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Effect of cyclohexenonic long-chain fatty alcohol on rat overactive bladder induced by bladder neck obstruction

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Abstract

We attempted to clarify the preventive effects of cyclohexenonic long-chain fatty alcohol on detrusor overactivity induced by mild bladder neck obstruction. Bladder neck obstruction was created by partial ligation of the urethra. Female Sprague–Dawley rats were divided into three groups: those with bladder neck obstruction treated without long-chain fatty alcohol, those with bladder neck obstruction with long-chain fatty alcohol (8 mg/kg, i.p., every day) and the sham-operated control group (A, B, and C groups, respectively). Six weeks after the induction of bladder neck obstruction, voiding behavior was observed in the metabolic cage, and a cystometrogram was performed in the experimental animals. Furthermore, Hematoxylin and Eosin, Azan-Mallory, and Bodian stainings were performed in these bladders. Bladder weight, voiding behaviors and a cystometry indicated that rats in the A group showed detrusor overactivity, which was improved by treatment with long-chain fatty alcohol. The proportion of connective tissue and the density of bundles of neurofibers in the bladder of the A group was significantly less than that in the other bladders. Mild bladder neck obstruction induces detrusor overactivity, which is improved by treatment with long-chain fatty alcohol.

Keywords: Bladder; Long-chain fatty alcohol; (Rat); Bladder neck obstruction; Detrusor overactivity

1. Introduction

Although benign prostate hyperplasia is common in elderly males, its etiology and clinical symptoms are not fully understood (MacConnell, 1998). Common symptoms of benign prostate hyperplasia include increased urinary frequency, urgency, nocturia, and dysuria. The clinical manifestations of benign prostate hyperplasia are thought to be primarily due to bladder neck obstruction, which may be caused by compression of the prostatic urethra (Lepor and Machi, 1992; MacConnell, 1998). However, the mechanisms of bladder neck obstruction remain unclear. Moreover, studies have shown a poor correlation between clinical symptoms of the lower urinary tract and either prostate volume or histological prevalence of prostate hyperplasia (Dorflinger et al., 1988; Bosh et al., 1995). Clinically, it is well known that benign prostate hyperplasia induces urinary frequency and myogenic bladder overactivity. Morphological examination of the bladder after obstruction reveals increased collagen production and smooth muscle hypertrophy in the bladder. Steers et al. (1991) reported that some of the neuro-

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anatomical changes result from increased levels of nerve growth factor (NGF) in the bladder. Autoimmunization to NGF is reported to prevent some of the morphologic alterations (Steers, 1994). Thus, peripheral neurons play an important role in the bladder dysfunction induced by bladder neck obstruction.

The tropical plant, *Hygrophilia erecta* Hochr., has been shown to contain some cyclohexenonic long-chain fatty alcohols 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 the neurite extension as well as the biochemical differentiation of these neurons. It has also been reported that the peripheral administration of this compound prevents neuronal death in the rat brain (Borg et al., 1987, 1990; De Arguir et al., 2001; Luu et al., 2000). These reports indicated that 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). We have reported the preventive effect of long-chain fatty alcohol on diabetes-induced neuropathy and ischemia–reperfusion injury in the rat bladder (Watanabe and Miyagawa, 2002; Saito et al., 2002). Our previous reports indicate that cyclohexenonic long-chain fatty alcohol has a beneficial effect on not only central nerve system disorders, but also peripheral neuropathy. In this study, we investigated the preventive effect of cyclohexenonic long-chain fatty alcohol on rat detrusor overactivity induced by mild bladder neck obstruction.

