Effect of NS-21, an Anticholinergic Drug with Calcium Antagonistic Activity, on Lower Urinary Tract Function in a Rat Model of Urinary Frequency

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Background: NS-21 is under development for the treatment of urinary frequency and urinary incontinence. The purpose of this study was to investigate the effects of NS-21 and its active metabolite, RCC-36, on lower urinary tract function in an experimental rat model of urinary frequency.
Methods: Cystometrograms were recorded in anesthetized rats with bilaterally transected hypogastric nerves. All drugs were administered intraduodenally.
Results: In sham-operated rats, NS-21 (≥ 50 mg/kg) significantly increased the bladder capacity without significantly decreasing micturition pressure, while RCC-36 (100 mg/kg) significantly increased bladder capacity, and at a dose of ≥ 30 mg/kg, also caused a decrease in micturition pressure. This increase in bladder capacity appeared at lower doses of both NS-21 and RCC-36 in the hypogastric nerve-transected rats. Propiverine (100 mg/kg) increased bladder capacity and at ≥ 30 mg/kg, decreased micturition pressure in both sham-operated and nerve-transected rats. Oxybutynin (100 mg/kg) and atropine (30 mg/kg) decreased the micturition pressure in both sham-operated and nerve-transected rats without increasing the bladder capacity, while a similar anticholinergic calcium antagonist, terodiline (100 mg/kg) had no effect on bladder capacity in either sham-operated or nerve-transected rats. Flavoxate (500 mg/kg) significantly increased bladder capacity without significantly decreasing micturition pressure in both sham-operated and nerve-transected rats, while 50 mg/kg of verapamil significantly increased bladder capacity without significantly decreasing the micturition pressure in nerve-transected rats.
Conclusions: NS-21 and RCC-36 increased bladder capacity at lower doses in hypogastric nerve-transected rats than in sham-operated rats. Furthermore, NS-21 increased the bladder capacity without suppressing micturition pressure, suggesting that NS-21 may be a more effective therapeutic drug than propiverine, oxybutynin or flavoxate for the treatment of urinary frequency and urinary incontinence.
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Key words: NS-21, bladder capacity, hypogastric nerve transection, rat

INTRODUCTION

(±)-4-diethylamino-1,1-dimethylbut-2-yn-1-yl 2-cyclohexyl-2-hydroxy-2-phenylacetate monohydrochloride monohydrate (NS-21; Fig. 1) is a novel compound intended for the treatment of urinary frequency and urinary incontinence. This compound was designed to possess both calcium antagonistic and anticholinergic activities, and is predicted to have a beneficial effect on bladder dysfunction caused by hyperexcitability of the bladder smooth muscle.[1] The effects of drugs that have been clinically used to treat urinary frequency and urinary incontinence on urinary bladder function, including propiverine and oxybutynin, have been evaluated in various species such as rats, rabbits, cats and dogs.[2–7] However, there are very few studies using these drugs in animal urinary frequency models that reflect the clinical symptoms of impaired bladder function.

In this study, the effects of NS-21 and its active metabolite, RCC-36 [(±)-4-ethylamino-1,1-dimethylbut-2-yn-1-yl 2-cyclohexyl-2-hydroxy-2-phenylacetate monohydrochloride], were cystometrically examined in a rat model of urinary frequency produced by bilateral transection of the hypogastric nerves on lower urinary tract function, and their effects compared with those of propiverine, oxybutynin and terodiline (all of which have both anticholinergic and calcium antagonistic activity), flavoxate (a drug which acts on the micturition center), atropine (an anticholinergic agent) and verapamil (a calcium antagonist).

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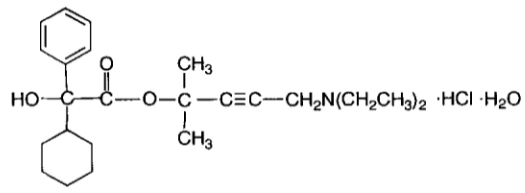

Fig. 1. Chemical structure of NS-21.

