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Effects of TAK-802, a novel acetylcholinesterase inhibitor, on distension-induced rhythmic bladder contractions in rats and guinea pigs

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Abstract

In the present study, we investigated the effects of 8-[3-[1-[(3-fluorophenyl)methyl]-4-piperidinyl]-1-oxopropyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (TAK-802), a novel acetylcholinesterase inhibitor, on distension-induced rhythmic bladder contractions in urethane-anesthetized rats and guinea pigs. TAK-802 potently inhibited human-erythrocyte-derived acetylcholinesterase activity with an IC_{50} value of 1.5 nM, which represented a potency 30 and 250 times greater than that of the two carbamate acetylcholinesterase inhibitors, neostigimine and distigmine, respectively. Unlike the carbamate acetylcholinesterase inhibitors, TAK-802 exhibits high selectivity for acetylcholinesterase inhibition over butyrylcholinesterase inhibition. In an assay conducted to measure the muscarinic and nicotinic actions, TAK-802 was found to exhibit higher selectivity for muscarinic actions over nicotinic actions in comparison to distigmine. Both TAK-802 and distigmine increased isovolumetric bladder contractions in rats and guinea pigs in a dose-dependent manner, with a minimum effective dose (MED) of 0.01 and 0.03 mg/kg i.v., respectively, in rats, and 0.01 and 0.1 mg/kg i.v., respectively, in guinea pigs. The effects of both the drugs were completely abolished by atropine. These results suggest that TAK-802 and other acetylcholinesterase inhibitors can effectively increase reflex bladder contractions by increasing the efficacy of acetylcholine released by nerve impulses. On the other hand, bethanechol, a muscarinic agonist, markedly changed the pattern of distension-induced bladder contractions when administered at the dose of 1 mg/kg i.v., and it did not necessarily augment well-coordinated bladder contractions. Thus, considering that it has some selectivity for muscarinic action, TAK-802 might be expected to be useful in the treatment of voiding dysfunction caused by impaired detrusor contractility.

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

1. Introduction

Acetylcholine released from parasympathetic postganglionic neurons plays an essential role in the contraction of the urinary bladder during the voiding phase (Anderson, 1993). The released acetylcholine is rapidly hydrolyzed by the acetylcholinesterase localized pre- and post-junctionally (McConnell et al., 1982). Administration of acetylcholinesterase inhibitors has been reported to increase acetylcholine release (Somogyi and de Groat, 1992) and the nerve-mediated contractile responses in the bladder strips of various species, including humans (Brading and Mostwin, 1989; 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.

It is well known that the pelvic nerve is fully activated during the voiding phase, but silent during the storage phase of the bladder. Therefore, it would be relevant, from the physiological point of view, to use acetylcholinesterase inhibitors to improve the voiding function of the bladder; the drugs would theoretically act only during the voiding phase and not during the storage phase, that is, they would not interfere with the storage function of the bladder, but only increase detrusor muscle contractility during the voiding phase. Distigmine, a long-acting carbamate acetylcholinesterase inhibitor, has been clinically used to treat patients with voiding dysfunction associated with impaired detrusor

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contractility (Wein et al., 1994), however the efficacy of this drug remains controversial (Hameed and Charles, 1994; Philp and Thomas, 1980; Shah et al., 1983; Tanaka et al., 2001).

Some carbamate acetylcholinesterase inhibitors have been reported to also non-selectively inhibit butyrylcholinesterase (Ogura et al., 2000). Butyrylcholinesterase is also known to hydrolyze the acetylcholine released from nerve endings, and is distributed in a wide variety of tissues. While butyrylcholinesterase has been actually demonstrated to be present in the bladder (Roshani et al., 1996), the role of butyrylcholinesterase and the clinical advantages of inhibition of both acetylcholinesterase and butyrylcholinesterase have not been elucidated, because no selective acetylcholinesterase inhibitor has been studied experimentally or used clinically. Carbamate acetylcholinesterase inhibitors are also known to have some direct excitatory effects on nicotinic receptors besides effecting acetylcholinesterase inhibition (Pereira et al., 1993; Sung et al., 1998). The nicotinic effects of carbamate acetylcholinesterase inhibitors have been put to favorable use in the treatment of myasthenia gravis (Ishikawa et al., 1969), but not for voiding dysfunction, because the nicotinic effects potentially increase urethral resistance by inducing contraction of the external urethral sphincter muscles (von Heyden et al., 1995).