2. Material 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. Eight-week-old female Sprague–Dawley rats weighing 240–260 g (SLC, Shizuoka, Japan) were used in this study. A modification of the technique described by Mattiasson and Uvelius (1982) was used to produce mild partial bladder neck obstruction. After anesthesia with intraperitoneal sodium pentobarbital (50 mg/kg), the abdomen was opened through a midline suprapubic incision, and the bladder and the proximal urethra were exposed. A constant degree of urethra obstruction was produced by tying a ligature of 2–0 silk around the urethra and a catheter with an outer diameter of 1.70 mm, and then the catheter was removed. To investigate bladder function in vitro, the rats were divided randomly into three groups: Those receiving surgical treatment without or with treatment with cyclohexenonic long-chain fatty alcohol, 3-(15 hydroxypentadecyl)-2,4,4-trimethyl-2-cyclohexen 1-one (8 mg/kg), and age-matched sham-operated control groups (in each group, n=6–8) (A, B and C groups, respectively). In the B group, cyclohexenonic long-chain fatty alcohol, 3-(15 hydroxypentadecyl)-2,4,4-trimethyl-2-cyclohexen 1-one (8 mg/kg) was injected i.p. every day, since our previous reports indicated that this dose was effective in causing a significant improvement in the nerve damage induced by ischemia–reperfusion or diabetes mellitus in the rat urinary bladder (Watanabe and Miyagawa, 2002; Saito et al., 2002). Six weeks after the surgery, micturition behavior in the metabolic cage, and cystometry were performed. Immediately after these experiments, all rats were sacrificed and their bladders were removed and used for histological examination.

2.2. Voiding behavior studies

The rats were placed in a metabolic cage containing a urine collection funnel and placed over an electronic balance (HF200, A.N.D., Tokyo, Japan) to measure micturition behavior. The balances were connected to a personal computer (Macintosh 5300 CS, Apple Computer, Cupertino, CA) via a multiport controller (Maclab/400, AD Instruments, Castle Hill, Australia) to monitor the cumulative weight of the collected urine. All rats received water ad libitum when initially placed in the cage. Each monitoring period started at 18:00. The following parameters of the micturition reflex were obtained: urine volume per micturition, micturition frequency and total urine output.

2.3. Cystometrogram

Cystometric studies were performed according to Watanabe and Miyagawa (2002). A cystometry was performed under urethane anesthesia (1.0 g/kg, subcutaneously). In short, rat's abdomen was opened through lower midline incision and bladder was exposed, and the cystometry was done using a 24-G catheter inserted into the apex of the bladder dome for the purpose of recording pressure and filling the bladder with physiological saline (0.9% NaCl). External bladder filling was carried out using an infusion pump (5200, TOP, Tokyo) at a constant rate of 0.4 ml/min until micturition was detected. A cystometory catheter was connected to an external pressure transducer (P2310, Gould, Eastlake, OH) for the measurement of intravesical pressure. Intravesical pressure was recorded on the personal computer (Macintosh 5300 CS, Apple Computer) via a bridge amplifier (ML112, AD Instruments) and multiport controller (Maclab/400). The following parameters were evaluated: bladder capacity, bladder compliance, maximum detrusor pressure during void, voided volume and residual urine volume. The maximum detrusor pressure was defined as instantaneous pressure minus post-contraction resting pressure. In each animal, approximately 10–12 voiding cycles were recorded and then the means of the voiding were calculated.

2.4. Histological examination of the rat bladder

The bladders were cut free of surrounding tissue and weighed on a Mettler Basbal scale (Delta Range, Tokyo). After each bladder was transected at the level of the ureteral orifice, it was immediately fixed with 20% formalin. After fixation, the tissues were embedded in paraffin. Five micron-thick tissue sections were cut from these paraffin blocks. All of the bladder specimens were stained using Hematoxylin and Eosin (H&E) staining in accordance with previous reports (Saito et al., 1998; Saito and Miyagawa, 1999), Azan-Mallory, and Bodian staining. Each section was viewed under a light microscope at a magnification of 40–400.

2.5. Color-assisted quantitative image analysis

The stained tissue sections were viewed under a microscope (BX50, Olympus, Tokyo, Japan) using a digital camera (HC-3000, Fujix, Tokyo, Japan). The automated analysis was performed using the National Institutes of Health (NIH) Image 1.55 (Research Service Branch, NIH). NIH Image 1.55 is an image analysis system, which discriminates the color differences of stained smooth muscle and connective tissue elements. At least 10 different fields were examined from each tissue section. The ratio of the percent area density of connective tissue was determined for each subject.

