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Effects of TAK-802, a novel acetylcholinesterase inhibitor, and various cholinomimetics on the urodynamic characteristics in anesthetized guinea pigs

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Abstract

In the present study, we investigated the effects of cholinomimetic drugs on the urodynamic characteristics in anesthetized guinea pigs. 8-[3-[1-[(3-fluorophenyl)methyl]-4-piperidinyl]-1-oxopropyl]-1,2,5,6-tetrahydro-4H-pyrrolo[3,2,1-ij]quinolin-4-one (TAK-802), a novel acetylcholinesterase inhibitor, (0.003–0.03 mg/kg, i.v.) increased the voided volume and the maximum flow rate without affecting either the intravesical pressure or the bladder compliance. Distigmine (0.03–0.3 mg/kg, i.v.) and neostigmine (0.01–0.1 mg/kg, i.v.), both carbamate acetylcholinesterase inhibitors, while not increasing the maximum flow rate, increased the intravesical pressure at the maximum flow rate. They also decreased the bladder compliance. Bethanechol (0.1–1 mg/kg, i.v.), a muscarinic receptor agonist, decreased the voided volume and the bladder compliance but did not affect the maximum flow rate. TAK-802 did not affect the intraurethral pressure at doses of up to 0.03 mg/kg in anesthetized guinea pigs. Distigmine increased the intraurethral pressure when administered at the dose of 0.3 mg/kg, and the effect was completely abolished by pretreatment with *d*-tubocurarine. These results suggest that TAK-802 reinforces the bladder-voiding functions by increasing the bladder contractility without decreasing the storage function. On the other hand, carbamate acetylcholinesterase inhibitors not only deteriorate the voiding function by inducing contraction of the external urethral sphincter muscle, resulting in increasing the urethral resistance, but also cause deterioration of the storage function. Bethanechol obviously decreased the bladder capacity, possibly due to a direct contractile effect on the detrusor smooth muscle. TAK-802 may therefore be a more useful drug than either carbamate acetylcholinesterase inhibitors or muscarinic receptor agonists in the treatment of voiding dysfunction associated with impaired detrusor contractility.

Keywords: TAK-802; Acetylcholinesterase inhibitor; Urodynamics; (Guinea pig); Bladder

1. Introduction

Impaired detrusor muscle contractility is considered to be one of the important causes of voiding dysfunction in both men (Ameda et al., 1999) and women (Groutz et al., 1999). Detrusor contractility becomes weakened with aging (Malone-Lee and Wahedna, 1993) and also in the presence of chronic diseases such as benign prostatic hypertrophy (Akino et al., 1996; Eckhardt et al., 2001), diabetes mellitus (Hunter and Moore, 2003; Ueda et al., 1997), and multiple sclerosis (Litwiller et al., 1999). Although clean intermittent catheterization is the treatment of first choice for impaired detrusor contractility, it is associated with some problems, such as urinary tract infections and bladder injury (Madjar and Appell, 2002).

It has been reported that the density of acetylcholinesterase-positive nerve endings in the bladder is decreased with increasing age in humans (Gilpin et al., 1986), under the condition of overdistension of the bladder in rats (Lasanen et al., 1992) and experimental bladder outlet obstruction in rabbits (Elbadawi et al., 1989), and in rats with diabetes mellitus (Lincoln et al., 1984). These observations suggest that impaired detrusor contractility may be caused partly by a decrease in the cholinergic innervation of the bladder. Therefore, it has been proposed that cholinomimetic agents, such as muscarinic receptor agonists and acetylcholinesterase inhibitors, may be useful in the pharmacological treatment of lower urinary tract symptoms associated with impaired detrusor contractility (Wein et al., 1994). The rationale of the treatment is that the activation of

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the parasympathetic cholinergic system induced by these drugs may increase the detrusor muscle contractility. Muscarinic agonists and acetylcholinesterase inhibitors have been demonstrated to induce and enhance, respectively, the contraction of isolated bladder strips in various species (Brading and Mostwin, 1989; King et al., 1998; Levin et al., 1983; Longhurst et al., 1995; Maggi et al., 1985; Sibley, 1984). Maggi et al. (1987) reported that physostigmine, a carbamate acetylcholinesterase inhibitor, increased the voiding efficiency in urethane-anesthetized guinea pigs. In spite of the accumulated experimental evidence, clinical use of cholinomimetic agents has not yet become widely accepted, and the efficacy of these drugs remains controversial (Barrett, 1981; Hameed and Charles, 1994; Philp and Thomas, 1980; Shah et al., 1983; Tanaka et al., 2001; Wein et al., 1980).

