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Possible site of action of TAK-637, a tachykinin NK_1 receptor antagonist, on the micturition reflex in guinea pigs

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Abstract

TAK-637((*aR*,9*R*)-7-[3,5-bis(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-methyl-5-(4-methylphenyl)-7*H*-[1,4]diazocino[2,1-*g*][1,7]naphthyridine-6,13-dione) is a novel tachykinin NK_1 receptor antagonist that has been shown to inhibit the micturition reflex in guinea pigs. The aim of this study was to clarify its mechanism of action in guinea pigs. TAK-637 inhibited the spinal vesico-vesical reflex induced by electrical stimulation of the proximal cut end of the pelvic nerve in spinal animals, but not bladder contractions induced by electrical stimulation of the distal cut end of the nerve. Furthermore, TAK-637 had no effect on carbachol- or electrical field stimulation-induced contractions of isolated bladder muscle strips in an organ bath, whereas drugs used for abnormally frequent micturition inhibited both contractions. These results suggest that TAK-637 inhibits the micturition reflex by acting, at least in part, on the spinal cord, and its mechanism of action clearly differs from those of antimuscarinics or spasmolytics.

Keywords: TAK-637; Tachykinin NK_1 receptor antagonist, non-peptide; Micturition reflex; Spinal cord; Electrical stimulation

1. Introduction

Substance P is a major neuropeptide in capsaicin-sensitive primary afferent neurons, some of which innervate the mammalian urinary bladder (Sharkey et al., 1983) and are thought to play an important role in the regulation of micturition (Maggi, 1991). Depletion of substance P by the administration of a high dose of capsaicin increased bladder capacity in rats (Holzer-Petsche and Lembeck, 1984; Maggi, 1991) and guinea pigs (Maggi et al., 1987), suggesting that substance P participates in the distension-induced micturition reflex. Intrathecal administration of several non-peptide tachykinin NK_1 receptor antagonists increased the volume threshold for micturition in anesthetized rats (Lecci et al., 1993a) and inhibited bladder hyperactivity in conscious rats (Ishizuka et al., 1995).

TAK-637((*aR*,9*R*)-7-[3,5-bis(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-methyl-5-(4-methylphenyl)-7*H*-[1,4]diazocino[2,1-*g*][1,7]naphthyridine-6,13-dione) is a potent tachykinin NK_1 receptor antagonist with a higher affinity for guinea pig and human tachykinin NK_1 receptors than for the rat tachykinin NK_1 receptor. Its receptor affinity is 0.45 nM ($= IC_{50}$) in human IM-9 cells, and its in vivo activity is 33 $\mu g/kg$, p.o. ($= ID_{50}$) in capsaicin-induced trachea extravasation in guinea pigs (Natsugari et al., 1999). Systemic administration of TAK-637 increases bladder capacity in both urethane-anesthetized and conscious guinea pigs (Doi et al., 1999). In neuraxially intact animals, TAK-637 increased the intervals between distension-induced rhythmic bladder contractions without reducing the contractile force, indicating that TAK-637 affects the afferent rather than the efferent components of the micturition reflex. TAK-637 also increased the intervals between distension-induced rhythmic bladder contractions in spinal animals, suggesting that the spinal cord is an important site of action of this drug (Doi et al., 2000).

In order to make the mechanisms of TAK-637 clearer, we focused on the site of action and compared the effects of drugs for abnormally frequent micturiton.

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2. Materials and methods

2.1. Animals

Male Hartley guinea pigs were used in all the experiments, which were approved by Takeda's Experimental Animal Care and Use Committee.

2.2. Effects on bladder contractions induced by electrical stimulation of the pelvic nerve

Animals weighing 250–400 g were anesthetized with an i.p. injection of urethane (1.2 g/kg). The urinary bladder was exposed through an abdominal incision, the urethra was ligated and a 23-gauge needle connected to a polyethylene tube (PE-90) was inserted into the bladder dome to record the intravesical pressure. Warmed physiological saline (39°C) was injected into the bladder in amounts such that the volume was just below the threshold for isovolumetric bladder contractions.

