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Short communication

Preventive effect of long-chain fatty alcohol on ischemia–reperfusion injury in the rat bladder

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Abstract

We attempted in the present study to clarify the preventive effects of cyclohexenonic long-chain fatty alcohol on ischemia–reperfusion injury in the rat bladder. Rat bladders were exposed to 30 min of ischemia and a subsequent 30 min of reperfusion with or without several doses of cyclohexenonic long-chain fatty alcohol (0.5, 2, 8 mg/kg). Muscle-bath studies were performed, and malonaldehyde concentrations were measured in the bladder. Bladder dysfunction and lipid peroxidation caused by ischemia–reperfusion were prevented by cyclohexenonic long-chain fatty alcohol in a dose-dependent manner. Our data indicate that cyclohexenonic long-chain fatty alcohol can prevent the production of free radicals and ischemia–reperfusion injury in the bladder.

Keywords: Bladder; Long-chain fatty alcohol; Ischemia–reperfusion injury; Free radical; Malonaldehyde

1. Introduction

It is well known that patients with benign prostatic hyperplasia and those with acute and/or chronic urinary retention are often observed to have bladder dysfunction and bladder instability. The etiology of this mechanism may be due, at least in part, to bladder ischemia and subsequent reperfusion in the bladder (Levin et al., 1998; Greenland and Brading, 2001; Parekh et al., 2001). We have reported that ischemia and subsequent reperfusion significantly damage bladder function, as measured by organ-bath techniques and histological study (Saito et al., 1998; Saito and Miyagawa, 1999). We have also demonstrated that peroxynitrite, reacting with NO and superoxide radicals, plays an important role as a cell/tissue-damaging agent during ischemia–reperfusion in the rat bladder (Saito et al., 1998; Saito and Miyagawa, 1999, in press). It has recently been reported that, after overdistension, catheterization induces reperfusion injury in the bladder and that reactive oxygen species are one of the main contributing factors in this injury (Lin et al., 2000; Saito and Miyagawa, 2001). Based on these reports, it appears that one of the most important issues in preventing bladder dysfunction in these patients is to reduce the production of free radicals in the bladder.

The tropical plant, *Hygrophilia erecta* Hochr., has been shown to contain some cyclohexenonic long-chain fatty alcohol that have neurotrophic activities on cultured neurons from the cerebral cortex (Borg et al., 1987, 1990). The C26-alcohol, *n*-hexacosanol, has been found to directly increase neurite extension as well as biochemical differentiation of these neurons directly. It has also been reported that the peripheral administration of this compound prevents neuronal death in rat brain (Borg et al., 1987, 1990; Luu et al., 2000). These findings are particularly interesting because long-chain fatty alcohols have been shown to be synthesized by the rat brain as well as by sciatic nerves during development (Luu et al., 2000). Watanabe and Miyagawa (2002) recently reported that cyclohexenonic long-chain fatty alcohol has beneficial effects on peripheral neuropathy and cystopathy in streptozotocin-induced diabetic rats. Based on these reports, cyclohexenonic long-chain fatty alcohol seems to have various pathophysiological effects on many different conditions.

We now studied the preventive effects of cyclohexenonic long-chain fatty alcohol on ischemia–reperfusion injury in the rat bladder. Furthermore, we attempted to measure malonaldehyde as a marker of lipid peroxidation in the bladder (Saito and Miyagawa, 2001).

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2. Materials and methods

2.1. Production of the animal model

All animal experiments were performed in accordance with the guidelines set by the Tottori University Committee for Animal Experimentation. To investigate bladder function in vitro, 8-week-old male Wistar rats weighing 250–300 g (SLC, Shizuoka, Japan) were divided into five groups: those receiving 30 min of ischemia followed by 30 min with or without treatment with cyclohexenonic long-chain fatty alcohol, 3-(15-hydroxypentadecyl) 2,4,4-trimethyl cyclohex 2-en 1-one (0.5, 2, 8 mg/kg), and age-matched control groups (in each group, $n=6–8$). The urinary bladders were subjected to ischemia–reperfusion as described previously (Saito et al., 1998; Saito and Miyagawa, 1999). Under pentobarbital anesthesia (50 mg/kg, i.p.), the abdominal aorta was clamped just above its bifurcation, using a small clip (Sugita standard aneurysm clip, holding force 145 g; Mizuho Ikakogyo, Tokyo), for 30 min. To remove the clip, reperfusion in the bladder was performed. Our previous study had demonstrated that blood flow in the bladder of aorta-clamped rats is approximately 10% that of controls (Saito and Miyagawa, 1999). In the cyclohexenonic long-chain fatty alcohol-treated groups, cyclohexenonic long-chain fatty alcohol, 3-(15-hydroxypentadecyl) 2,4,4-trimethyl cyclohex 2-en 1-one (0.5, 2, 8 mg/kg) was injected intraperitoneally 30 min before the induction of ischemia.