We previously reported that both TAK-802 (Fig. 1), a novel non-carbamate acetylcholinesterase inhibitor, and distigmine, a carbamate acetylcholinesterase inhibitor used clinically, augmented bladder contractions induced by the micturition reflex in the same manner. However, TAK-802 showed a higher selectivity for muscarinic actions over nicotinic actions than distigmine (Nagabukuro et al., in press). Some carbamate acetylcholinesterase inhibitors are known to have some direct effects on nicotinic receptors, besides acting on them via acetylcholinesterase inhibition (Pereira et al., 1993; Sung et al., 1998). These nicotinic effects are not desirable in the treatment of voiding dysfunction because they are associated with potential increase in the urethral resistance due to contraction of the external urethral sphincter muscle (Von Heyden et al., 1995). Therefore, we hypothesized that TAK-802 may show superior efficacy for the potentiation of bladder-voiding functions than distigmine.

Simultaneous measurements of urinary flow and intravesical pressure and analysis of the relationship between the maximum flow rate and detrusor pressure at the maximum flow rate, namely, pressure–flow studies, have been undertaken for assessing the severity of bladder outlet obstruction and detrusor contractility and for evaluating the treatment outcome (Griffiths, 1980; Schafer, 1990). Evaluation of the urodynamic characteristics has been reported in animal models, including rats (Watanabe and Constantinou, 1996), guinea pigs (Van Asselt et al., 1995), and minipigs (Guan et al., 1995). In these studies, although the authors have shown the pressure–flow characteristics and also evaluated the severity of bladder outlet obstruction, detrusor contractility, and pharmacological modulation of various urodynamic parameters, the effects of cholinomimetics have not yet been elucidated.

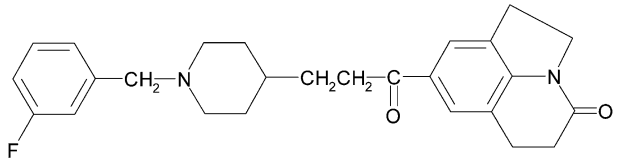

Fig. 1. Chemical structure of TAK-802.

In the present study, we compared the effects of TAK-802 and other cholinomimetics on the urodynamic characteristics in urethane-anesthetized guinea pigs and demonstrated that TAK-802 is highly efficacious for potentiation of bladder-voiding functions.

2. Materials and methods

2.1. Animals

All the animal experiments conducted in this study were approved by Takeda's Experimental Animal Care and Use Committee. Male and female Hartley guinea pigs weighing 250–380 g and 300–350 g, respectively, were used for the study. All the animals were housed in a temperature- and light-controlled (12-h light/dark cycle) room and were allowed access to food and water ad libitum.

2.2. Urodynamic study in urethane-anesthetized guinea pigs

Male guinea pigs were anesthetized with urethane (1.5 g/kg, ip). A lower abdominal midline incision was made to expose the bladder, and two 20-gauge needles connected to a polyethylene tube (PE-100) were inserted into the bladder dome for recording the intravesical pressure and for intravesical infusion of physiological saline at the rate of 0.3 ml/min, respectively. The intravesical pressure and voided volume were measured using a pressure transducer (TP-400T, Nihon Kohden, Tokyo, Japan) and an electronic balance (HX-400, A&D, Tokyo, Japan), and each signal was concomitantly recorded using a multiple-channel data acquisition system (MP-100A-CE, Biopac Systems, Santa Barbara, CA, USA) at a sampling rate of 10 Hz. The voided volume signal was filtered (low pass filter, 0.5 Hz) to remove noises and then differentiated to obtain the flow rate. The delay (0.1 s) of the voided volume and flow rate signal was adjusted to the pressure signal. After confirming three successive micturition reflexes induced by intravesical infusion of physiological saline, the bladder was completely drained off, and infusion was then restarted. Bladder filling was continued until micturition was observed. The following urodynamic parameters were obtained (Fig. 2): maximum flow rate, bladder capacity, voided volume, maximum intravesical pressure, intravesical pressure at the maximum flow rate, average flow rate (voided volume/voiding duration), and bladder compliance. The volume threshold and pressure threshold were determined as the points of intersection of the linearly fitted curves during the storage phase and the micturition-reflex phase. Bladder compliance was defined as the ratio of the volume threshold to pressure threshold (volume threshold/pressure threshold). Each urodynamic parameter was measured twice, and then the drugs were administered intravenously. The postdrug values of the urodynamic parameters were measured 10 min after the drug administration, except in the case of distigmine, in