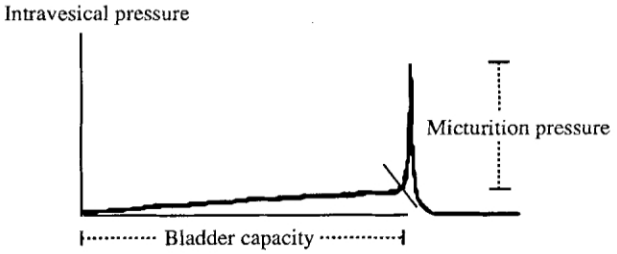

Fig. 2. Schematic cystometrogram. \, threshold pressure.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats 9–12 weeks old and weighing 300–380 grams (Japan SLC, Shizuoka, Japan) were housed in groups of 4 to 6 in a room maintained at 21–25°C and 45–65% humidity with an alternating 12-hour light/dark cycle. Food and water were given ad libitum.

Transection of the Hypogastric Nerves

The method of Maggi et al.[8] was used to transect the hypogastric nerves. Rats under ketamine anesthesia (25 mg/kg, im) were placed in a supine position. A midline incision was made in the abdomen, both hypogastric nerves transected about 5 mm from the pelvic plexus, and the wound sutured. At the same time, a group of rats was subjected to laparotomy alone (sham-operated rats). Two to 3 days after surgery, cystometry was performed.

Cystometry

Under urethane anesthesia (900 mg/kg, sc), rats were placed in a supine position. The bladder was exposed through a midline incision in the abdomen, and a polyethylene catheter (PE50; Clay Adams, Parsippany, NJ, USA) inserted into the apex of the bladder dome and connected to a three-way stopcock. One outlet was connected to a pressure transducer (TP-200T; Nihon Kohden, Tokyo, Japan) for recording the intravesical pressure. The other outlet was connected to an infusion pump (STC-521; Terumo, Tokyo, Japan) for infusion of physiological saline. After the bladder was emptied, reflex micturition was induced by filling the bladder with warm saline at a rate of 2.8 mL/h with the infusion pump. The flow of saline was terminated when reflex micturition occurred. The bladder capacity and the micturition pressure were read from the cystometrogram (Fig. 2). After the bladder capacity and micturition pressure stabilized, drugs were injected into the duodenum and cystometric recordings were taken at 30, 60, 120, and 180 minutes after injection in order to evaluate the effects of the drugs on bladder capacity and micturition pressure.

Drugs

NS-21, RCC-36, propiverine (propiverine hydrochloride), oxybutynin (oxybutynin hydrochloride) and terodiline (terodiline hydrochloride) were synthesized in our laboratories. Flavoxate (flavoxate hydrochloride) was obtained from Recordati (Milan, Italy). Atropine (atropine sulfate) and urethane were purchased from Sigma Chemical (St. Louis, MO, USA), verapamil (verapamil hydrochloride) from Nacalai Tesque (Kyoto, Japan), and ketamine (ketamine hydrochloride) from Sankyo (Tokyo, Japan). The test drugs were suspended in 0.5% methyl cellulose (Shinetsu Chemical, Tokyo, Japan) at concentrations appropriate for an injection volume of 1 mL/kg.

Statistical Analysis

Data for the bladder capacity and micturition pressure are expressed as the mean ± SEM. The significance of differences between the values before and after drug administration was determined by a paired *t* test (data for animals that died during the experiments were excluded from analysis).

RESULTS

The bladder capacity of the hypogastric nerve-transected rats was significantly less than that of the sham-operated rats, but there was no significant difference in micturition pressure between the 2 groups (Table 1). Figures 3 and 4 show representative recordings before and after treatment with NS-21 or RCC-36 in sham-operated rats and hypogastric nerve-transected rats, and the time courses for the effects of these drugs on the bladder capacity are shown in Figs. 5 and 6. Figures 7 (sham-operated rats) and 8 (hypogastric nerve-transected rats) show the changes in the bladder capacity and micturition pressure at the time when the mean increases in bladder capacity was maximized for each group after drug administration. Although the micturition pressure varied among individual rats, it was fairly constant in the same animal after injection of vehicle (data not shown).