2.2. Measurement of contractile response

The contractile function of the bladder muscles was examined as described previously (Saito et al., 1998; Saito and Miyagawa, 1999, 2001, in press). Just after ischemia–reperfusion, the bladder dome was removed to yield smooth muscle strips which were then mounted in 3.0-ml organ baths and maintained at 37 °C in Krebs–Henseleit solution gassed with 95% O_2 and 5% CO_2. The K–H solution had the following composition in millimoles/liter: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 24.9; KH_2PO_4, 1.2; glucose, 5.6; and sodium pyruvate, 2.0. Isometric force was monitored with a TB-612T transducer (Nihon Koden, Tokyo) and processed by an AP-621G (Nihon Koden, Tokyo). The contractile response to carbachol is expressed as the force per cross-sectional area of muscle (g/mm^2) (Saito et al., 1998). Cumulative dose-response curves were made in a stepwise manner after the response to the previous concentration had reached a plateau. The contractile responses of the same muscle strips to 100 mM KCl were also monitored (in each group, $n=6–8$).

2.3. Measurement of malonaldehyde

For biochemical studies, the rest of the bladder was chopped, frozen in liquid nitrogen and stored at −80 °C until used. Malonaldehyde concentrations were measured by colorimetric assay in bladders from these five groups as described in our previous report (Saito and Miyagawa, 2001).

2.4. Data analysis

The data for contractions were calculated as grams of active force per cross-sectional area in square millimeters. The cross-sectional area was calculated using the following equation:

$$\text{cross–sectional area} = \text{weight}/(\text{length} \times 1.05),$$

where 1.05 is the assumed density of the muscle (Saito et al., 1998; Saito and Miyagawa, 1999, 2001). ED_{50} values (the concentration of agonist producing half-maximal contractile responses) were calculated as geometric means, whereas E_{max} values were calculated as arithmetic means. Statistical comparison of differences between groups was performed using analysis of variance and Fisher's multiple comparison test. $P<0.05$ was regarded as the level of significance.

2.5. Drugs and chemicals

3-(15-Hydroxypentadecyl) 2,4,4-trimethyl cyclohex 2-en 1-one was obtained from Meiji Milk Products, Tokyo, Japan. Carbachol and pentobarbital were purchased from Sigma (St. Louis, MO). A kit for colorimetric assay of lipid peroxidation was purchased from OXIS International (Portland, OR). All other chemicals were of reagent grade.

3. Results

3.1. Measurement of contractile response

The E_{max} and ED_{50} values for the contractile response of bladder strips to carbachol and the response to KCl (100 mM) were determined, and the results are shown in Table 1. Bladder dysfunction caused by ischemia–reperfusion was prevented by cyclohexenonic long-chain fatty alcohol in a dose-dependent manner. E_{max} values for the response of

Table 1
Functional data for ischemia–reperfusion of rat bladder

	E_{max}, g/mm^2	pED_{50}, M	KCl (100 mM), g/mm^2
Control	9.0 ± 0.9	6.19 ± 0.09	6.2 ± 0.6
I–R	3.2 ± 0.4[a]	5.57 ± 0.30[a]	2.8 ± 0.4[a]
I–R (FA;0.5)	4.5 ± 0.5[a]	6.26 ± 0.11[b]	3.0 ± 0.4[a]
I–R (FA;2)	5.4 ± 0.4[a,b]	6.19 ± 0.05[b]	3.9 ± 0.3[a,b]
I–R (FA;8)	6.5 ± 0.5[a,b]	6.10 ± 0.09[b]	3.9 ± 0.4[a,b]

Data are shown as means ± S.E.M. of six to eight separate determinations in each group. E_{max} and pED_{50} values for carbachol effect in experimental rat bladder strips. I–R; 30-min ischemia + 30-min reperfusion group. I–R (FA; 0.5, 2, 8) indicate I–R groups treated with cyclohexenonic long-chain fatty alcohol, 0.5, 2, 8 mg/kg, respectively.

[a] Significantly different from control group.

[b] Significantly different from I–R group.

Table 2
Measurement of malonaldehyde production in the experimental bladders

	Malonaldehyde (μM/g tissue)	Malonaldehyde (nM/mg protein)
Control	6.1 ± 1.4	2.5 ± 0.4
I–R	14.2 ± 2.5[a]	7.6 ± 1.5[a]
I–R (FA;0.5)	10.1 ± 1.0[a]	4.9 ± 0.6[a]
I–R (FA;2)	8.1 ± 1.9[b]	3.9 ± 0.7[b]
I–R (FA;8)	6.5 ± 0.9[a]	3.1 ± 0.5[b]

Data are shown as means ± S.E.M. of six to eight separate determinations in each group. I–R; 30-min ischemia + 30-min reperfusion group. I–R (FA; 0.5, 2, 8) indicate I–R groups treated with cyclohexenonic long-chain fatty alcohol, 0.5, 2, 8 mg/kg, respectively.
[a] Significantly different from control group.
[b] Significantly different from I–R group.