In order to induce the spinal vesico-vesical reflex by electrically stimulating the central cut end of the pelvic nerve, the spinal cord was severed at the level of Th13-L1, and the hypogastric nerves on both sides and the pelvic nerve on one side were severed. The proximal cut end of the pelvic nerve was mounted on bipolar platinum wire electrodes, and covered with liquid paraffin to keep it moistened. Rectangular electric pulses (30 Hz, 0.5 ms width pulse trains lasting 10 s) were delivered every 15 min by an electrical stimulator (Nihon Kohden, SEN-3301) connected to an isolater (Nihon Kohden, SS-202J) and the strength of the stimuli was set just above supramaximal thresholds (40–60 V). When the evoked contractions were stable, TAK-637 or vehicle (dimethylsulfoxide (DMSO)) was injected i.v.

In order to stimulate the peripheral cut end of the pelvic nerve, both the pelvic nerves and the hypogastric nerves were severed bilaterally. The peripheral cut end of one pelvic nerve was mounted on bipolar platinum wire electrodes, and covered with liquid paraffin. Rectangular electric pulses (30 Hz, 0.5 ms width pulse trains lasting 5 s) were delivered every 5 min by the electrical stimulator connected to an isolator and the strength of the stimuli was adjusted just above supramaximal thresholds (40–60 V). When the evoked contractions were stable, the required drug or vehicle was injected i.v.

TAK-637 was dissolved in DMSO and injected in a volume of 0.05 ml/100 g body weight and other drugs were dissolved in isotonic saline and injected in a volume of 0.1 ml/100 g body weight.

Differences between mean (±S.E.M.) pre- and post-drug values were compared with Student's paired *t*-test. Differences at $P < 0.05$ were considered significant.

2.3. Effects on guinea pig isolated bladder strips

Animals weighing 250–600 g were killed by decapitation and strips of detrusor muscle (8-mm long, 3-mm wide) were cut from the dome of the bladder, avoiding the area surrounded by the urethra and ureters. Each strip was placed in a 20-ml organ bath containing Krebs solution maintained at 37°C and bubbled continuously with a mixture of 95% O_2 and 5% CO_2. The strip was connected to an isometric transducer (Minebea, UL-10 GR) with a tension of 1 g, allowed to equilibrate and then used in the experiments described below.

Cumulative concentration–response curve to carbachol was obtained and, 60 min later, a 20-μl aliquot of the test drug or vehicle solution was added to the bath. Then, 5 min later, another concentration–response curve to carbachol was obtained. Each contractile response was expressed as a percentage of the maximal contraction achieved in the first concentration–response curve.

In order to induce contractions with electrical field stimulation, two rectangular-shaped platinum electrodes were placed on both sides of the strip, connected to an electrical stimulator (Nihon Kohden, SEN-3301) and transmural stimulation (50 V, 15 Hz, 1 ms width pulse trains lasting 10 s) was carried out every 5 min. When two successive reproducible responses had been achieved, the test drug or vehicle solution was added to the bath. Each contractile response was expressed as a percentage of the mean of the last two contractions before the drug or vehicle was added. The effects of the drugs and vehicle were compared using Dunnett's test. Differences at $P < 0.05$ were considered significant.

2.4. Chemicals

TAK-637, inaperisone hydrochloride (inaperisone) and tolterodine hydrogen tartrate (tolterodine) were synthesized in Takeda's Pharmaceutical Research Laboratories. Oxybutynin hydrochloride (oxybutynin, Kodama) and propiverine hydrochloride (propiverine, Taiho) were extracted from the respective commercially available tablets in Takeda's Pharmaceutical Research Laboratories. Urethane was obtained from Aldrich and carbachol, tetrodotoxin and atropine were purchased from Sigma.

3. Results

3.1. Effect on the spinal vesico-vesical reflex

The effect of TAK-637 on the spinal vesico-vesical reflex induced by stimulating the proximal cut end of the pelvic nerve was investigated. In order to exclude supraspinal effects on micturition, acutely spinalized animals were used and both sides of the hypogastric nerves were cut to eliminate any effect mediated by the sympathetic nerve. The respective mean ± S.D. ($n = 6$) baseline intravesical pressures before stimulation of the nerve ranged from 1.6 ± 1.5 to 2.3 ± 1.7 mm Hg. Electrical stimulation of the central cut end of the pelvic nerve elicited transient bladder contractions (Fig. 1).

