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Short communication

Rolipram, a cyclic AMP-selective phosphodiesterase inhibitor, reduces neuronal damage following cerebral ischemia in the gerbil

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Abstract

We examined the effects of rolipram, a cyclic AMP-selective phosphodiesterase inhibitor, on cerebral ischemia-induced neuronal damage in Mongolian gerbils. Transient forebrain ischemia was induced by 3-min occlusion of bilateral common carotid arteries. Rolipram, at a dose of 0.3 or 3 mg/kg, was injected i.p. 30 min before ischemia. Histopathological observations showed that neuronal damage to the hippocampal CA1 subfield, which was seen 7 days after ischemia in vehicle-treated animals, was reduced in animals treated with the higher dose of rolipram.

Keywords: Cerebral ischemia; Rolipram; cAMP; Phosphodiesterase; Hippocampus; (Gerbil)

1. Introduction

Stroke is one of the most common causes of physical and intellectual impairment in the elderly. The mechanisms of ischemic brain damage, however, have not been fully elucidated. One of the intriguing phenomena in experimental cerebral ischemia is the delayed neuronal death following transient cerebral ischemia (Kirino, 1982). Pyramidal neurons in the CA1 subfield of the hippocampus, which are most vulnerable to ischemia, are destroyed 3–4 days after cerebral ischemia in rodents. The mechanism of the neuronal damage has not been fully elucidated, but several factors are suggested to participate in the process, such as excitotoxicity induced by excitatory amino acids, overload of intracellular Ca^{2+}, and the formation of free radicals (Rothman and Olney, 1986; Siesjö et al., 1989).

Rolipram, which has been known as an antidepressant, inhibits a Ca^{2+}/calmodulin-independent cyclic adenosine monophosphate (cyclic AMP)-selective phosphodiesterase isozyme (Schwabe et al., 1976), leading to an increase in brain cyclic AMP levels (Schneider, 1984). Specific binding sites for [^{3}H]-rolipram have been visualized and quantified by in vitro autoradiography, which showed high binding site densities in the CA1 subfield of the hippocampus (Kaulen et al., 1989). Furthermore, [^{3}H]rolipram binding decreases rapidly in the CA1 region following cerebral ischemia (Araki et al., 1993). However, the effect of rolipram treatment in cerebral ischemia has not been studied. The purpose of this study was therefore to examine the effects of rolipram treatment on ischemia-induced hippocampal CA1 neuronal damage with a widely used experimental model of cerebral ischemia in Mongolian gerbils.

2. Materials and methods

We used male Mongolian gerbils (*Meriones unguiculatus*, Seiwa Experimental Animals, Fukuoka, Japan), 12 weeks old and weighing 65–75 g. They were allowed free access to food and water before and after surgery. Under anesthesia with 2% halothane in a mixture of 30% oxygen and 70% nitrous oxide, bilateral common carotid arteries were gently exposed. One minute after discontinuation of anesthesia, the arteries were occluded with aneurysm clips for 3 min. This procedure is

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Table 1
Rectal and temporalis muscle (in parentheses) temperatures before and after ischemia

Groups	Before ischemia	After ischemia		
		30 min	1 h	2 h
Sham-operated	37.1 ± 0.06 (36.6 ± 0.17)	37.1 ± 0.69 [a]	37.5 ± 0.31[a]	37.6 ± 0.17
Vehicle	37.0 ± 0.12 (36.6 ± 0.21)	38.8 ± 0.24	38.4 ± 0.34	37.6 ± 0.21
Rolipram 0.3 mg/kg	37.1 ± 0.08 (36.6 ± 0.10)	38.8 ± 0.31	38.4 ± 0.15	37.9 ± 0.22
Rolipram 3 mg/kg	37.1 ± 0.16 (36.5 ± 0.11)	38.6 ± 0.50	38.5 ± 0.25	37.7 ± 0.34

Values are expressed as means ± S.D.
[a] $P < 0.01$ vs. vehicle-treated animals (Dunnett's multiple comparison test).

known to lead to severe reductions of blood flow in the forebrain (Kato et al., 1990). When an animal was not considered to have been rendered severely ischemic (e.g., absence of loss of consciousness during and immediately after ischemia), it was discarded (< 10% of animals). Body and temporalis muscle temperatures were maintained at approximately 37.0°C with a lamp and a heat pad when ischemia was induced, and the body temperature was monitored for 2 h after ischemia. Rolipram (ME3167; donated by Meiji Seika Kaisha, Tokyo, Japan) dissolved in distilled water with 2% dimethyl sulfoxide was injected intraperitoneally (10 ml/kg) at a dose of 0.3 mg/kg ($n = 6$) or 3 mg/kg ($n = 9$) 30 min before ischemia. Animals treated with vehicle ($n = 8$) were prepared simultaneously. Sham-operated animals ($n = 4$) were injected with the vehicle and were treated in the same manner except for clipping of the arteries.