2.6. Density of bundles of neurofibers in the bladder

The density of bundles of neurofibers in the bladder was calculated. In the connective tissue, bundles of neurofibers were observed. At least 10 different fields were examined from each tissue section.

2.7. Data analysis

A statistical comparison of the differences between groups was performed using analysis of variance and Fisher's multiple comparison tests. $P<0.05$ was regarded as the level of significance.

Table 1
Bladder weight and voiding behaviours in the intact experimental animals

Group	Bladder weight	Micturion frequency (voids/day)	Total urine production (ml/day)	Voided volume (ml/void)
A	0.150±0.010[a]	21.3±2.5[b]	9.7±1.7	0.421±0.092[a]
B	0.138±0.012[a]	14.7±1.5	7.9±1.5	0.515±0.149
C	0.109±0.004	11.5±1.5	7.9±2.7	0.809±0.166

Group A: mild bladder neck obstruction rats, Group B: mild bladder neck obstruction rats treated with cyclohexenonic long-chain fatty alcohol, Group C: age-matched sham-operated rats. Data are shown as means± S.E.M. of six to eight separated determinations in each group.
[a] Significantly different from C group.
[b] Significantly different from B and C groups.

Table 2
CMG data in the experimental animals

Group	Pdet (cm H_2O)	One voided volume (ml)	Residual urine (ml)	Bladder compliance (ml/cm H_2O)
A	66.4±9.4[a]	0.276±0.049[a]	0.036±0.052[b]	0.07±0.02
B	56.2±6.9[a]	0.447±0.155	0.013±0.032	0.06±0.01
C	36.9±2.9	0.450±0.050	0.010±0.050	0.06±0.01

Group A: mild bladder neck obstruction rats, Group B: mild bladder neck obstruction rats treated with cyclohexenonic long-chain fatty alcohol, Group C: age-matched sham-operated rats. Data are shown as means± S.E.M. of six to eight separated determinations in each group. Pdet means maximum contraction pressure of the detrusor.
[a] Significantly different from C group.
[b] Significantly different from B and C groups.

2.8. Drugs and chemicals

3-(15 Hydroxypentadecyl)-2,4,4-trimethyl-2-cyclohexen 1-one was obtained from Meiji Milk Products, Tokyo, Japan. All other chemicals were of reagent grade.

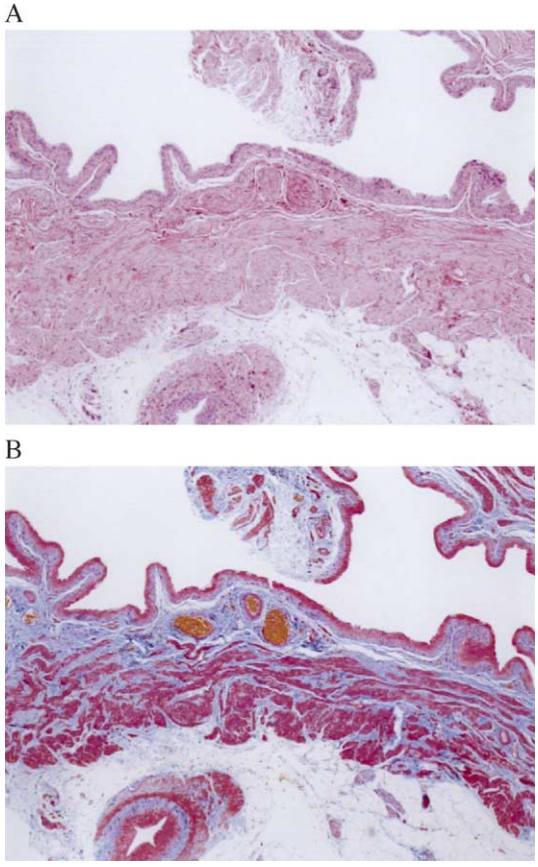

Fig. 1. H&E staining (A) and Azan-Mallory (B) staining of control rat bladder. Smooth muscle is stained as red and connective tissue is stained as blue in Azan-Mallory staining (B). ×40.