In sham-operated rats, NS-21 at a dose of 30 mg/kg

Table 1. Effect of bilateral transection of the hypogastric nerves on bladder capacity and micturition pressure in urethane anesthesized rats.

Treatment	Bladder capacity (mL)	Micturition pressure (mm Hg)
Sham operation	0.44 ± 0.04	28.3 ± 2.9
Hypogastric nerve transection	0.20 ± 0.02[a]	26.2 ± 2.0

Data are presented as the mean ± SEM of 6 animals. [a]$P < 0.01$ between treatment groups.

had no effect on the bladder capacity. However, at 50 and 100 mg/kg, it caused a significant increase in bladder capacity without significantly affecting the micturition pressure, with the increase in bladder capacity reaching a maximum by 60 or 120 minutes after dosing. In hypogastric nerve-transected rats, NS-21 at 30 mg/kg or more increased the bladder capacity in a dose-dependent manner. At 30 and 50 mg/kg, it caused small decreases in micturition pressure which

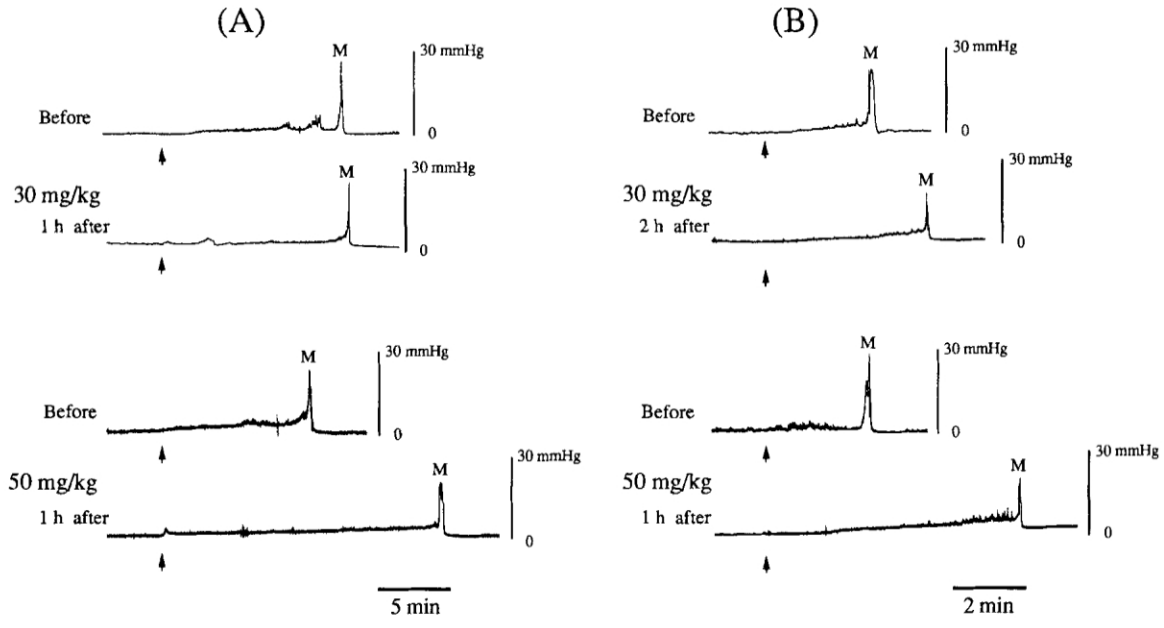

Fig. 3. Representative cystometric recordings showing the effect of intraduodenally administered NS-21 on the urodynamics in a (A) sham-operated rat or (B) rat with transected hypogastric nerves. ↑, start of saline infusion; M, micturition.