We therefore hypothesized that an acetylcholinesterase inhibitor with a chemical structure different from that of carbamate acetylcholinesterase inhibitors might cause less potent activation of nicotinic transmission and improve bladder voiding function more effectively than carbamate acetylcholinesterase inhibitors.

In the present study, we report on the profile of 8-[3-[1-[(3-fluorophenyl)methyl]-4-piperidinyl]-1-oxopropyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (TAK-802) as a selective acetylcholinesterase inhibitor with relatively weak nicotinic actions, and also on the effect of TAK-802 on isovolumetric contractions in urethane-anesthetized rats and guinea pigs.

2. Materials and methods

2.1. Acetylcholinesterase and butyrylcholinesterase inhibition activity

Acetylcholinesterase and butyrylcholinesterase activities were measured by the thiocholine method (Ellman et al., 1961) using human-erythrocyte-derived acetylcholinesterase and human-serum-derived butyrylcholinesterase, respectively. Thirty microliters of 0.3% bovine serum albumin–80 mM Tris–HCl (pH 7.4), 50 μl of acetylcholinesterase or butyrylcholinesterase solution (0.2 IU/ml) and 20 μl of drug solution were added to the well of a microplate. After incubation for 1 h, 50 μl of 5 mM 5,5-dithio-bis(2-nitrobenzoic acid) and 50 μl of 4 mM acetylthiocholine iodide or butyrylthiocholine chloride were added, and the absorbance at 412 nm was read every 30 s for 10 min using a microplate reader (Spectra rainbow thermo, TECAN, Maennedorf, Switzerland), followed by calculation of the reaction velocities. The IC_{50} value and 95% confidence interval of each drug was calculated by the logistic regression analysis.

2.2. Muscarinic and nicotinic actions

Male Sprague–Dawley rats weighing 150–190 g were fasted for 24 h before the experiment. Enhancement of the intestinal transit of a charcoal meal and fasciculation were used as the indices of muscarinic and nicotinic actions, respectively. The drugs were first administered intravenously, followed by oral administration of a suspension of charcoal (5% suspended in a 10% gum arabic solution) in a volume of 1 ml/animal, 15 and 30 min after the administration of TAK-802 and distigmine, respectively. The rats were sacrificed by placing them in a dry ice chamber 10 min after the administration of charcoal. The intestinal transit of the charcoal meal was calculated as follows:

$$\text{Transportation}\ (\%) = \text{distance traveled from the pyloric sphincter} / \text{the total length of the intestine}$$

The behavior of each animal was observed immediately before it was sacrificed, and scored from 0 to 3, according to the severity of the fasciculation: score 0, normal; score 1, weak fasciculation of some limbs, but not of all limbs; score 2, moderate fasciculation of all limbs and the tail; score 3, rigorous fasciculation of all limbs and the tail. Statistical analysis was performed by comparison with the corresponding vehicle-treated group using Dunnett's test for the intestinal charcoal transit ratio and fasciculation score, and Fisher's exact probability test for the incidence of fasciculation.

2.3. Effect on distension-induced rhythmic bladder contractions in urethane-anesthetized rats

Male Sprague–Dawley rats weighing 220–300 g were anesthetized with an intraperitoneal injection of urethane (1.2 g/kg). The urinary bladder was exposed through an incision in the abdomen, and the urethra was ligated. A 23-gauge needle attached to a polyethylene tube (PE-50) was inserted into the bladder dome to record the intravesical pressure using a pressure transducer (TP-400T, Nihon-Kohden, Tokyo, Japan) and a multiple-channel data acquisition system (MP-30, Biopac systems, Santa Barbara, CA, USA). Warmed physiological saline (39 °C) was injected into the bladder until regular isovolumetric bladder contractions appeared. Although the thresholds of intravesical volume to induce rhythmic bladder contractions were different for each animal, we confirmed the linear relationship between the acetylcholinesterase inhibitory