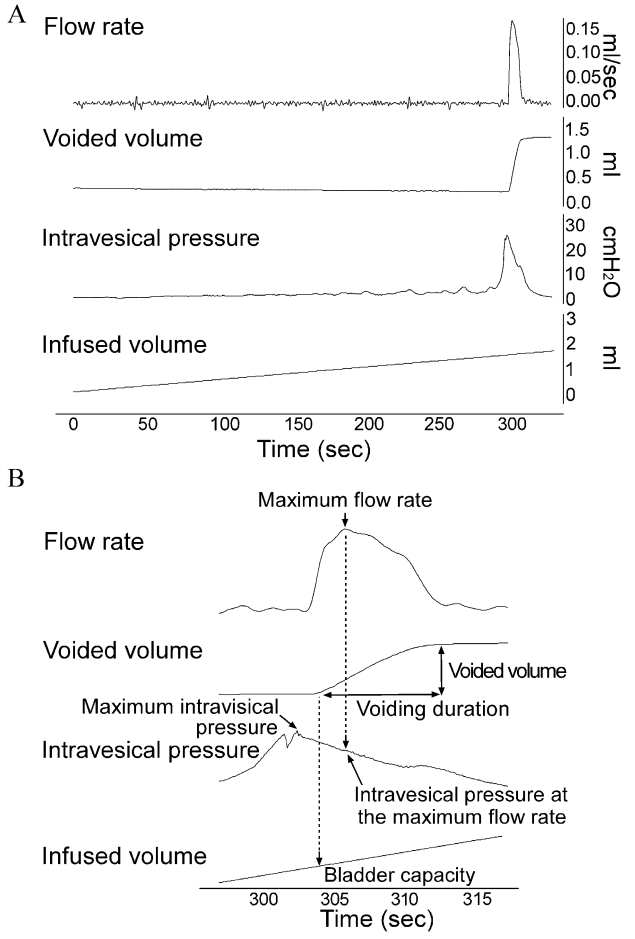

Fig. 2. Representative cystmetrogram of the urodynamic study in a guinea pig (A) and schematic presentation of the urodynamic parameters obtained (B).

which the measurements were made 30 min after the drug administration, because a latent period was observed in our previous study before the maximum effect of distigmine was reached (Nagabukuro et al., in press). Statistical analysis was performed by comparison of the results expressed as % of the predrug values (the mean of two sessions) with the corresponding values in the vehicle-treated group, using Dunnett's test. Differences were considered significant when $P < 0.05$. Changes after the drug administration in both the maximum flow rate and the intravesical pressure at the maximum flow rate were calculated, and the relationship of both parameters was analyzed.

2.3. Measurement of the intraurethral pressure in urethane-anesthetized guinea pigs

Female guinea pigs were anesthetized with urethane. A small incision was made in the bladder dome to empty the contents. A pressure transducer (size 3F, Mikro-tip®, Millar Instruments, Houston, TX, USA) was inserted from the external orifice of the urethra into the bladder, and it was then slowly withdrawn using a control module (AU-601G, Nihon Kohden) while monitoring the urethral pressure to construct a urethral pressure profile. The pressure signals were recorded using a multiple-channel data acquisition system (MP-30, Biopac Systems) at the rate of 5 Hz. The transducer was put in place so as to record the highest urethral pressure in the distal part of the external urethral sphincter. After measuring the predrug urethral pressure, the postdrug pressures were measured 30 min after the administration of the drugs. Statistical analysis of the differences between the predrug values and the postdrug values was performed using the paired *t*-test.

2.4. Chemicals

TAK-802 is relatively insoluble in aqueous solvents. Therefore, the hydrochloride form of TAK-802 was used. TAK-802 hydrochloride and distigmine bromide were synthesized in Takeda's Medicinal Chemistry Research Laboratories. Neostigmine bromide and bethanechol chloride were purchased from RBI (Natick, MA, USA). *d*-tubocurarine chloride was purchased from Yoshitomi Pharmaceutical Industries (Saga, Japan). All the drugs were dissolved in distilled water and administered intravenously at a volume of 0.5 ml/kg.