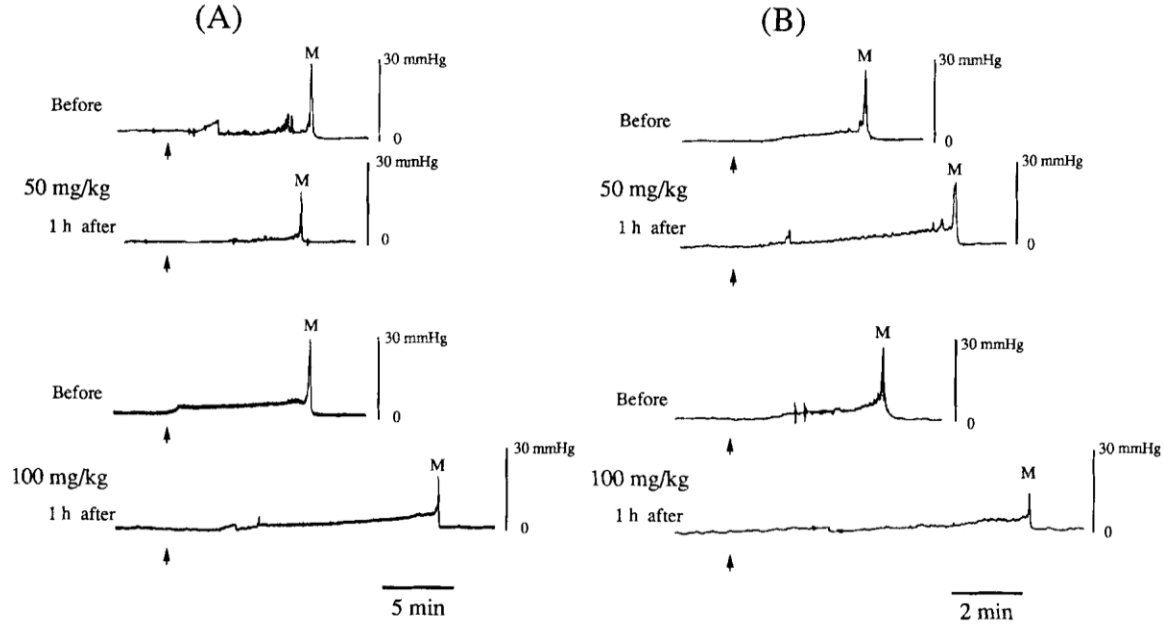

Fig. 4. Representative cystometric recordings showing the effect of intraduodenally administered RCC-36 on the urodynamics of (A) sham-operated rats and (B) rats with transected hypogastric nerves. ↑, start of saline infusion; M, micturition.

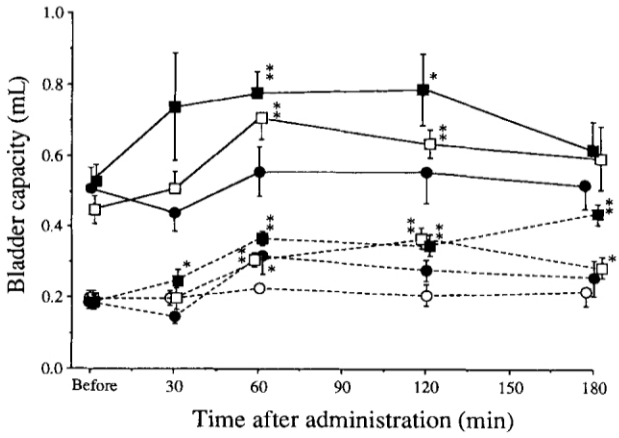

Fig. 5. Effect of intraduodenally administered NS-21 on the bladder capacity of sham-operated rats (solid lines) and rats with transected hypogastric nerves (broken lines). ○, 10 mg/kg; ●, 30 mg/kg; □, 50 mg/kg; ■, 100 mg/kg. Each point represents the mean ± SEM for 6 animals. $*P < 0.05$; $**P < 0.01$; significance determined between pre- and postdrug administration (paired *t* test).