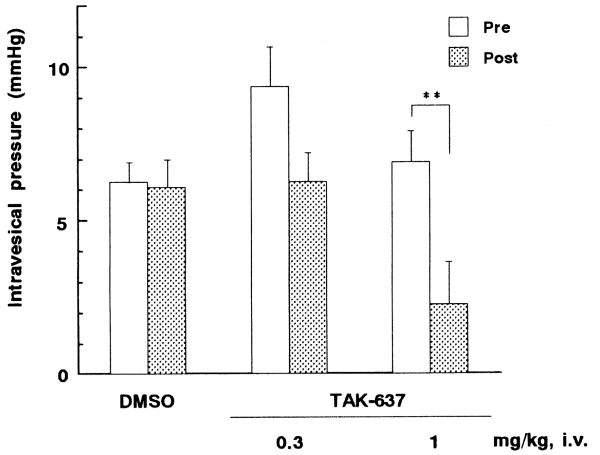

Fig. 1. Effect of TAK-637 on the spinal vesico-vesical reflex. Drug and vehicle (DMSO) were administered intravenously. The open and hatched columns represent the bladder contractile pressure before and after drug treatment, respectively. Each bar represents the mean ± S.E.M. for six animals. ** $P < 0.01$ compared with the pretreatment value (Student's paired *t*-test).

As TAK-637 at 1 mg/kg, i.v. inhibited the isovolumetric bladder contractions in guinea pigs (Doi et al., 2000), the doses of 0.3 and 1 mg/kg were tested on the spinal vesico-vesical reflex 15 min after the i.v. administration. The vehicle (DMSO) alone had no significant effect on this vesico-vesical reflex, whereas TAK-637 reduced the contractile pressure and its effect at 1 mg/kg, i.v. was significant (Fig. 1).

3.2. Effect on bladder contractions induced by electrical stimulation of the peripheral cut end of the pelvic nerve

Electrical stimulation of the peripheral cut end of one pelvic nerve induced transient bladder contractions and their mean amplitudes before drug administration ranged from 10 to 25 mm Hg. Intravenous injection of vehicle had no effect on these bladder contractions. The respective mean ± S.E.M. ($n = 6$) contractile bladder pressures before and after vehicle administration were 16.7 ± 2.1 and 19.6 ± 3.2 mm Hg for the DMSO-treated group and 14.3 ± 0.6 and 14.8 ± 1.1 mm Hg for the saline-treated group.

TAK-637, at a dose of 3.0 mg/kg, i.v., had no effect on the contractions induced by electrical stimulation (Table 1), whereas the drugs used for abnormally frequent micturition such as oxybutynin, tolterodine and propiverine reduced the responses. Their respective minimum effective doses were 0.3, 0.1 and 10 mg/kg, i.v. (Table 1).

Inaperisone, a centrally acting muscle relaxant, which inhibits the micturition reflex in rats (Morikawa et al., 1992), also reduced the contractile response and its minimum effective dose was 1 mg/kg, i.v. (Table 1).

Table 1
Effects of intravenously injected drugs on the contractions induced by electrical stimulation of the peripheral cut end of the pelvic nerve. Pre and post indicate the intravesical pressure, mean ± S.E.M., before and after drug administration. *n* represents the number of animals

Drug	Dose (mg/kg)	*n*	Intravesical pressure (mm Hg)	
			Pre	Post
TAK-637	3	6	10.8 ± 1.5	11.7 ± 1.3
Oxybutynin	0.1	6	21.3 ± 2.0	17.4 ± 1.8
	0.3	6	19.7 ± 2.0	11.3 ± 1.9[a]
Tolterodine	0.03	6	16.1 ± 1.9	17.0 ± 2.5
	0.1	6	18.8 ± 3.1	14.6 ± 2.0[b]
	0.3	6	23.2 ± 2.6	15.0 ± 1.6[a]
Propiverine	3	5	11.5 ± 1.1	10.6 ± 1.1
	10	8	12.1 ± 2.1	8.6 ± 1.6[a]
Inaperisone	0.3	6	19.4 ± 1.6	19.9 ± 2.9
	1.0	6	24.0 ± 2.3	21.8 ± 2.5[b]

[a] Mean difference between the pre and post value is significant at $P < 0.01$ (Student's paired *t*-test).
[b] Mean difference between the pre and post value is significant at $P < 0.05$ (Student's paired *t*-test).