3. Results

3.1. Voiding behavior studies

Bladder weight and voiding behaviors in the experimental animals are shown in Table 1. Bladder weight in the group A was significantly larger than that in the group C (0.15 and 0.11 g, respectively). In the rat bladder in the group B, treatment with cyclohexenonic long-chain fatty alcohol slightly but not significantly prevented the increase in the bladder weight. In the behavior studies, micturion frequency in the group A was significantly larger than that in the groups B or C (21.3, 14.7, 11.5 per day, respectively), and one voided volume in the group A was significantly smaller than that in the group C (0.421 and 0.809 ml, respectively). There were no significant differences in voided volume per day among groups. Our data indicated that treatment with cyclohexenonic long-chain fatty alcohol improved voiding behaviors in rats with bladder neck obstruction rats.

3.2. Cystometrogram

The cystometrogram data are shown in Table 2. Six weeks after the induction of bladder neck obstruction, the maximum detrusor pressure and the residual urine significantly increased in the group A rats compared to control rats. The maximum detrusor pressure was slightly but not significantly increased, and the residual urine was significantly reduced in the group B rats compared to the group A rats. One voided volume in the group A was significantly smaller than that in the groups B or C (0.276, 0.450 and 0.447 ml, respectively). The residual urine in the group A was significantly larger than the other groups. There were no significant differences in bladder compliance among the experimental groups.

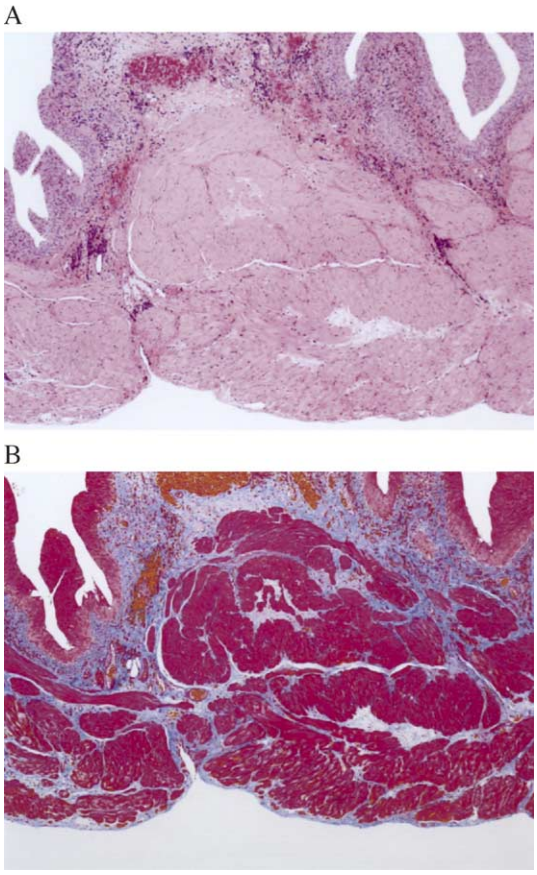

Fig. 2. H&E staining (A) and Azan-Mallory (B) staining of mild bladder neck obstruction rat bladder. Smooth muscle is stained as red and connective tissue is stained as blue in Azan-Mallory staining (B). ×40. Detrusor hypertrophy is observed in the rat bladder with mild bladder neck obstruction.