3. Results

3.1. Effects of the drugs on the urodynamic parameters

The values of the urodynamic parameters before the drug administration are shown in Table 1. The voiding efficiency (voided volume/bladder capacity) was approximately 60% in the urethane-anesthetized guinea pigs. The doses of the drugs used in this study were determined in accordance with their potencies for acetylcholinesterase inhibition in vitro and for enhancing the isovolumetric bladder contraction in urethane-anesthetized guinea pigs (Nagabukuro et al., in press). The results are presented in Fig. 3 and Table 2. TAK-802 significantly increased the voided volume and the maximum flow rate when adminis-

Table 1
Preadministration values of various urodynamic parameters in the vehicle-treated group in the TAK-802 administration experiment

Parameter (unit)	Value	Parameter (unit)	Value
Bladder capacity (ml)	2.00 ± 0.12	Intravesical pressure at the maximum flow rate (cmH_2O)	21.26 ± 0.73
Voided volume (ml)	1.18 ± 0.11	Average flow rate (ml/s)	0.13 ± 0.02
Maximum intravesical pressure (cmH_2O)	26.83 ± 0.91	Bladder compliance (ml/cmH_2O)	0.47 ± 0.05
Maximum flow rate (ml/s)	0.20 ± 0.03		

Mean ± S.E.M. $N = 10$.

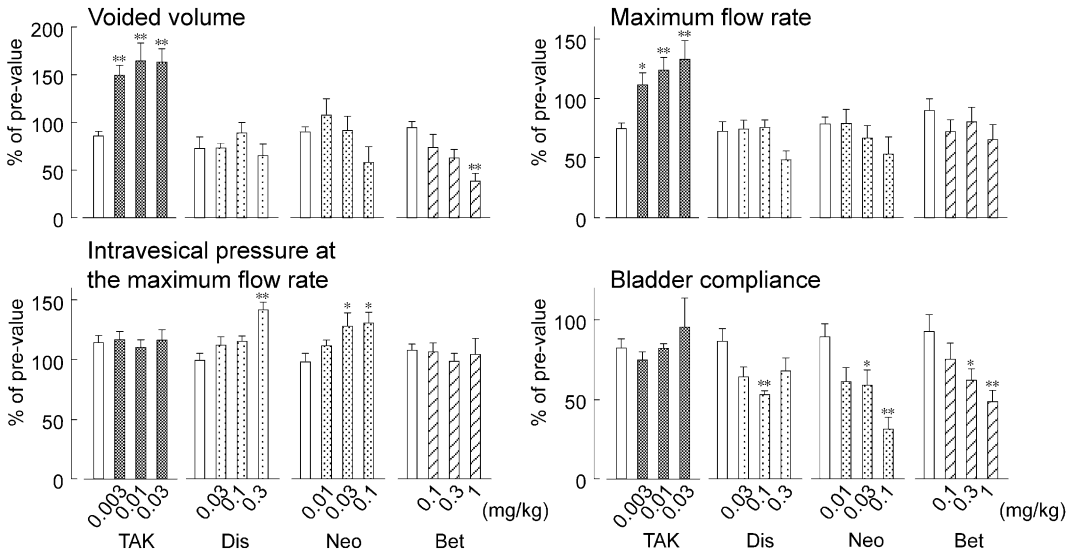

Fig. 3. Changes in various urodynamic parameters after administration of TAK-802 (TAK), distigmine (Dis), neostigmine (Neo), and bethanechol (Bet) in guinea pigs. Values are presented as % of the predrug values and the mean ± S.E.M. *$P<0.05$, **$P<0.01$ versus vehicle (white column)-treated group (Dunnett's test). $N=7-10$.

tered intravenously at a dose exceeding 0.003 mg/kg. The drug had no effect on the maximum intravesical pressure, the intravesical pressure at the maximum flow rate, or the bladder compliance. The bladder capacity was significantly increased when the drug was administered at the dose of 0.03 mg/kg, i.v. Distigmine and neostigmine showed a similar effect on the urodynamic profile. Neither of these drugs showed any definite effects on either the voided volume or the maximum flow rate, but they significantly increased the maximum intravesical pressure and the intravesical pressure at the maximum flow rate. Neostigmine significantly decreased the bladder compliance at the two higher doses. Distigmine also significantly decreased the bladder compliance at the dose of 0.1 mg/kg, i.v. Bethanechol significantly decreased the voided volume at the dose of 1.0 mg/kg, i.v., and also decreased the bladder compliance at a dose exceeding 0.3 mg/kg, i.v. This drug also decreased the bladder capacity in a dose-dependent manner. Bethanechol had no effect on the maximum flow rate, maximum intravesical pressure, or the intravesical pressure at the maximum flow rate.