3.3. Comparison with drugs for overactive bladder in organ bath

3.3.1. Effect on carbachol-induced contractions of guinea pig isolated bladder muscle strips

The effect of each drug was assessed by comparing the concentration–response curve to carbachol with that of the vehicle-treated group. As shown in Fig. 2, TAK-637 had no clear effect on carbachol-induced contractions. The maximal contraction of the bladder strips was reduced by 18.8% in the presence of the 10^{-5} M concentration, but this reduction was not significant.

Atropine, oxybutynin and tolterodine all evoked rightward shifts of the cumulative concentration–response curve for carbachol (Fig. 3). Propiverine showed mixed antagonism: a parallel rightward shift of the concentration–re-

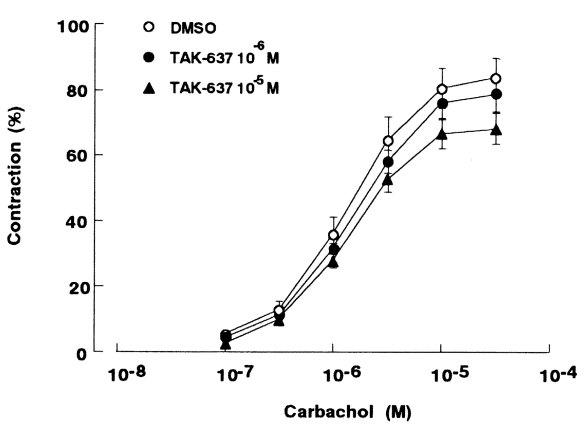

Fig. 2. Effect of TAK-637 on carbachol-induced contractions of guinea pig isolated bladder strips. Each point represents the mean ± S.E.M. for six experiments.