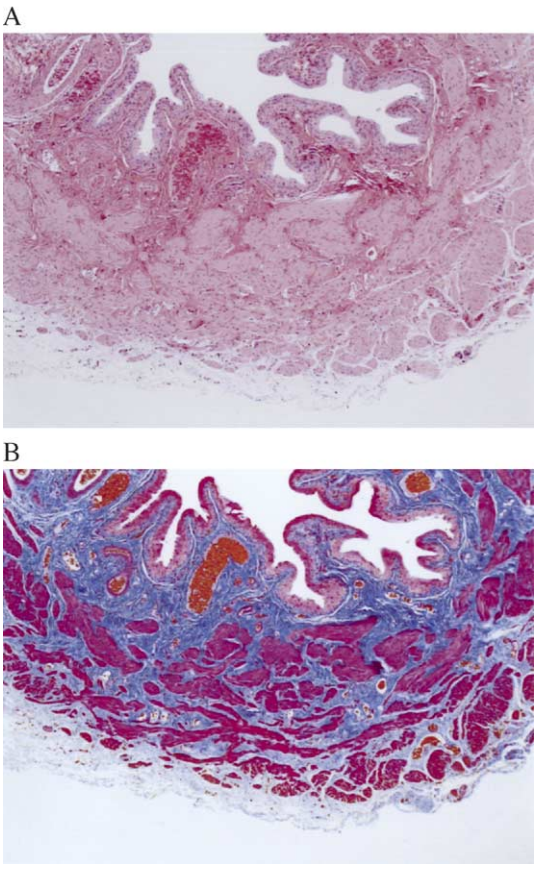

Fig. 3. H&E staining (A) and Azan-Mallory (B) staining of mild bladder neck obstruction rat bladder treated with cyclohexenonic long-chain fatty alcohol. Smooth muscle is stained as red and connective tissue is stained as blue in Azan-Mallory staining (B). ×40. Detrusor hypertrophy is not observed in the rat bladder treated with cyclo-hexenonic long-chain fatty alcohol.

3.3. Histological examination of the rat bladder

The typical H&E staining and Azan-Mallory staining in the histological examinations is shown in Figs. 1–3. Bladder neck obstruction induces bladder smooth muscle hypertrophy, which was normalized by treatment with cyclohexenonic long-chain fatty alcohol. The typical pictures of bundles of neurofibers in the bladder were shown in Figs. 4 and 5. The bundles of neurofibers in the bladder were mainly observed in connective tissue.

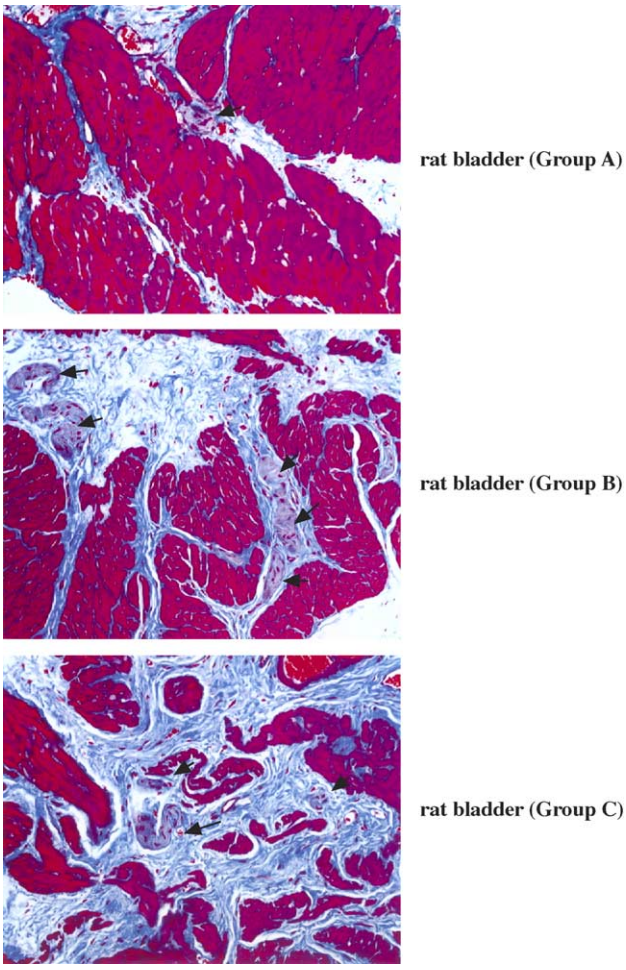

Fig. 4. Azan-Mallory staining of the experimental rat bladder. Smooth muscle is stained as red and connective tissue is stained as blue in Azan-Mallory staining (A). ×200. Bundles of neurofiber were shown as allowed.

