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Short communication

Late preconditioning in rat retina: involvement of adenosine and ATP-sensitive K^+ channel

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Abstract

To determine whether stimulation of adenosine receptors and opening of ATP-sensitive K^+ channels were involved in the protective effect of late preconditioning in the rat retina, rats were subjected to 60 min of retinal ischemia, and ischemic preconditioning was achieved by applying 5 min of ischemia 24 h before 60 min of ischemia. In non-preconditioned rats, cell loss in the ganglion cell layer and thinning of the inner plexiform and inner nuclear layer were observed 7 days after 60 min of ischemia. Ischemic preconditioning completely prevented the retinal tissue damage and 8-phenyltheophylline or 5-hydroxydecanoate reduced the protective effect of ischemic preconditioning. Therefore, stimulation of adenosine receptors and opening of ATP-sensitive K^+ channels might be involved in the mechanism of histological protection by late preconditioning in the retina. © 2001 Published by Elsevier Science B.V.

Keywords: Retinal ischemia; Reperfusion; K^+ current; Adenosine receptor

1. Introduction

Several retinal diseases, for example glaucoma and diabetic retinopathy, lead to neuronal cell death. The mechanism of cell death induced by retinal ischemia is not completely understood. Endogenous substrates such as glutamate, oxygen-free radicals, nitric oxide and Ca^{2+} are regarded as the pathophysiological cause of ischemia–reperfusion injury in the retina (Choi, 1995). It has been reported that N^{ω}-nitro-L-arginine, a nitric oxide synthase inhibitor, and dizocilpine (MK-801), an NMDA-receptor inhibitor, reduced ischemia–reperfusion injury in retina (Adachi et al., 1998). However, these compounds have little protective effect on the injury in inner plexiform layer (Adachi et al., 1998), and were not suitable for clinical use due to their pharmacological and toxicological properties.

Brief period of ischemia and reperfusion protects heart (Murry et al., 1986) and brain (Kitagawa et al., 1990) against subsequent ischemia and reperfusion and this phenomenon is known as ischemic preconditioning. The protective effects of "classical" ischemic preconditioning, i.e. early ischemic preconditioning, occur within a few minutes of its application and dissipate within 2 h in heart (Murry et al., 1991). In addition, ischemic stress may also initiate a slower form of adaptation after the early ischemic preconditioning has worn off, i.e. the late ischemic preconditioning (Marber et al., 1993). Because preconditioning harnesses the endogenous protective action of the tissue, the research of ischemic preconditioning is very important for the search for effective methods to protect the retinal tissue against ischemia–reperfusion injury. Recently, late ischemic preconditioning was reported to occur also in rat retina (Roth et al., 1998; Li and Roth, 1999; Li et al., 2000).

The mechanism of late preconditioning in the retina has not yet been clarified completely. According to the previous reports on preconditioning in the heart (Richard et al., 1996) and the brain (Heurteaux et al., 1995), stimulation of adenosine receptors and opening of ATP-sensitive K^+ channels are involved in the mechanism of late ischemic preconditioning. Experiments using electroretinogram indicated that adenosine A_1 and A_{2A} receptors and ATP-sensitive K^+ channels were critical components in the induction of ischemic tolerance by late preconditioning in the rat

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retina (Li and Roth, 1999; Li et al., 2000). However, there is no histological data showing protective effect of late ischemic preconditioning in the retina.

The aim of the present study was to determine whether stimulation of adenosine receptors and opening of ATP-sensitive K^+ channels are involved in protection by late ischemic preconditioning examined by histological methods in the rat retina. We tested the effects of 8-phenyltheophylline, an adenosine receptor antagonist, and 5-hydroxydecanoate, an ATP-sensitive K^+ channel blocker, on the neuroprotection by late ischemic preconditioning.