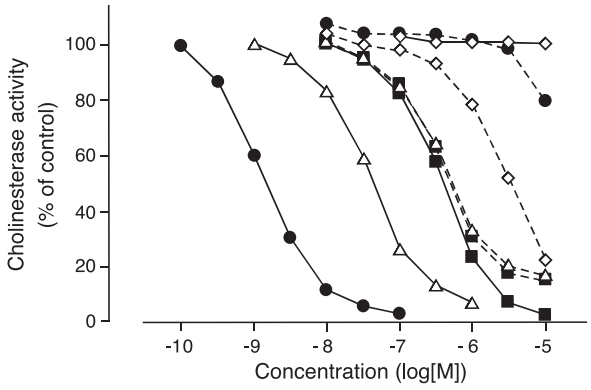

Fig. 1. Effect of TAK-802 (closed circle), distigmine (closed square), neostigmine (open triangle) and iso-OMPA (open rhombus) on human acetylcholinesterase (solid line) or butyrylcholinesterase (dotted line) activity. Values are represented by % of control and mean of two experiments.

activities in vitro and the potency for increasing bladder contractions in the course of the optimization of TAK-802 analogs. TAK-802 and distigmine bromide were administered intravenously after confirming stable rhythmic contractions. The areas under the curve (AUC) of the vesical contractions vs. time were calculated. The effects of the drugs were estimated by the AUC of the maximum contractile response for 30 min after the administration of the drugs. The ratio of the change of the AUC after the drug administration was calculated and compared with that in the vehicle-treated group using Dunnett's test for statistical analysis.

2.4. Effect on distension-induced rhythmic bladder contractions in urethane-anesthetized guinea pigs

Male Hartley guinea pigs weighing 260–400 g were anesthetized and the urinary bladder was exposed through an incision in the abdomen, followed by urethral ligation. A 20-gauge needle attached to a polyethylene tube (PE-100) was inserted into the bladder dome to record the intravesical pressure. Warmed physiological saline (39 °C) was injected until regular isovolumetric bladder contractions appeared. Drugs were administered intravenously after confirming stable rhythmic contractions. The AUC of the vesical contractions vs. time was calculated. The effects of TAK-802 were estimated from the AUC of the vesical contractions about 15 min after the administration of the drug. The effect of distigmine was measured about 30 min after its administration, because there is a latent period before the

Table 1
Acetylcholinesterase and butyrylcholinesterase inhibition activity

Compound	Acetylcholinesterase		Butyrylcholinesterase	
	IC_{50} (μM)	(95% CI)	IC_{50} (μM)	(95% CI)
TAK-802	0.00149	(0.00138–0.00162)	>10	
Distigmine	0.380	(0.366–0.394)	0.537	(0.447–0.647)
Neostigmine	0.0427	(0.0394–0.0463)	0.598	(0.492–0.728)
iso-OMPA	>10		3.21	(3.00–3.44)

IC_{50} values calculated from the results of two experiments conducted in duplicate. 95% confidence interval (CI) for each IC_{50} value is represented in brackets.

Table 2
Muscarinic and nicotinic effects of TAK-802 and distigmine in rats

Drug	Dose (mg/kg i.v.)	*N*	Transportation (%) (muscarinic)	Fasciculation (nicotinic)	
				Incidence	Score
Vehicle		6	46 ± 2	0/6	0
TAK-802	0.001	6	50 ± 2	0/6	0
	0.003	6	59 ± 3[a]	0/6	0
	0.01	6	56 ± 2	1/6	0.2 ± 0.2
	0.03	6	53 ± 4	6/6[b]	2.5 ± 0.2[c]
Vehicle		6	40 ± 3	0/6	0
Distigmine	0.01	6	52 ± 3	0/6	0
	0.03	6	58 ± 4[a]	4/6	1.0 ± 0.4
	0.1	6	55 ± 3	6/6[b]	3.0 ± 0.0[c]
	0.3	5[d]	34 ± 9	6/6[b]	3.0 ± 0.0[c]

Each value represents the mean ± S.E.M.
[a] $P<0.05$ vs. the vehicle-treated group (Dunnett's test).
[b] $P<0.01$ vs. the vehicle-treated group (Fisher's exact probability test).
[c] $P<0.01$ vs. the vehicle-treated group (Dunnett's test, ranked data).
[d] One animal died prior to the scheduled sacrifice.