Table 2
Effects of TAK-802 and other cholinomimetics on the urodynamic parameters

	Dose (mg/kg, i.v.)	N	Bladder capacity	% of pre-value	
				Maximum intravesical pressure	Average flow rate
Vehicle		10	91.9 ± 3.2	109.3 ± 5.5	77.7 ± 4.4
TAK-802	0.003	10	94.6 ± 5.8	118.5 ± 4.8	122.8 ± 11.6[a]
	0.01	10	101.7 ± 5.0	104.4 ± 5.0	136.0 ± 14.6[a]
	0.03	10	117.2 ± 9.0[b]	103.3 ± 5.3	115.5 ± 16.8
Vehicle		10	83.9 ± 5.0	94.1 ± 5.9	68.6 ± 10.8
Distigmine	0.03	10	80.6 ± 3.3	106.8 ± 7.4	63.9 ± 6.9
	0.1	10	83.6 ± 5.9	112.2 ± 6.6	77.9 ± 11.4
	0.3	9	80.0 ± 4.5	145.8 ± 6.6[a]	47.4 ± 7.7
Vehicle		8	97.2 ± 5.3	101.7 ± 4.4	74.7 ± 8.4
Neostigmine	0.01	7	81.1 ± 8.9	112.2 ± 4.3	82.3 ± 11.7
	0.03	8	86.4 ± 7.5	137.5 ± 17.9[b]	69.9 ± 15.0
	0.1	8	66.5 ± 14.7	133.1 ± 9.3[b]	50.6 ± 15.9
Vehicle		8	96.0 ± 4.4	103.5 ± 4.1	84.7 ± 8.5
Bethanechol	0.1	8	86.0 ± 2.5	110.8 ± 7.3	69.3 ± 8.9
	0.3	8	85.3 ± 6.3	98.5 ± 5.0	71.6 ± 11.5
	1	7	66.3 ± 6.0[a]	111.5 ± 9.5	55.0 ± 13.2

Mean ± S.E.M.
[a] $P<0.01$ versus vehicle (Dunnett's test).
[b] $P<0.05$ versus vehicle (Dunnett's test).

3.2. Analysis of the pressure–flow characteristics

The changes in the pressure–flow relationships after the administration of TAK-802 and other cholinomimetics are represented in Fig. 4. TAK-802 increased the maximum flow rate without affecting the intravesical pressure. Distigmine decreased the maximum flow rate and increased the intravesical pressure at the dose of 0.3 mg/kg, i.v. Neostigmine did not affect the maximum flow rate but increased the intravesical pressure at doses of 0.03 and 0.1 mg/kg, i.v. Bethanechol affected neither the maximum flow rate nor the intravesical pressure.

3.3. Effects on the intraurethral pressure

Administration of vehicle was not observed to have any effect on the urethral pressure. The mean predrug urethral pressure value in each experimental group was in the range of 2.5 – 4.5 mmHg. The results are shown in Fig. 5. TAK-802 did not have any effect on the urethral pressure when administered at the dose of 0.01 mg/kg, which is the minimum effective dose (MED) for augmentation by the