2. Materials and methods

2.1. Animals and induction of retinal ischemia

In the present study, experimental procedures conformed to the Guiding Principles for the Care and Use of Laboratory Animals, approved by the Japanese Pharmacological Society. Male Sprague–Dawley rats weighing 230–300 g (Japan SLC, Hamamatsu, Japan) were anesthetized with pentobarbital sodium (50 mg/kg i.p.; Nembutal® injection, Abbott Laboratories, North Chicago,

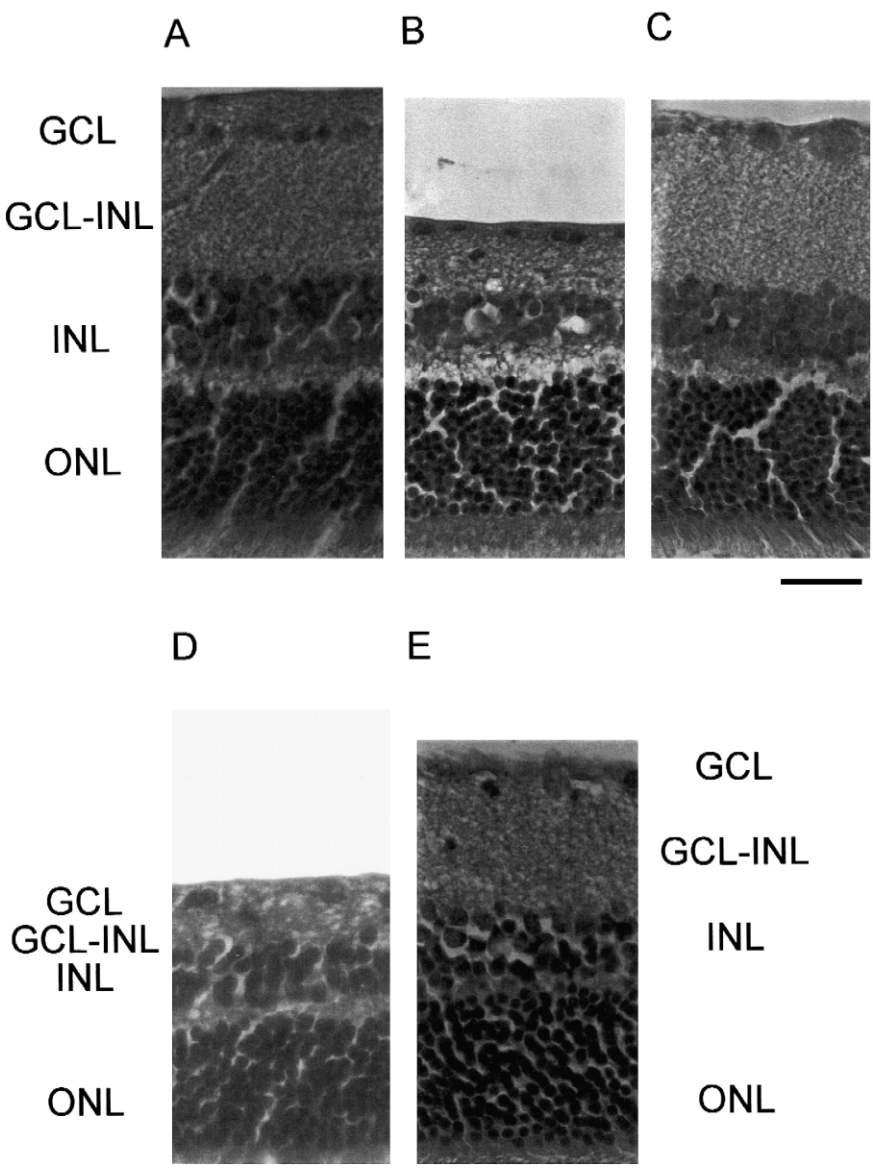

Fig. 1. Representative photomicrographs showing histological appearance of the non-ischemic control (A), and ischemic retinae 7 days after 60 min of ischemia (B, C, D). Severe damage is shown in the ganglion cell layer (GCL), the inner plexiform layer (GCL–INL) and the inner nuclear layer (INL) of the non-preconditioned retina, whereas the outer nuclear layer (ONL) is not injured (B). In the late preconditioned group, retinal structure is completely preserved (C). The protection by preconditioning is almost totally blocked by 10 mg/kg 5-hydroxydecanoate (D). The histological protection by late preconditioning is blocked by 10 mg/kg 8-phenyltheophylline in GCL, not but in GCL–INL or INL (E). Scale bar = 50 μm. Original magnification is ×200.