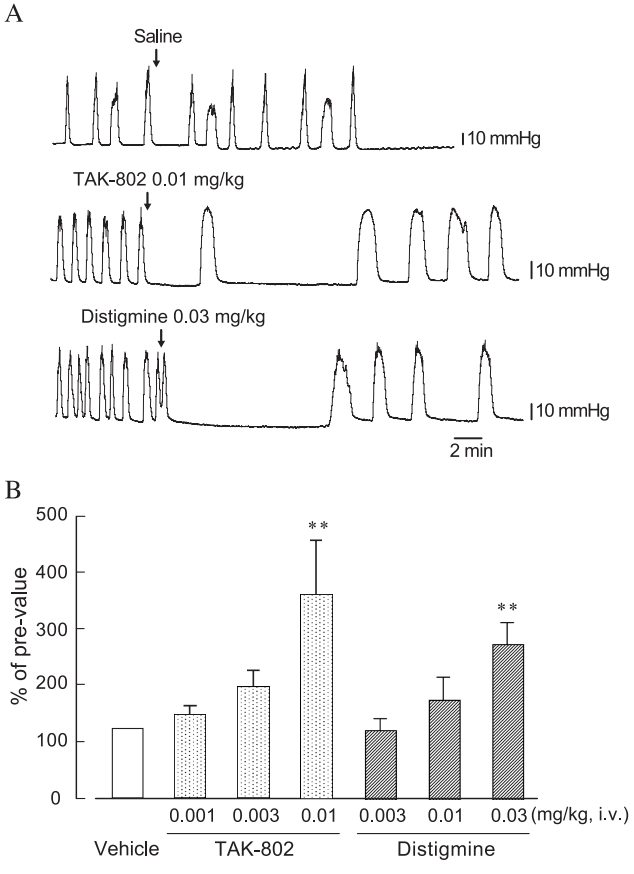

Fig. 2. (A) Representative cystometrograms of distention-induced rhythmic bladder contractions in rats. After confirming stable rhythmic contractions, saline (vehicle), TAK-802 or distigmine was administered intravenously. (B) Effect of TAK-802 and distigmine on distension-induced bladder contractions in rats. Values are represented by % of pre-drug value and mean ± S.E.M. $^{**}P<0.01$ vs. vehicle-treated group (Dunnett's test). $N=6$.

onset of action of this drug. Statistical analysis was performed by comparison of the % of the pre-drug values with the corresponding values in the vehicle-treated group using Dunnett's test.

All the animal experiments were conducted with the approval of Takeda's Experimental Animal Care and Use Committee.

2.5. Chemicals

TAK-802 is relatively insoluble in aqueous solvents. Therefore, the hydrochloride form of TAK-802 was used for the in-vivo studies. TAK-802, TAK-802 hydrochloride and distigmine bromide were synthesized in Takeda's medicinal chemistry research laboratories. Acetylcholinesterase