After 7 days of survival, the brains were perfusion-fixed with 10% formalin in phosphate buffer (pH 7.4) under anesthesia with pentobarbital (50 mg/kg i.p.). After post-fixation in the same fixative overnight at 4°C, the brains were routinely embedded in paraffin, and coronal sections at the level of the dorsal hippocampus were cut at a thickness of 5 μm for histopathology. Neuronal density of the hippocampal CA1 subfield, i.e. the number of intact CA1 pyramidal neurons per 1-mm linear length of the pyramidal cell layer, was counted with a microscope using sections stained with cresyl violet as reported previously (Kirino et al., 1986). Briefly, photographs of left and right dorsal hippocampi were taken and the total linear length of the CA1 sector (approximately 2.5–3.0 mm) was measured. Using the photograph as a guide, the number of living neurons was counted under a microscope, beginning from the medial border of the CA1 to the lateral border of the CA1. Since the neuronal damage is seen similarly throughout the rostral-caudal extent of the dorsal hippocampus in this model (Kirino et al., 1986), one section from each animal was used for counting. The neuronal densities of left and right sides were averaged and expressed as mean values ± S.D. Statistical analysis was performed with the Kruskal-Wallis non-parametric analysis of variance followed by the Williams-Wilcoxon multiple rank sum test, and the analysis of variance followed by the Dunnett's multiple comparison test.

3. Results

There were no differences in body and cranial temperatures among three ischemic groups (Table 1). Post-ischemic hyperthermia was observed in all animals subjected to ischemia. Immediately after rolipram injection, mild hypothermia (−1 to 1.5°C) and hypoactivity, which were more severe in animals treated with

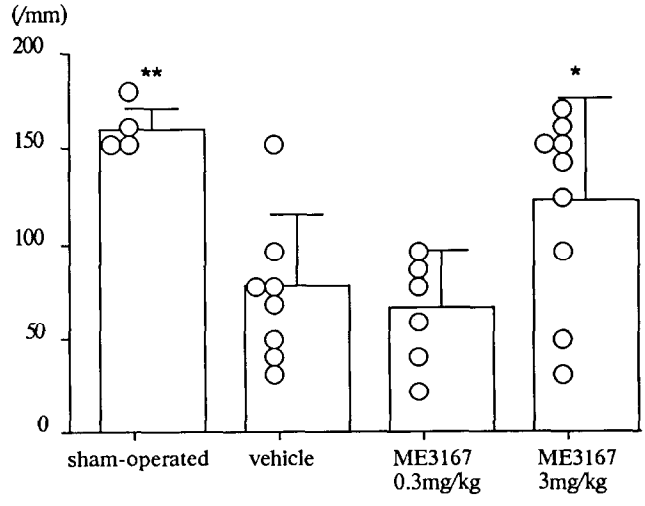

Fig. 1. Neuronal density (/mm) of the CA1 subfield of the hippocampus 7 days after 3 min of forebrain ischemia in the gerbil. Individual values and the mean ± S.D. are shown. * $P < 0.05$, ** $P < 0.01$ vs. vehicle-treated animals (Williams-Wilcoxon multiple rank sum test).

the higher dose, were seen as reported previously (Wachtel, 1982), but the hypothermia was adjusted by external heating. However, the hypothermic reactions were transient and external heating was not necessary after ischemia.

No neuronal damage was observed in sham-operated animals. Many CA1 pyramidal neurons were destroyed in the vehicle-treated animals 7 days after ischemia. The animals treated with 0.3 mg/kg of rolipram showed similar CA1 neuronal damage. In contrast, most of the CA1 neurons survived in animals treated with the higher dose (3 mg/kg) of rolipram. Neuronal densities of the hippocampal CA1 subfield were 160 ± 11.0 (157)/mm (mean $\pm$ S.D. (median)) in the sham-operated animals and 78 ± 37.1 (76)/mm ($P < 0.01$ vs. sham-operated animals) in the vehicle-treated animals. The gerbils treated with rolipram at the dose of 0.3 mg/kg and 3 mg/kg showed neuronal densities of 66 ± 30.6 (73)/mm (not significant vs. vehicle-treated animals) and 122 ± 54.0 (146)/mm ($P < 0.05$ vs. vehicle-treated animals), respectively, as shown in Fig. 1.