IL), and the body temperature of animals was maintained at 37°C during experiments. The anterior chamber of the one eye, the pupil of which had been dilated with 1% atropine sulfate (Nihon Tengan-yaku, Nagoya, Japan), was cannulated with a 27-gauge needle connected to a bottle filled with saline. Retinal ischemia was induced by raising intraocular pressure to 130 mm Hg by lifting the bottle for 60 min. The opposite eye of each animal served as a non-ischemic control.

In the present study, there were 12 experimental groups: vehicle of 5-hydroxydecanoate ($n = 5$), vehicle of 5-hydroxydecanoate—preconditioning ($n = 5$), 10 mg/kg 5-hydroxydecanoate ($n = 5$), 2.5 mg/kg 5-hydroxydecanoate—preconditioning ($n = 10$), 5 mg/kg 5-hydroxydecanoate—preconditioning ($n = 5$), 10 mg/kg 5-hydroxydecanoate—preconditioning ($n = 6$), vehicle of 8-phenyltheophylline ($n = 5$), vehicle of 8-phenyltheophylline—preconditioning ($n = 5$), 10 mg/kg 8-phenyltheophylline ($n = 5$), 1 mg/kg 8-phenyltheophylline—preconditioning ($n = 9$), 3 mg/kg 8-phenyltheophylline—preconditioning ($n = 8$), 10 mg/kg 8-phenyltheophylline—preconditioning ($n = 7$).

In the preconditioning group, rats were subjected to 5 min of ischemia followed by 24 h of reperfusion and 60 min of sustained ischemia. The non-preconditioned groups were subjected to 5 min of sham preconditioning 24 h before 60 min of ischemia.

All drugs were administered intravenously 15 min before preconditioning or sham preconditioning. We dissolved 5-hydroxydecanoate (2.5, 5, and 10 mg/kg) in