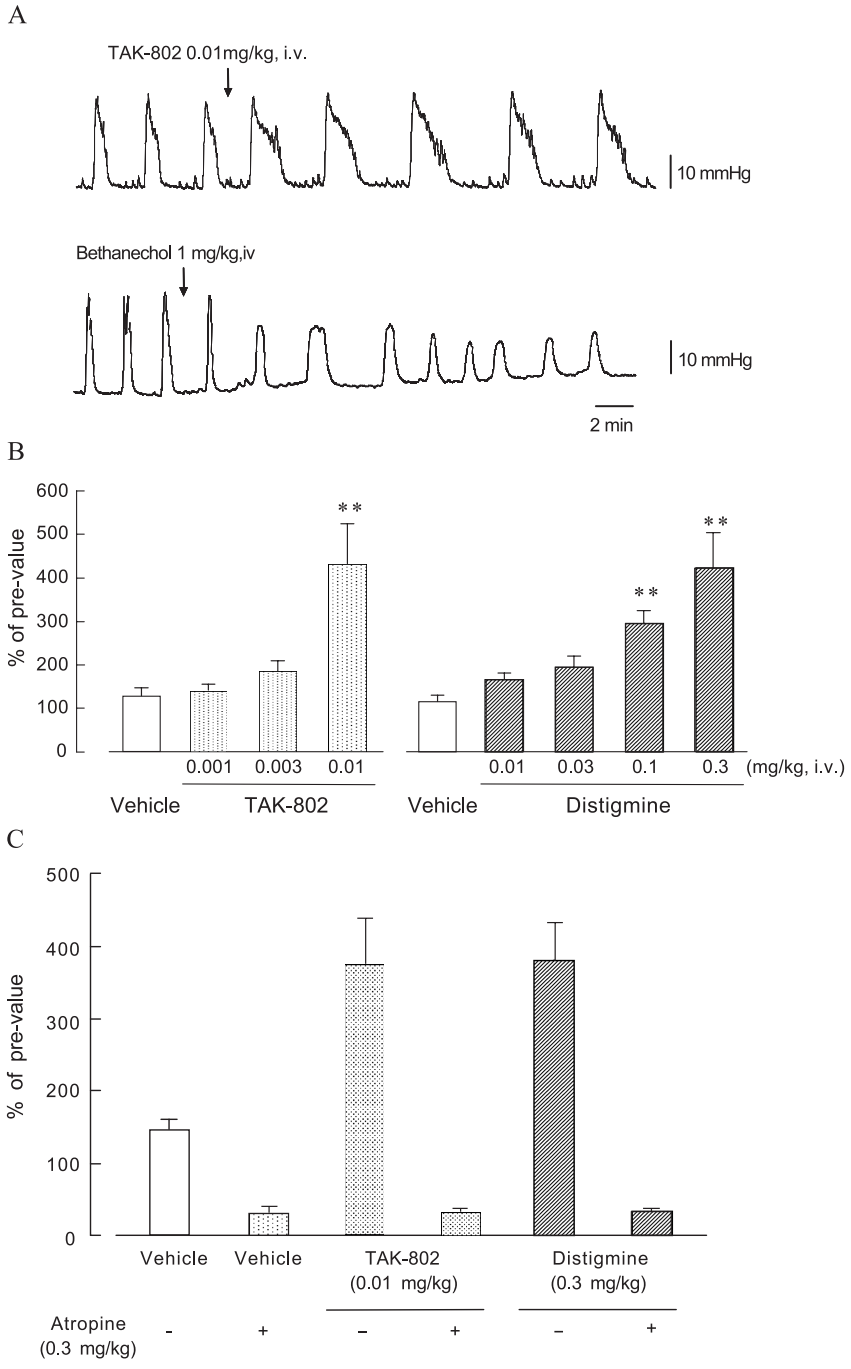

Fig. 3. (A) Representative cystmetrograms of distention-induced rhythmic bladder contractions in guinea pigs. After confirming stable rhythmic contractions, TAK-802 or bethanechol was administered intravenously. (B) Effect of TAK-802 and distigmine on the isovolumetric rhythmic bladder contractions in guinea pigs. Values are represented by % of pre-value and mean ± S.E.M. $^{**}P < 0.01$ vs. vehicle-treated group (Dunnett's test). $N = 8$, except for vehicle-treated group; $N = 7$. (C) Antagonism of the effects of TAK-802 and distigmine by atropine. Atropine was administered concomitantly with acetylcholinesterase inhibitors. Values are mean ± S.E.M. $N = 6$, except for the animal that received co-administration of TAK-802 and atropine; $N = 5$.

derived from human erythrocytes, 5,5-dithio-bis(2-nitrobenzoic acid), acetylthiocholine iodide, butyrylthiocholine chloride, neostigmine bromide, tetraisopropyl pyrophosphoramide (iso-OMPA) and atropine were obtained from Sigma (St. Louis, MO, USA). Butyrylcholinesterase derived from human serum was purchased from Biogenesis (Poole, UK). Bethanechol chloride was purchased from RBI (Natick, MA, UAS). The drugs were dissolved in dimethyl sulfoxide (Wako, Osaka, Japan) for the in-vitro studies and in saline or distilled water for in-vivo studies.

3. Results

3.1. Acetylcholinesterase and butyrylcholinesterase inhibition activity

Both TAK-802 and the carbamate acetylcholinesterase inhibitors distigmine and neostigmine inhibited acetylcholinesterase activity in a concentration-dependent manner (Fig. 1). While both distigmine and neostigmine also inhibited butyrylcholinesterase activity, TAK-802 did not. Iso-OMPA, a selective butyrylcholinesterase inhibitor, inhibits only the activity of butyrylcholinesterase. The IC_{50} values of the drugs are shown in Table 1. Inhibition of the acetylcholinesterase activity by TAK-802 was 250 and 30 times more potent than that by distigmine and neostigimine, respectively. Unlike carbamate acetylcholinesterase inhibitors, TAK-802 did not inhibit butyrylcholinesterase activity even up to the concentration of 10 μM, thus exhibiting high selectivity (more than 6700 times) for acetylcholinesterase inhibition over that of butyrylcholinesterase.