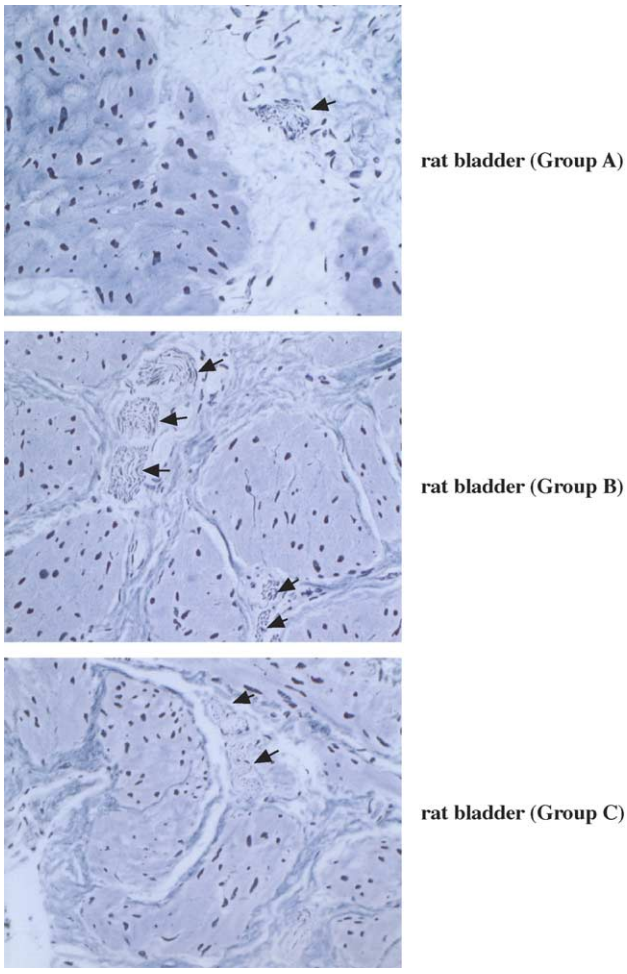

Fig. 5. Bodian staining of the experimental rat bladder. Smooth muscle is stained as red and connective tissue is stained as blue in Azan-Mallory staining (B). ×400. Bundles of neurofiber were shown as allowed.

3.4. Color-assisted quantitative image analysis

The data from the histological examinations are shown in Table 3. The ratio of connective tissue in the group A bladder was significantly smaller than that in the group B or C bladder (23.1%, 29.8%, 27.6%, respectively). The density of bundles of neurofibers in the group A bladder was significantly smaller than those in the groups B or C (2.58, 5.62, 5.01/mm^2, respectively).

Our data indicated that mild bladder neck obstruction induces detrusor hypertrophy and detrusor overactivity, which were improved by treatment with cyclohexenonic long-chain fatty alcohol.

4. Discussion

In the present study, we demonstrated that the preventive effects of cyclohexenonic long-chain fatty alcohol on mild bladder neck obstruction induced bladder overactivity. In the present experiments, mild bladder neck obstruction induced detrusor overactivity, which was improved by treatment with cyclohexenonic long-chain fatty alcohol. We also demonstrated that long-chain fatty alcohol normalized the density of bundles of neurofibers in the bladder. As cyclohexenonic long-chain fatty alcohol has preventive effects on nerve injury, it is possible that this effect on bladder dysfunction may be caused by a preventive effect on nerve alterations in the bladder.

Table 3
H&E staining, Azan-Mallory staining, and Bodian staining data in the experimental animals

Group	% Connective tissue area	Density of bundles of neurofibers (/mm^2)
A	23.1±1.4[a]	2.58±0.51[a]
B	29.8±2.0	5.62±0.90
C	27.6±1.2	5.01±0.63

Group A: mild bladder neck obstruction rats, Group B: mild bladder neck obstruction rats treated with cyclohexenonic long-chain fatty alcohol, Group C: age-matched sham-operated rats. Data are shown as means± S.E.M. of six to eight separated determinations in each group. In each animal, at least 10 different fields are observed.