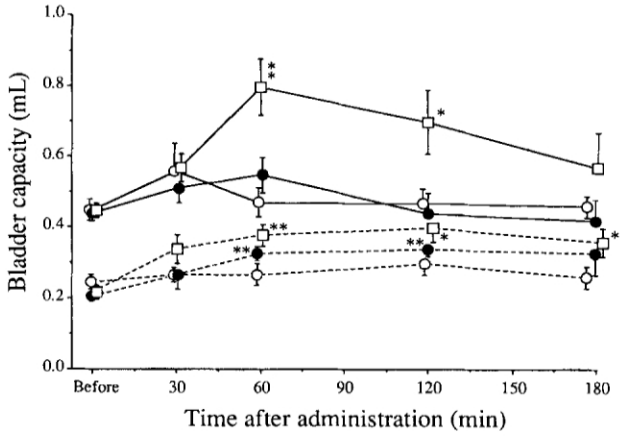

Fig. 6. Effect of intraduodenally administered RCC-36 on the bladder capacity of sham-operated rats (solid lines) and rats with transected hypogastric nerves (broken lines). ○, 30 mg/kg; ●, 50 mg/kg; □, 100 mg/kg. Each point represents the mean ± SEM for 6 animals. $*P < 0.05$; $**P < 0.01$; significance determined between pre- and postdrug administration (paired *t* test).

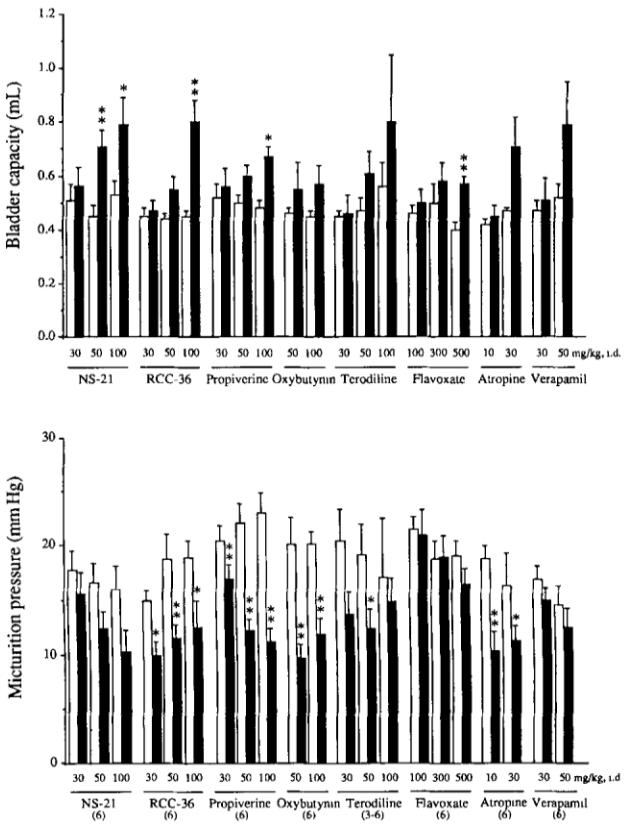

Fig. 7. Effect of NS-21, RCC-36 and reference drugs on the bladder capacity and micturition pressure in sham-operated rats. □, before drug administration; ■, after. Each value represents the mean ± SEM, with the number of animals used for each experiment shown in parentheses. $*P < 0.05$; $**P < 0.01$; significance determined between pre- and postdrug administration (paired *t* test).

were not statistically significant, but caused a significant decrease at 100 mg/kg.

In sham-operated rats, RCC-36 at 100 mg/kg caused a significant increase in bladder capacity, and at 30–100 mg/kg, a significant decrease in micturition pressure. In hypogastric nerve-transected rats, RCC-36 at 50 and 100 mg/kg caused significant increases in bladder capacity and at 30–100 mg/kg, caused a significant decrease in micturition pressure.