3.2. Muscarinic and nicotinic actions

The minimum effective doses (MEDs) of TAK-802 and distigmine administered intravenously for their muscarinic actions were 0.003 and 0.03 mg/kg, respectively, as revealed by the intestinal charcoal meal transit assay (Table 2). The enhancement of the charcoal meal transit decreased at doses beyond the MED concomitant with the emergence of fasciculation. Fasciculation, an index of drugs' nicotinic actions, was induced in a dose-dependent manner. The MEDs of TAK-802 and distigmine for the induction of fasciculation were 0.03 and 0.1 mg/kg i.v., respectively. The potency of the nicotinic actions of TAK-802 relative to that of its muscarinic actions in terms of the MED was three times less than that of the corresponding potency ratio of distigmine in rats.

3.3. Effect on distension-induced rhythmic bladder contractions in urethane-anesthetized rats

After administration of TAK-802 and distigmine, the frequency of rhythmic bladder contractions tended to decrease. Both of drugs augmented bladder contractions mainly by prolonging the duration of the contractions (Fig. 2A). Both TAK-802 and distigmine caused a dose-dependent increase in the AUC of distension-induced bladder contractions (Fig. 2B); the MEDs were 0.01 and 0.03 mg/kg i.v., respectively.

3.4. Effect on distension-induced rhythmic bladder contractions in urethane-anesthetized guinea pigs

TAK-802 and distigmine augmented well-coordinated bladder contractions, and both of drugs increased the AUC of the bladder contractions in a dose-dependent manner when administered intravenously, with a MED of 0.01 and 0.1 mg/kg, respectively (Fig. 3A,B). The effects were completely abolished by the muscarinic antagonist atropine (0.3 mg/kg i.v.) (Fig. 3C). On the other hand, bethanechol, a muscarinic agonist, markedly changed the pattern of the cystometrogram when administered at the dose of 1 mg/kg i.v. ($N = 5$); an irregular contraction rhythm, increase in basal intravesical pressure and decrease in the maximum contractile pressure were observed (Fig. 3A).

4. Discussion

Two transmitters released from parasympathetic postganglionic nerve endings are believed to play an important role in mediating bladder contractions in most mammalian species. One is acetylcholine, which activates muscarinic receptors, induces contraction of the detrusor smooth muscle of the bladder, and sustains the contraction to empty the bladder, and the other is ATP, which activates the P2X purinoceptor, causes rapid contractions of the detrusor muscle, and may participate in initiating micturition (Anderson, 1993).

Impaired detrusor contractility is one of the causes of voiding dysfunction associated with aging (Malone-Lee and Wahedna, 1993) and chronic diseases such as benign prostatic hypertrophy (Akino et al., 1996; Eckhardt et al., 2001), diabetic mellitus (Ueda et al., 1997), multiple sclerosis (Litwiller et al., 1999). It has been reported that the density of acetylcholinesterase-positive nerve endings in the bladder decreases with overdistension of the bladder in rats (Lasanen et al., 1992), in the case of bladder outlet obstruction in rabbits (Elbadawi et al., 1989) and in rats with diabetes (Lincoln et al., 1984). These observations suggest that impaired detrusor contractility may be caused partly by a decrease in the cholinergic innervation to the bladder. Cholinomimetic agents, such as muscarinic agonists and acetylcholinesterase inhibitors, have been used in the pharmacologic treatment of lower urinary tract symptoms associated with impaired detrusor contractility (Wein et al., 1994). The rationale of the treatment is to increase the detrusor muscle contractility by boosting the parasympathetic cholinergic system. 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).