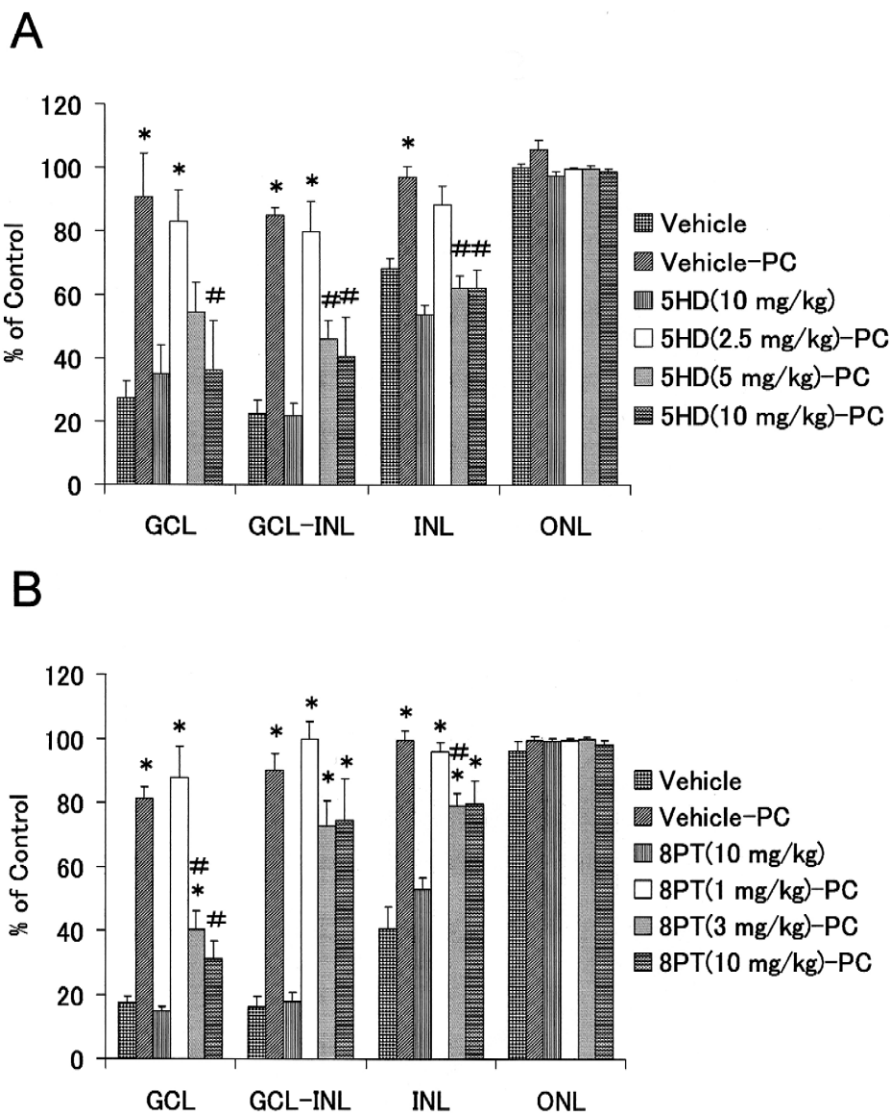

Fig. 2. Effect of late ischemic preconditioning (PC) on morphology 7 days after 60 min of ischemia. The following four parameters of the ischemic eyes were normalized to those of the intact eyes (opposite side of the ischemic eye) and are presented as percentages: linear cell density in the ganglion cell layer (GCL); thickness of the inner plexiform layer (GCL–INL), the inner nuclear layer (INL) and the outer nuclear layer (ONL). (A) The 5-hydroxydecanoate (5HD)-treated group. (B) The 8-phenyltheophylline (8PT)-treated group. $^{*}P < 0.05$ vs. non-preconditioned retina. $^{\#}P < 0.05$ vs. preconditioned retina.

saline and 8-phenyltheophylline (1, 3 and 10 mg/kg) in a solution of dimethylsulfoxide (DMSO):1 N NaOH:saline = 1:1:8. According to the previous reports (Hide and Thiemermann, 1996; Miura et al., 1992), the maximum doses of the drugs used in the present study are considered to be sufficient for blocking their target receptors or channels. We found in preliminary experiments that the doses of the drugs used in the present study did not affect blood pressure and heart rate.

2.2. Histological evaluation

Animals were sacrificed by overdosage of pentobarbital sodium 7 days after 60 min of ischemia and both eyes were enucleated. Enucleated eyes were fixed with 1% glutaraldehyde and 4% formalin for 24 h at 4°C. Horizontal sections through the optic disk of the eye were subjected to morphometry. Retinal specimens were embedded in paraffin, sectioned 5 μm thick, stained with hematoxylin and eosin, and examined by a light microscope.

Values were obtained as an average of measurements in five adjacent areas within 1 mm of the optic disk in the inferior peripapillary region. Measurements were performed in the same topographic region of the retina to avoid possible regional anatomic variations in the results. In a blind fashion, we determined thickness of the inner plexiform layer, the inner nuclear layer and the outer nuclear layer. Manual cell counts of the ganglion cell layer were performed across a length of 100 μm. These parameters of each eye subjected to ischemia were normalized with those of the corresponding intact opposite eyes and are presented as percentages.

2.3. Statistical analysis

The data were expressed as mean ± S.E.M. One-way analysis of variance followed by Bonferroni's multiple *t*-test was used for multiple comparisons. Differences were considered to be statistically significant when the P values were less than 0.05.

3. Results

Fig. 1A is a representative photomicrograph of the non-ischemic control group. Application of ischemia for 60 min induced typical histopathological changes in the saline-treated and non-preconditioned retina. Marked reduction of the cell density of the ganglion cell layer and the thinning of the inner plexiform and the inner nuclear layer were observed. The thickness of outer nuclear layer did not change (Figs. 1B and 2A). In contrast, preconditioning dramatically reduced these ischemia-induced retinal damages (Figs. 1C and 2A).

Intravenous injection of 5-hydroxydecanoate did not affect the retinal damage induced by 60 min of ischemia. A dose of 2.5 mg/kg of 5-hydroxydecanoate did not affect histological protection induced by preconditioning. 5-Hydroxydecanoate (5 mg/kg) blocked the protective effect partially in the ganglion cell and the inner plexiform layer, and completely in the inner nuclear layer. 5-Hydroxydecanoate (10 mg/kg) inhibited the protective effect completely in the ganglion cell layer and the inner nuclear layer, and partially in the inner plexiform layer (Figs. 1D and 2A).