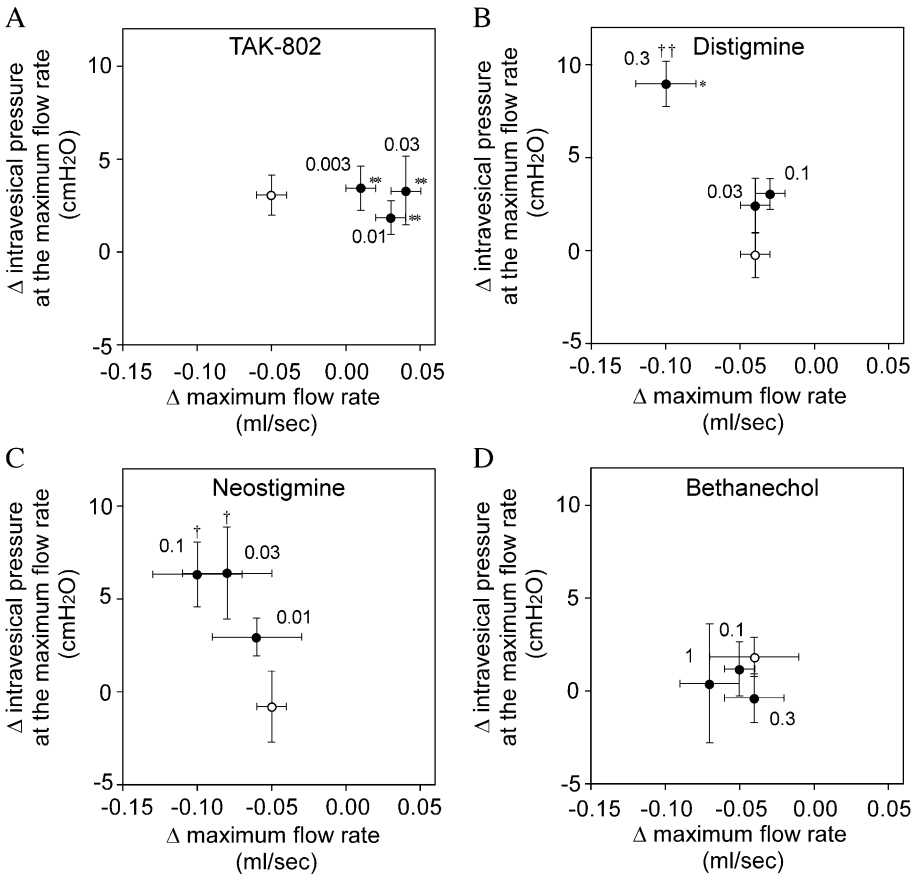

Fig. 4. Effects of TAK-802 (A), distigmine (B), neostigmine (C), and bethanechol (D) on the pressure–flow characteristics in guinea pigs. Values are represented as the differences between the predrug and postdrug values and the mean ± S.E.M. The numbers beside the filled circles indicate dosage (mg/kg, i.v.). The values in the vehicle-treated group are denoted by open circles. *$P<0.05$, **$P<0.01$ versus Δ maximum flow rate in the vehicle-treated group (open circles), †$P<0.05$, ††$P<0.01$ versus Δ intravesical pressure at the maximum flow rate in the vehicle-treated group (Dunnett's test).

drug of isovolumetric bladder contractions in the guinea pig (Nagabukuro et al., in press), or even at the dose of 0.03 mg/kg, i.v. Distigmine did not show any significant effect on the urethral pressure when administered at the dose of 0.1 mg/kg, i.v., which is the MED of the drug for inducing bladder contractions. However, it significantly increased the urethral pressure at doses of 0.3 and 1.0 mg/kg, i.v. The increasing effect of distigmine at the dose of 0.3 mg/kg was completely abolished by pretreatment with *d*-tubocurarine (0.34 mg/kg, s.c.).

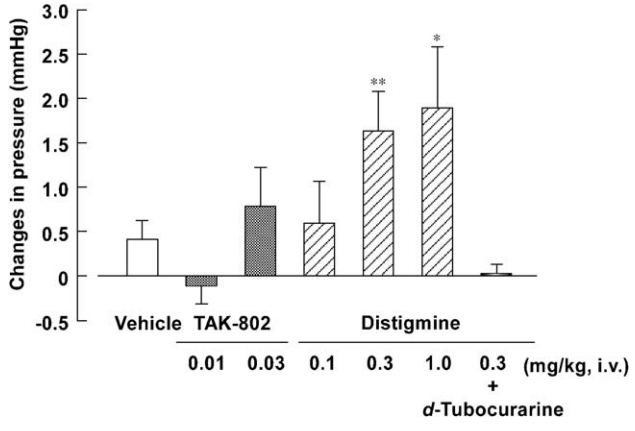

Fig. 5. Effect of TAK-802 and distigmine on the urethral pressure in guinea pigs. The ordinate represents the difference between the predrug and postdrug values. *d*-tubocurarine (0.34 mg/kg, s.c.) was given subcutaneously 30 min prior to the intravenous injection of distigmine (0.3 mg/kg, i.v.). Each vertical bar represents the mean ± S.E.M. *$P<0.05$, **$P<0.01$ versus the predrug values (paired *t*-test). $N=8$.

4. Discussion

Damage of the detrusor smooth muscle itself (detrusor myopathy) or of its nerve supply leads to impaired detrusor contractility, resulting in inadequate bladder emptying (Madjar and Appell, 2002). The etiology of impaired detrusor contractility includes postsurgical conditions (Anderson and Grant, 1991), benign prostatic hypertrophy (Akino et al., 1996; Eckhardt et al., 2001), diabetes mellitus (Hunter and Moore, 2003; Ueda et al., 1997), and multiple sclerosis (Litwiller et al., 1999). Detrusor contractility can also become weakened with aging (Malone-Lee and Wahedna, 1993) or idiopathically. Although clean intermittent catheterization is the most commonly used treatment

method, it is associated with some problems such as urinary tract infections and bladder injury (Madjar and Appell, 2002). Cholinomimetic agents, e.g., muscarinic receptor agonists and acetylcholinesterase inhibitors, have been proposed as being potentially useful for the treatment of voiding dysfunction associated with impaired detrusor contractility (Wein et al., 1994), but they have not yet become widely accepted as the first choice of therapy, probably because of their controversial efficacy (Barrett, 1981; Hameed and Charles, 1994; Philp and Thomas, 1980; Shah et al., 1983; Tanaka et al., 2001; Wein et al., 1980).

