in accordance with previous findings by others (Ide et al., 1996) and ourselves (Maeda et al, 2002; Furuichi et al., 2003). The present investigation reveals for the first time that tacrolimus is also protective on ischemia-induced locomotor hyperactivity. As we have recently proposed that tacrolimus exerts the ability to inhibit apoptotic cell death and the inflammatory reaction by microglial activation and neutrophil infiltration in the ischemic brain area, which may also contribute to its neuroprotective action, further studies will be needed to depict the mechanism by which tacrolimus reduces the early events such as locomotor hyperactivity following global ischemia. Important contribution of inflammatory reactions to propagation of ischemic brain injury has been repeatedly emphasized to date. Activated glial cells such as microglia, astrocyte and infiltrated blood cells (e.g., neutrophils and macrophage) release substances harmful to neuronal cells, such as cytokines, chemokines, nitric oxide, arachidonic acid metabolized by cyclooxygenase and lipoxygenase (Iadecola and Alexander, 2001), although they are also known to secrete putative neurotrophic or neuroprotective factors (Matsuda et al., 1996). In the present study, a cyclooxygenase inhibitor piroxicam exerted the neuroprotective actions, partly supporting the previous finding with another conventional cyclooxygenase inhibitor indomethacin (Nakagomi et al., 1989).

Hypermotility induced by global cerebral ischemia in gerbils results from deficits in the animal's ability to habituate or spatially map to a novel testing environment, rather than general hyperactivity or dopamine stimulant-like stereotypy (Wang and Corbett, 1990). Thus, the increased motor activity directly results from cell loss in the hippocampus (Gerhardt and Boast, 1988). However, these two events do not correspond temporally. As shown in the present study, motor activation peaks 1 day after the ischemia, whereas neuronal degeneration takes place gradually over several days. However, despite this temporal difference, we also demonstrate for the first time that neuroprotective agents ameliorate both delayed neuronal death and the increase in locomotor activity. Furthermore, potencies for two parameters were similar and strongly correlated, suggesting hippocampal delayed neuronal death could be a sequelae of locomotor hyperactivity. These results support the hypothesis that global ischemia leads to acute excitotoxicity in CA1 neurons, which consequently results in disruption of hippocampal function (such as spatial mapping) leading to hypermotility. Although further investigation will be required to clarify the relationship of these sequential events, the present study provides compelling pharmacological evidence for close correlation in the mechanisms underlying locomotor hyperactivation and subsequent delayed neuronal death in the hippocampus.

In conclusion, the present study clearly demonstrates that diverse classes of putative neuroprotective agents can dose-dependently ameliorate both delayed neuronal death in the hippocampus and locomotor hyperactivity with similar potencies. These results suggest that post-ischemic delayed neuronal death and hypermotility share common mechanisms. Furthermore, post-ischemic hypermotility can be used as a screening method for neuroprotective drugs with higher throughput than histological measurement of CA1 injury.

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