Effect of 8-phenyltheophylline on the protective effects of preconditioning are also shown in Figs. 1E and 2B. Vehicle (DMSO:1 N NaOH:saline = 1:1:8) did not affect the protective effects of preconditioning (Fig. 2B). Intravenous injection of 10 mg/kg did not change the histological damage induced by 60 min of retinal ischemia. 8-Phenyltheophylline (1 mg/kg) did not affect the histological protection induced by preconditioning. Although 3 and 10 mg/kg 8-phenyltheophylline have little effects on the histological protection observed in the inner plexiform and the inner nuclear layer by late preconditioning (Figs. 1E and 2B), they decreased preconditioning effect on preservation of the cell number in the ganglion cell layer.

4. Discussion

In the present study, we confirmed development of late ischemic preconditioning in the rat retina and obtained data suggesting that stimulation of adenosine receptors and opening of ATP-sensitive K^+ channels are involved in the mechanism underlying this phenomenon. Therefore, the mechanism of developing late preconditioning seems, at least in part, common among the heart, brain and retina.

In the present study, 8-phenyltheophylline completely inhibits preconditioning effect only in the ganglion cell layer. However, the blocking effect of 8-phenyltheophylline on the inner and the outer nuclear layer is weaker than that on the ganglion cell layer. These data suggest that the drug hardly reaches the inner retina due to their pharmacokinetic properties, or the cell layer in the retina may have its own endogenous mechanisms of the late ischemic preconditioning. Though experiments using electroretinogram indicated that adenosine A_1 and A_{2A} receptor antagonists inhibit late preconditioning completely in the rat retina (Li and Roth, 1999), there is no contradiction between the data in the previous report and those in the present study. The ganglion cell layer contains ganglion cells, which transmit the visual information from neurons in other retinal layers to brain. Even if other retinal layers should be intact, the damage of ganglion cells leads to the loss of visual function.

Increase in extracellular adenosine concentration has been demonstrated after a brief ischemic insult in the heart (Mei et al., 1998) and retina (Roth et al., 1997). However, it is currently unknown how stimulation of adenosine receptors leads to protection against ischemia–reperfusion

injury. In heart muscle cells, activation of protein kinase C induced by adenosine A_1 receptors (Henry et al., 1996) is thought to be necessary for the protective effect of late ischemic preconditioning (Richard et al., 1996). Further study is necessary to clarify whether activation of protein kinase C induced by stimulation of adenosine receptor is needed for the protective effect of late ischemic preconditioning in rat retinal cells.

In the present study, 5-hydroxydecanoate almost completely inhibited the protective effect of late ischemic preconditioning in rat retina. Therefore, opening of ATP-sensitive K^+ channels is involved in the mechanism of late ischemic preconditioning in rat retina. ATP-sensitive K^+ channels are known to exist on the sarcolemmal and mitochondrial membrane (Inoue et al., 1991). Recently, 5-hydroxydecanoate was reported to block specifically mitochondrial ATP-sensitive K^+ channel in rabbit heart muscle cells (Liu et al., 1998). However, 5-hydroxydecanoate has been reported to block sarcolemmal ATP-sensitive K^+ channel in the heart of different animal species, such as guinea-pig (Notsu et al., 1992; Sakamoto et al., 1998) and dog (Miyoshi et al., 1996). Therefore, the study about the electrophysiological property of 5-hydroxydecanoate in retina is needed to determine the target of the inhibitory effect of the very drug on late ischemic preconditioning.

Based on the effect of cycloheximide, involvement of de novo protein synthesis has been suggested in the late preconditioning in the rat retina (Roth et al., 1998). Probably, the protective effect induced by the late preconditioning in the heart may be related to the expression of stress proteins, such as heat shock proteins, superoxide dismutase and catalase (Richard et al., 1996). Further studies that clarify the mechanism of the late ischemic preconditioning in the retina will provide clinically useful strategies for treatment of ischemic retinal diseases, such as glaucoma.

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