[a] Significantly different from B and C groups.

Naturally occurring neurotrophic factors induce the differentiation, maturation, and survival of neurons through a wide variety of cellular responses, including proliferation on neuroblasts, survival of developing neurons, induction of neurite outgrowth, and changes in their physiological features (DiCicco-Bloom et al., 1993; Segal et al., 1995; Wright et al., 1992; Wong et al., 1993). From this hypothesis, neurotrophic factors were used in the treatment of neurodegenerative disease. NGF was infused into the brain to ameliorate the cholinergic deficits in Alzheimer's disease patients (Olson et al., 1992), and several neurotrophic factors have been shown to prevent cell death in axotomy or murine mutant models of motoneuron degeneration (Mitsmoto et al., 1994; Wright et al., 1992). However, these treatments have some disadvantages, since proteic neurotrophic factors cannot cross the blood–brain barrier, are rapidly degraded by endogenous peptidase when administered peripherally, and have undesirable side effects.

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. They also demonstrated that diabetes induced alteration of motor sciatic nerve conduction is normalized by treated with long-chain fatty alcohol. These data demonstrated that cyclohexenonic long-chain fatty alcohols can exhibit NGF-like activity on not only the central nervous system but also on the peripheral nervous system. Previously, we demonstrated 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 (Saito et al., 2002).

It has been considered that the bladder neck obstruction influences the function and nerve innervations of the bladder (Steers, 1994). Detrusor hypertrophy after bladder neck obstruction can induce changes in the morphology and function of afferent and efferent neurons. Afferent and efferent neurons undergo hypertrophy with increased labeling of sensory projections to preganglionic neurons in the sacral spinal cord following bladder neck obstruction (Steers, 1994). Steers et al. (1991) reported that parenchymal cells in the hypertrophied bladder can synthesize NGF and possibly other molecular messengers that act to alter the size and function of neurons in adult animals and man. Recently, Seki et al. (2004) reported that an increase in the levels of NGF in the spinal cord was involved in dyssynergic urethral sphincter activity during reflex bladder contractions in spinal-cord-injured rats, and that abnormal sphincter activity was induced by C-fiber bladder afferents. The suppression of NGF levels in afferent pathways is useful for treating detrusor-sphincter dyssynergia under spinal cord injury.

In this study, we demonstrated that mild bladder neck obstruction decreased in the density of bundles of neurofibers in the bladder, and that treatment with long-chain fatty alcohol normalized the density of bundles of neurofibers. There is a possibility that this effect on the bladder is caused by a preventive effect on nerve alterations in the bladder. Previous reports indicated that in bladder neck obstruction bladder weight increased several fold the weight in the controls (Steers et al., 1991). In our study, however, the bladder weight in the group A was about 1.4-fold that in the group C. This finding can be mainly explained by the fact that our ligation was looser than the ligation in the other reports, because in this study we attempted to create "mild" bladder neck obstruction. In the present study, 6 weeks after the induction of mild bladder neck obstruction, rats in the group A were clearly shown to be in detrusor overactivity condition. In the group A, urinary frequency, decreased single-void volume, and increased residual urine were observed. In the histological examination, detrusor hypertrophy was observed in the group A. Our data indicated that all of the evaluated parameters in the voiding behavior, cytometrogram, and histological examinations were significantly improved or tended to be improved by treatment with cyclohexenonic long-chain fatty alcohol. As cyclohexenonic long-chain fatty alcohol has a preventive effect on nerve injury and NGF-like effects on the central and peripheral nervous systems, it is possible that this effect on bladder dysfunction may be caused by a modulating effect of the peripheral nerve alterations in the bladder.

The physiological and pharmacological role of cyclohexenonic long-chain fatty alcohol, however, remains unclear and warrants further study.

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