Propiverine caused a significant increase in bladder capacity at 100 mg/kg in both sham-operated and hypogastric nerve-transected rats. The micturition pressure was decreased using a dose of at least 30 mg/kg. Oxybutynin caused a significant decrease in micturition pressure using at least 50 mg/kg in sham-operated rats, and at least 30 mg/kg in hypogastric nerve-transected rats but had no effect on the bladder capacity at any dose tested. In sham-operated rats, terodiline had no effect on the bladder capacity at 30 and 50 mg/kg, but caused a significant decrease in micturition pressure at 50 mg/kg. At 100 mg/kg, it showed a tendency to increase bladder capacity, however, 3 of 6 rats died within 180 minutes after receiving this dose. In hypogastric nerve-transected rats, terodiline had no effect on bladder capacity at 30 or 50 mg/kg, but at 100 mg/kg, it showed the same tendency to increase bladder capacity as it did in sham-operated rats. The micturition pressure was significantly decreased with 30 mg/kg terodiline, but this effect was not significant at 50 or 100 mg/kg. As with sham-operated rats, 3 of 6 rats died within 180 minutes after receiving a dose of 100 mg/

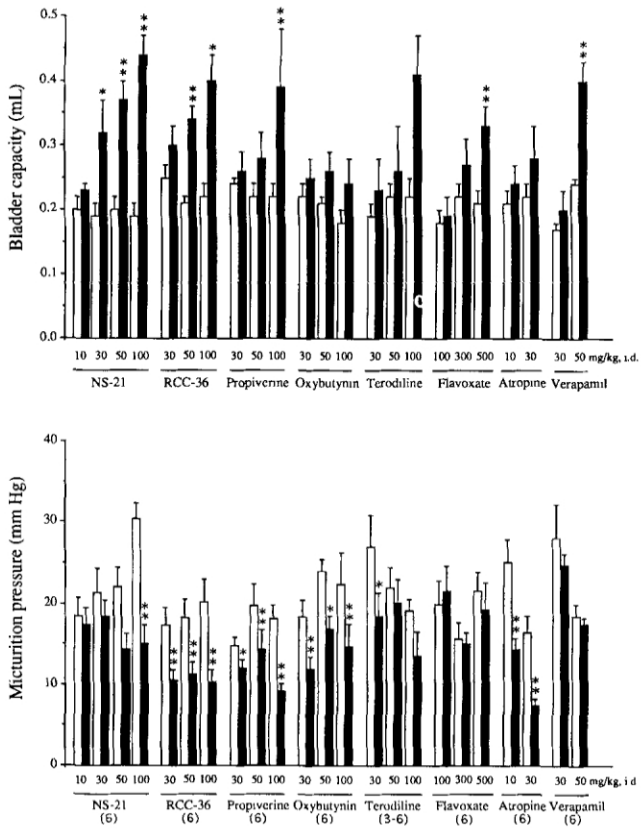

Fig. 8. Effect of NS-21, RCC-36 and reference drugs on bladder capacity and micturition pressure in rats with transected hypogastric nerves. □, before drug administration; ■, after. Each value represents the mean ± SEM, with the number of animals for each experiment shown in parentheses. $*P < 0.05$, $**P < 0.01$; significance determined between pre- and postdrug administration (paired t test).

kg. Flavoxate had no effect on the bladder capacity in either sham-operated or hypogastric nerve-transected rats at 100 or 300 mg/kg. However, at 500 mg/kg, it caused a significant increase in bladder capacity without affecting micturition pressure. In both sham-operated and hypogastric nerve-transected rats, atropine had no effect on bladder capacity even at 30 mg/kg, but it caused a significant decrease in micturition pressure. Verapamil at 30 or 50 mg/kg had no significant effect on either bladder capacity or micturition pressure in sham-operated rats, but in hypogastric nerve-transected rats, a dose of 50 mg/kg caused a significant increase in bladder capacity without any significant effect on the micturition pressure.

DISCUSSION

Maggi et al.[8] reported that bilateral transection of the hypogastric nerves abolishes the sympathetic inhibitory control of the urinary bladder and induces a decrease in the bladder capacity of rats. In this study, we confirmed the decrease in bladder capacity in rats with bilateral transection of the hypogastric nerves and, using this model, performed a cystometrical evaluation of the effects of NS-21, RCC-36 and reference drugs on bladder function.