Pressure–flow studies of voiding have been clinically used for assessment of the grade of bladder outlet obstruction and detrusor contractility (Griffiths, 1980; Schafer, 1990) and have clearly revealed that impaired detrusor contractility is frequently associated with lower urinary tract symptoms in men. Weak detrusor contractility accounted for voiding dysfunction in 50% of patients awaiting transurethral prostatectomy (Javle et al., 1996). While lower urinary tract symptoms in men are significantly correlated with the detrusor contractility, they are not related to the prostate volume or obstruction grade (Eckhardt et al., 2001). Te and Kaplan (1996) reported that 17% of men with lower urinary tract symptoms had impaired detrusor contractility. In women with lower urinary tract symptoms, voiding difficulties were diagnosed in 25.5% of patients older than the age of 65 years (Stanton et al., 1983). Pressure–flow studies revealed that impaired detrusor contractility is also a major cause of voiding difficulties in women (Groutz et al., 1999).

Analyses of urodynamic characteristics have also been carried out in animal models: rats (Watanabe and Constantinou, 1996), guinea pigs (Van Asselt et al., 1995), and minipigs (Guan et al., 1995). By a comparative study of voiding in the rat and the guinea pig, Van Asselt et al. (1995) showed that high-frequency oscillations in urinary flow were observed in rats but not in guinea pigs. High-frequency oscillations are caused by relaxation and contraction of the external urethral sphincter muscle and may have a role in territory marking. Because high-frequency oscillations are not observed in human urinary flow, we chose guinea pigs for our assessment of urodynamics in this study.

TAK-802 is a novel synthetic acetylcholinesterase inhibitor with a chemical structure different from that of carbamate acetylcholinesterase inhibitors. In our previous study (Nagabukuro et al., in press), we showed that unlike carbamate acetylcholinesterase inhibitors, which inhibit both acetylcholinesterase and butyrylcholinesterase activity, TAK-802 selectively inhibits acetylcholinesterase activity. Both TAK-802 and carbamate acetylcholinesterase inhibitors augmented bladder contractions induced by the micturition reflex in urethane-anesthetized rats and guinea pigs in the same manner. However, to our surprise, the effects of TAK-802 and carbamate acetylcholinesterase inhibitors on the urodynamic characteristics were clearly different. TAK-802 increased the voided volume and the maximum flow rate without affecting the intravesical pressure, while distigmine and neostigmine showed no definite effects on either the voided volume or the maximum flow rate, but increased the intravesical pressure at the maximum flow rate. These results suggest that both TAK-802 and carbamate acetylcholinesterase inhibitors augmented the detrusor contractility during the voiding phase but that the detrusor-contractile force was utilized for the increase of urinary flow in the case of TAK-802, while it acted to further increase the intravesical pressure in the case of carbamate acetylcholinesterase inhibitors. One reason for this difference between TAK-802 and carbamate acetylcholinesterase inhibitors might be that carbamate acetylcholinesterase inhibitors increase the urethral resistance while TAK-802 does not. Actually, distigmine significantly increased the intraurethral pressure in urethane-anesthetized guinea pigs at a dose exceeding 0.3 mg/kg, i.v., which is the same dose to induce significant increase in the intravesical pressure at the maximum flow rate and is three times higher than the minimum effective dose of the drug for augmentation of isovolumetric bladder contractions determined in our previous study (Nagabukuro et al., in press) . On the other hand, TAK-802 did not affect the intraurethral pressure at an equivalent dose (0.03 mg/kg, i.v.). The effect of distigmine on the intraurethral pressure was completely abolished by pretreatment with *d*-tubocurarine, an antagonist of nicotinic receptors at the neuromuscular junction. These results imply that the dose-dependent increase in the effects of distigmine on the intraurethral pressure or urethral resistance may be attributable to a mechanism other than the drug's inhibition of acetylcholinesterase activity. Indeed, distigmine showed three times higher selectivity for nicotinic actions than for muscarinic actions, as compared to TAK-802 (Nagabukuro et al., in press). Carbamate acetylcholinesterase inhibitors have been reported to exert direct excitatory modulation on nicotinic receptors (Pereira et al., 1993; Sung et al., 1998). While the direct modulatory effect of carbamate acetylcholinesterase inhibitors on nicotinic receptors may be ascribed not to their acetylcholinesterase inhibitory activity but to their chemical structures, TAK-802 might not have any clear direct excitatory action on nicotinic receptors, although this remains to be confirmed. The preferential activation of nicotinic receptors by carbamate acetylcholinesterase inhibitors is suitable for the clinical use of these drugs in the treatment of myasthenia gravis but is not favorable for the treatment of voiding dysfunction because nicotinic effects are associated with contraction of the external urethral sphincter muscle and increase of the urethral resistance (Von Heyden et al., 1995).