4. Discussion

In this study, we showed that rolipram protects against neuronal damage to the CA1 subfield of the hippocampus following transient cerebral ischemia. A 3-min period of ischemia destroyed more than 50% of the CA1 pyramidal neurons in the vehicle-treated animals by 7 days after ischemia. The lower dose of rolipram (0.3 mg/kg) failed to protect the CA1 neurons from ischemia. However, 76% of the CA1 neurons survived in animals treated with the higher dose of rolipram (3 mg/kg). As the body and cranial temperatures of the animals treated with rolipram were monitored and kept very similar to those of vehicle-treated animals, the neuroprotective effects of rolipram cannot be attributed to drug-induced hypothermia, which is a powerful way of neuroprotection against ischemic neuronal damage and is often induced by drug treatment (Buchan and Pulsinelli, 1990).

Because rolipram is a cyclic AMP-selective phosphodiesterase inhibitor, the mechanism of neuroprotection by rolipram treatment observed in this study may be explained by an increased level of brain cyclic AMP and activation of the cyclic AMP-dependent second messenger system. Actually, an increase in brain cyclic AMP levels after rolipram injection has been demonstrated (Schneider, 1984). Rolipram (0.3 mg/kg and 3 mg/kg) dose dependently increases the brain cyclic AMP levels in the rat. Therefore, we used these doses in this experiment on the basis of results of the earlier study. Rolipram at the dose of 0.3 mg/kg increases the cyclic AMP level but was not effective in this study. The reason for this failure may be that the lower dose of rolipram inhibited the enzyme only briefly.

An inhibitory neuromodulatory action of cyclic AMP may play a role in the inhibition of excitotoxic neurotransmission that leads to ischemia-induced neuronal damage (Rothman and Olney, 1986). Cyclic AMP or norepinephrine, when applied to cerebral cortical neurons, causes electrophysiological neuronal inhibition, and cyclic AMP functions as intracellular second messenger for norepinephrine (Stone et al., 1975). Furthermore, rolipram effectively increases the synthesis and release of norepinephrine in the brain, and potentiates noradrenergic transmission via inhibition of cyclic AMP degradation (Wachtel et al., 1986). These effects of rolipram may be one of the mechanisms of the neuroprotection observed in this study because norepinephrine protects hippocampal neurons against ischemia-induced neuronal damage (Koide et al., 1986). However, cyclic AMP is the second messenger system for many transmitters other than norepinephrine, and therefore, the link between cyclic AMP and neuroprotection should be studied further.

On the other hand, Hara et al. (1990) demonstrated that a cyclic AMP-dependent protein kinase inhibitor does not ameliorate ischemia-induced neuronal damage in the gerbil hippocampus. In their study, cerebral ischemia produced maximal damage to the CA1 subfield and no protective effect of the protein kinase inhibitor was observed. Their study suggests that the activation of the cyclic AMP-dependent protein kinase system does not play a role in the development of ischemia-induced neuronal death.

The CA1 subfield of the hippocampus has the highest density of [^{3}H]rolipram binding sites in the brain (Kaulen et al., 1989). Furthermore, [^{3}H]rolipram binding in the brain begins to decrease as early as 1 h after 10 min of ischemia in the gerbil, suggesting down-regulation of this enzyme after ischemia (Araki et al., 1993). However, recovery from the reduced [^{3}H]rolipram binding was observed in ischemia-resistant regions, but severe reductions occurred in the regions where neurons were destroyed. The pathogenesis of reduced [^{3}H]rolipram binding in the brain following ischemia remains to be elucidated, but these observations suggest implication of cyclic AMP and its related second messenger system in cerebral ischemia.

In conclusion, this study showed that rolipram has a protective effect against ischemia-induced neuronal damage in the gerbil. The neuroprotective effects may be related to an increased level of cyclic AMP and the activation of its second messenger system. Further studies are warranted and the findings of the present study suggest that rolipram has a potential neuroprotective effect in ischemic stroke in man.

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