Atropine, a potent muscarinic acetylcholine receptor antagonist, and oxybutynin, which has potent anticholinergic activity and direct, but weak, muscle relaxant activity,[9] significantly decreased the micturition pressure in both sham-operated and hypogastric nerve-transected rats, but had no effect on the bladder capacity in rats from either group, which is similar to results reported by Noronha-Blob et al. in anesthetized guinea pigs.[10] In a study using conscious rats, Guarneri et al. reported that oxybutynin decreased the micturition pressure in a dose-dependent manner, but increased the bladder capacity significantly only at low doses.[11] Therefore, the major effect of anticholinergic agents on bladder function in rats and guinea pigs is thought to be a decrease in micturition pressure.

In this study, verapamil, a calcium antagonist, significantly increased the bladder capacity at 50 mg/kg without decreasing the micturition pressure in hypogastric nerve-transected rats, but had no effect in sham-operated rats. In sympathectomized rats, the intravesical pressure during the urine storage phase was shown to be significantly higher than in sham-operated rats, and consequently the bladder capacity was reduced.[8] Taken together with the report that calcium antagonists decrease the basal tension of bladder smooth muscle,[12] the findings of this study suggest that a decrease in the basal tension of bladder smooth muscle induced by calcium antagonists in hypogastric nerve-transected rats, which is more pronounced than the decrease observed in sham-operated rats, and which leads to a depression of afferent neural activity in the pelvic nerves that contributes to the inhibition of the micturition reflex and a consequent increase in bladder capacity.

NS-21, which has both calcium antagonistic and anticholinergic activities, significantly increased the bladder capacity at 50 mg/kg in sham-operated rats and at 30 mg/kg in hypogastric nerve-transected rats without affecting the micturition pressure. RCC-36, which has similar drug activities to NS-21, also increased the bladder capacity in hypogastric nerve-transected rats at lower doses than in sham-operated rats. The increase in bladder capacity caused by NS-21 is likely to be mediated by its calcium antagonistic activity through the mechanism similar to that reported for verapamil, and its anticholinergic activity may act synergistically with its calcium antagonistic activity to potently inhibit the contraction of bladder

smooth muscle, leading to an increase in bladder capacity. It is possible that the decrease in micturition pressure caused by NS-21 at high doses may be mediated through its anticholinergic action. RCC-36, whose anticholinergic activity is exerted at much lower concentrations than its calcium antagonistic activity,[1] is likely to produce a decrease in micturition pressure at doses lower than those which produce an increase in bladder capacity.

Propiverine,[13] which has pharmacological properties similar to those of NS-21 and RCC-36, has both calcium antagonistic and anticholinergic activities. However, unlike NS-21 or RCC-36, the increase in bladder capacity caused by propiverine was elicited only at high doses in the both sham-operated and hypogastric nerve-transected rats. It has been reported that the calcium antagonistic activity of propiverine is about 10 times less than that of NS-21 in vitro.[1] Its weak calcium antagonistic activity may account for the weak effect of this drug on the bladder capacity. Although terodiline has both calcium antagonistic and anticholinergic activity,[14] it had no effect in either sham-operated or hypogastric nerve-transected rats, even at a toxic dose. This drug may have pharmacological activities other than its calcium antagonistic and anticholinergic activities, which may prevent the inhibition of bladder contraction by a calcium antagonistic or anticholinergic mechanism, and at the same time cause the cardiac side effects that led to the withdrawal of this drug from clinical use.

In this study, the effects of NS-21 and RCC-36 on lower urinary tract function were investigated in sham-operated and hypogastric nerve-transected rats. Both compounds significantly increased the bladder capacity in hypogastric nerve-transected rats at lower doses than in sham-operated rats. In particular, NS-21 caused an increase in bladder capacity without a significant effect on the micturition pressure. High doses of propiverine caused an increase in bladder capacity and a decrease in micturition pressure in both sham-operated and hypogastric nerve-transected rats, but terodiline had no effect even at toxic doses. Oxybutynin caused a marked decrease in micturition pressure but no increase in bladder capacity in hypogastric nerve-transected rats. Our findings suggest that NS-21 may be a useful drug for the treatment of urinary frequency and urinary incontinence, and has fewer side effects than the drugs in current clinical use.

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