The urinary flow should be measured precisely to evaluate the urodynamic parameters in small animals. Therefore, we used anesthetized animals to fix the position of the external orifice of the urethra. In this study, the mean predrug value of the voiding efficiency was approximately 60%, which was consistent with the finding in a previous study (Groen et al., 1996). Urethane has been known to develop a large amount of residual urine, probably by

causing premature fading of the bladder contractions. For actual evaluation of the efficacy of cholinomimetic agents, we should use an animal model with impaired detrusor contractility associated with a large volume of residual urine. In our preliminary study to examine the effects of TAK-802 and carbamate acetylcholinesterase inhibitors on the function of the lower urinary tract in conscious animals, we found that TAK-802 decreased the residual urine volume in rats with partial bladder outlet obstruction, while decrease in the residual urine volume as triggered by distigmine was observed only in a limited dose (unpublished data).

Distigmine and neostigmine decreased the bladder compliance in this study. This is consistent with clinical reports that they impair the bladder-storage function (Finkbeiner et al., 1977). Neostigmine evoked an increased desire to urinate and decreased the bladder capacity, and distigmine caused bladder contractions. Recently, Nakahara et al. (2003) reported that neostigmine increased the resting tension of the isolated detrusor muscle in rats. Carbamate acetylcholinesterase inhibitors have also been reported to cause contraction of other smooth muscle preparations by themselves. Physostigmine and neostigmine produced spasm in the isolated guinea pig ileum (Cox and Lomas, 1972). Tracheal smooth muscle strips showed contractions following treatment with carbamate acetylcholinesterase inhibitors (Norel et al., 1993; Shibata et al., 1998). It was suggested that neostigmine and physostigmine caused spasm of the tracheal muscle not only by inhibition of acetylcholine hydrolysis but also by stimulating the release of acetylcholine from nerve terminals (Kirkpatrick and Rooney, 1982). These observations suggest that carbamate acetylcholinesterase inhibitors might directly activate intramural ganglion cells in the bladder and induce the release of acetylcholine from pelvic nerve terminals by their direct effects on nicotinic receptors, besides the effects via acetylcholinesterase inhibition, resulting in the increase of the tonus of the detrusor smooth muscle in the resting state. Therefore, some carbamate acetylcholinesterase inhibitors may increase the tonus of the detrusor muscle in the storage phase, which results in a decrease of the bladder compliance. It is hoped that in vitro studies, which are now under way, would help to confirm these findings.

Bethanechol did not cause any improvement of the bladder-voiding function in this study. Bladder capacity and bladder compliance decreased after the administration of bethanechol, indicating that bethanechol elevated the tonus of the detrusor smooth muscle, irrespective of whether the bladder was in the storage phase or in the voiding phase. A report that bethanechol increased the micturition frequency and improved overflow incontinence in bilaterally pelvic nerve-resected rats (Hirotsu et al., 1998) suggests that subjects to be treated with muscarinic receptor agonists or acetylcholinesterase inhibitors might be divided into two groups: subjects in whom the micturition reflex is preserved should be treated with acetylcholinesterase inhibitors to potentiate cholinergic transmission during the voiding phase, whereas subjects with loss of the micturition reflex should be treated with muscarinic receptor agonists to induce bladder contractions to expel urine.

TAK-802 apparently increased bladder capacity when administered at the highest dose (0.03 mg/kg). Ishiura et al. (2001) reported that intrathecal and intracerebroventricular administration of oxotremorine-M, a muscarinic receptor agonist, significantly increased the bladder capacity in conscious rats and that the effect was completely abolished by the pretreatment with atropine. Because TAK-802 has been confirmed to penetrate into the central nervous system in pharmacokinetic studies, the bladder capacity might be increased by activation of the cholinergic systems in the central nervous system induced by this drug.

In conclusion, unlike carbamate acetylcholinesterase inhibitors and muscarinic receptor agonists, TAK-802 potentiates the bladder-voiding function without affecting the urethral resistance or bladder-storage function in urethane-anesthetized guinea pigs, suggesting that TAK-802 might be potentially useful in the treatment of voiding dysfunction associated with impaired detrusor contractility.

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