In the present study, we have shown that TAK-802, a novel non-carbamate acetylcholinesterase inhibitor, potently and selectively inhibits human-erythrocyte-derived acetylcholinesterase, and has higher specificity for muscarinic actions over nicotinic actions than distigmine, a carbamate acetylcholinesterase inhibitor. Carbamate acetylcholinesterase inhibitors were originally developed as drugs for the treatment of myasthenia gravis and have been reported to exert direct excitatory modulation on nicotinic receptors (Pereira et al., 1993; Sung et al., 1998). The direct modulating effect on nicotinic receptors may be related not to their acetylcholinesterase-inhibitory activity, but to their chemical structures. As TAK-802 is a non-carbamate acetylcholinesterase inhibitor, it might not have any direct excitatory action on nicotinic receptors, although this remains to be clarified. The activation of nicotinic receptors by carbamate acetylcholinesterase inhibitors is not favorable for the treatment of voiding dysfunction, because nicotinic effects are associated with contraction of the external urethral sphincter muscle and increase in urethral resistance (von Heyden et al., 1995). Actually, the clinical efficacy of distigmine is controversial (Hameed and Charles, 1994; Philp and Thomas, 1980; Shah et al., 1983; Tanaka et al., 2001). TAK-802 may therefore improve voiding function more effectively than carbamate acetylcholinesterase inhibitors.

Although TAK-802 inhibited human-erythrocyte-derived acetylcholinesterase about 250 times more potently than distigmine, the difference between the two drugs, in terms of their potency to activate muscarinic events (intestinal charcoal transit in rats and bladder contraction in rats and guinea pigs) in vivo was 3–10-fold. One reason for this discrepancy between the drug activities in vitro and in vivo might be that distigmine more effectively potentiates bladder contractions by inhibiting both acetylcholinesterase and butyrylcholinesterase. Butyrylcholinesterase is present in the bladder (Roshani et al., 1996) and may also contribute to the hydrolysis of endogenous acetylcholine, besides acetylcholinesterase. Another reason might be the kinetic differences for acetylcholinesterase inhibition between TAK-802 and distigmine. Carbamate acetylcholinesterase inhibitors have an optimal incubation time to reach the maximum activity (Ogura et al., 2000). In our preliminary studies, the estimated acetylcholinesterase inhibitory activity of TAK-802 did not depend on the preincubation time, whereas the activity of distigmine increased with preincubation. Actually, in in-vivo studies, there was a latency period before the onset of action of distigmine, even when the drug was administered intravenously (preliminary observation).

Both TAK-802 and distigmine increased reflex bladder contractions in rats and guinea pigs in the same manner, i.e. mainly by prolonging the duration of bladder contraction with no effect on the basal intravesical pressure, unlike bethanechol, in guinea pigs. Acetylcholinesterase inhibitors potentiate the contraction of the detrusor smooth muscle by increasing the amount of acetylcholine released endogenously during the micturition reflex. In contrast, muscarinic agonists directly activate muscarinic receptors and induce contractions of the detrusor smooth muscle, irrespective of the phase of the micturition reflex. This may be the cause for the marked change of the pattern of distention-induced rhythmic bladder contractions by bethanechol. Bethanechol has also been clinically used for voiding dysfunction, but its effect on the treatment outcome is still not clear (Barrett, 1981; Wein et al., 1980).

After administration of TAK-802 and distigmine, the frequency of rhythmic bladder contractions tended to decrease in rats. Two reasons for this are conceivable. There was a clear relationship between the magnitude of the bladder contractions and the intervals between the bladder contractions in this study. The intervals increased as the bladder contractions became stronger and vice versa. The second is that the frequency of contractions is regulated by the micturition center in the central nervous system. Inhibitory regulation of the micturition reflex by cholinergic mechanisms in the spinal cord and brain has been reported in the rat (Ishiura et al., 2001). It is therefore conceivable that the acetylcholinesterase inhibitors decreased the frequency of rhythmic bladder contractions via activation of the central cholinergic system in this study.

In the treatment of voiding dysfunction caused by impaired detrusor contractility, the pharmacologic agent used must not increase the urethral resistance in order to avoid high-pressure micturition (Wein et al., 1994). While in this study, we examined only the effect of cholinomimetics on bladder contractility in vivo, study of the actions of TAK-802 on the urodynamic characteristics is necessary for a full evaluation of the usefulness of the drug in lower urinary tract dysfunction and is now on-going.

In conclusion, TAK-802 was shown to selectively inhibit acetylcholinesterase derived from human erythrocytes, to facilitate muscarinic events rather than nicotinic events when compared with distigmine, and to increase reflex bladder contractions in urethane-anesthetized rats and guinea pigs. TAK-802 is expected to become a novel drug belonging to a new class useful for the treatment of voiding dysfunction caused by impaired detrusor contractility.

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