bladder strips to carbachol for the control, ischemia–reperfusion without cyclohexenonic long-chain fatty alcohol, and with 0.5, 2, and 8 mg/kg cyclohexenonic long-chain fatty alcohol groups were 9.0 ± 0.9, 3.2 ± 0.4, 4.5 ± 0.5, 5.4 ± 0.4, and 6.5 ± 0.5 g/mm^2, respectively. We have previously shown that reperfusion (30 min) produced the most significant reduction in contractile response to carbachol in the rat bladder (Saito et al.,1998). The treatment with cyclohexenonic long-chain fatty alcohol (0.5 mg/kg) reduced the extent of reperfusion injury slightly but not significantly. Treatment with cyclohexenonic long-chain fatty alcohol of 2 and 8 mg/kg, however, significantly prevented reperfusion injury. ED_{50} values of the ischemia–reperfusion group without cyclohexenonic long-chain fatty alcohol treatment were significantly greater than those of the other group (Table 1). In all groups, the contractile response induced by 100 mM KCl was similar to the E_{max} value for the response to carbachol. Our data suggest that the insult of ischemia and consequent reperfusion weaken the response of bladder smooth muscle to carbachol or KCl, and that this can be partially prevented by treatment with cyclohexenonic long-chain fatty alcohol.

3.2. Measurement of malonaldehyde

Malonaldehyde concentrations in bladder tissue from the control, ischemia–reperfusion without cyclohexenonic long-chain fatty alcohol, with 0.5, 2, and 8 mg/kg cyclohexenonic long-chain fatty alcohol groups were 6.1 ± 1.4, 14.2 ± 2.5, 10.1 ± 1.0, 8.1 ± 1.9, 6.5 ± 0.9 μM/g tissue, and were 2.5 ± 0.4, 7.6 ± 1.5, 4.9 ± 0.6, 3.9 ± 0.7, 3.1 ± 0.5 nM/mg protein, respectively (Table 2). Our data indicate that lipid peroxidation in the experimental bladder is prevented by treatment with cyclohexenonic long-chain fatty alcohol in a dose-dependent manner.

4. Discussion

We have demonstrated in the present study the preventive effects of cyclohexenonic long-chain fatty alcohol on ischemia–reperfusion injury in the rat bladder. Bladder dysfunction and lipid peroxidation in the bladder are caused by ischemia–reperfusion in the bladder, and this injury can be partially prevented by cyclohexenonic long-chain fatty alcohol in a dose-dependent manner. Malonaldehyde production, a marker of lipid peroxidation, is also reduced by cyclohexenonic long-chain fatty alcohol treatment in a dose-dependent manner.

Recently, Watanabe and Miyagawa (2002) have reported that cyclohexenonic long-chain fatty alcohol has beneficial effects on peripheral neuropathy and cystopathy in streptozotocin-induced diabetic rats. The mechanism that investigators suggested involves the preventive effects of the excessive flux of sugar alcohols (polyols) in Schwann cells and axons, nerve-trunk ischemia, hypoxia and oxidative stress, non-specific glycosylation of important structural nerve proteins such as neurofilaments, and specific deficiencies of neurotrophins or non-neurotrophin growth factors. Thus, cyclohexenonic long-chain fatty alcohol appears to play a variety of roles in pathophysiological conditions.

The bladder dysfunction seen after acute overdistension and decompression is likely to be due to ischemia–reperfusion. Lin et al. (2000) have demonstrated that acute overdistension and the subsequent decompression in the bladder induce bladder dysfunction and enhance lipid peroxidation. This bladder dysfunction can be prevented by mannitol, a free-radical scavenger. We have also demonstrated that bladder dysfunction is induced by urinary retention and subsequent catheterization, and that bladder dysfunction following catheterization is, in part, caused by free radicals (Saito and Miyagawa, 2001). Based on previous reports, one of the most important things in prevention of bladder dysfunction is to reduce the production of free radicals in the bladder.

We have demonstrated in the present study that ischemia–reperfusion leads to bladder dysfunction as well as to the induction of lipid peroxidation. Both bladder dysfunction, which was evaluated in organ-bath studies, and the induction of lipid peroxidation, which was evaluated from the malonaldehyde production, are prevented by cyclohexenonic long-chain fatty alcohol in a dose-dependent manner. Our data suggest that cyclohexenonic long-chain fatty alcohol works as a radical scavenger on ischemia–reperfusion injury in the bladder. The physiological and pharmacological role of cyclohexenonic long-chain fatty alcohol, however, remains unclear and warrants further study.

5. Conclusions

(1) Ischemia induced by clamping of the rat abdominal aorta causes a reduction in contractile responses to carbachol of the bladder dome, and subsequent reperfusion causes additional damage to smooth muscle, which is partially prevented by treatment with cyclohexenonic long-chain fatty alcohol; (2) lipid peroxidation upon ische-

mia–reperfusion injury can be prevented by treatment with cyclohexenonic long-chain fatty alcohol; and (3) cyclohexenonic long-chain fatty alcohol works as a radical scavenger on ischemia–reperfusion injury.

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