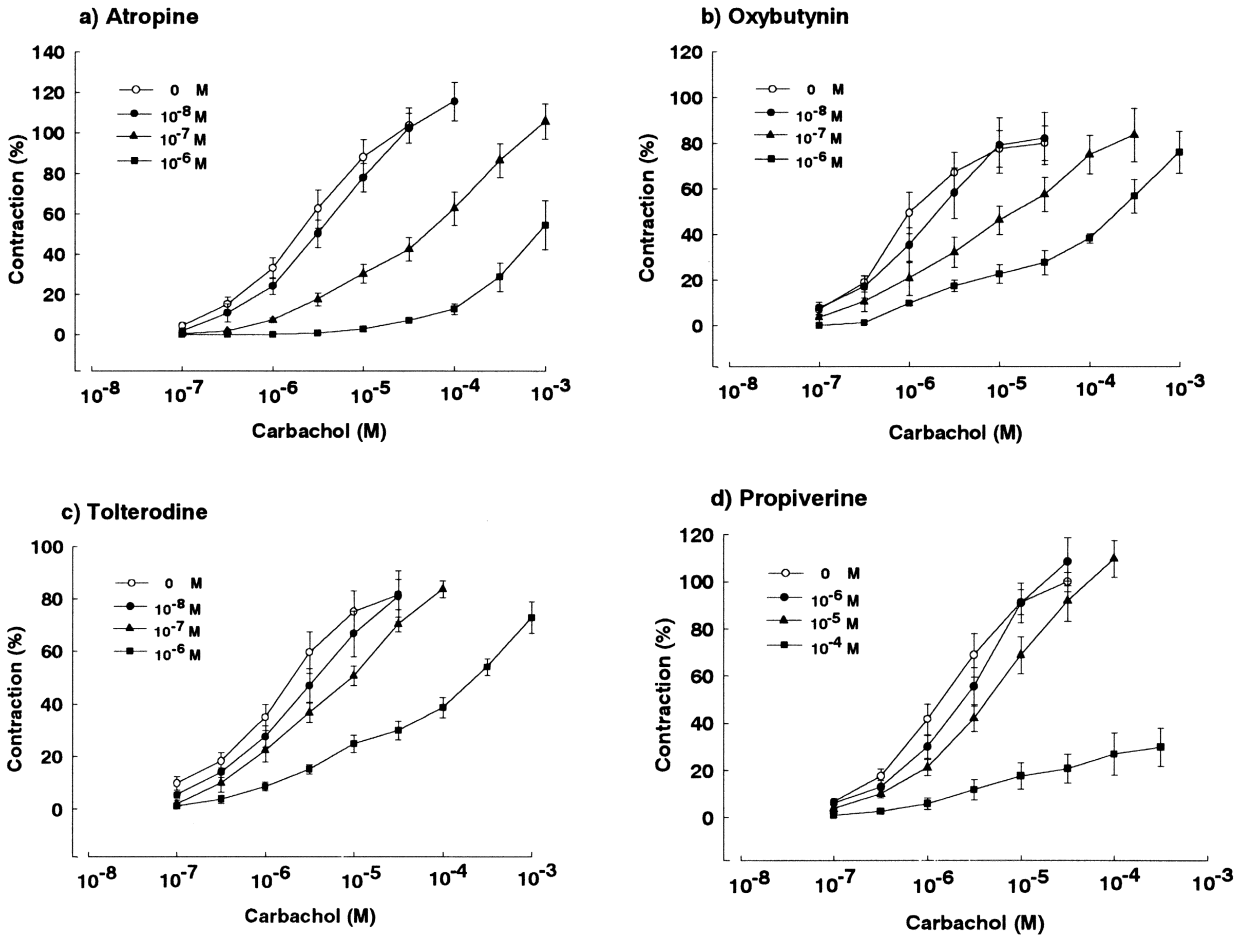

Fig. 3. Effect of atropine (a), oxybutynin (b), tolterodine (c) and propiverine (d) on carbachol-induced contractions of guinea pig isolated bladder strips. Each point represents the mean ± S.E.M. for five experiments.

sponse curve for carbachol was observed in the presence of 10^{-5} M, whereas the maximal contractions decreased in the presence of higher concentrations.

3.3.2. *Effect on electrical field stimulation-induced contractions of guinea pig isolated bladder strips*

Electrical field stimulation evoked frequency-dependent contractions of isolated bladder strips. The effects of the drugs were tested at 15 Hz, which evoked contractions of about 70% of the maximal responses. Tetrodotoxin, at a concentration of 10^{-6} M, abolished the contractions, showing they were neurogenic in nature ($n = 3$, data not shown). Atropine, at a concentration of 10^{-6} M, did not inhibit the contractions (Table 2), suggesting that any neurotransmitter other than acetylcholine was important mediator of this contraction.

As shown in Table 2, TAK-637 at a concentration of 10^{-5} M had no effect on transmural stimulation-evoked contractions. The effect of this drug at a concentration of 10^{-4} M could not be tested because of its lack of solubility.

The effects of the drugs for abnormally frequent micturition are shown in Table 2. Oxybutynin, tolterodine and propiverine, all at 10^{-5} M, did not significantly reduce the contractions, whereas they did so at 10^{-4} M. As a higher

Table 2
Effects of various drugs on EFS-induced contractions of guinea pig isolated bladder strips. Values are means ± S.E.M. for five experiments

Drug	Concentration (M)	%Control
DMSO		103.3 ± 3.6
TAK-637	10^{-6}	92.5 ± 7.9
	10^{-5}	94.9 ± 5.5
Saline		107.1 ± 7.5
Oxybutynin	10^{-5}	84.1 ± 5.6
	10^{-4}	50.3 ± 5.6[a]
Tolterodine	10^{-5}	91.2 ± 11.2
	10^{-4}	54.2 ± 8.5[a]
Saline		92.0 ± 6.7
Propiverine	10^{-5}	99.3 ± 4.2
	10^{-4}	52.6 ± 9.8[a]
Atropine	10^{-6}	94.1 ± 3.4

[a] $P < 0.01$ compared with the value for the corresponding vehicle-treated group (Dunnett's test).

concentration of drugs was required for inhibiting the nerve impulse-mediated contractions, it was likely that they reduced the contractions through their spasmolytic (Ca^{2+} antagonistic) properties (Anderson and Fredericks, 1977; Nilvebrant et al., 1997; Haruno, 1992).

4. Discussion

Our previous studies had shown that a novel tachykinin NK_1 receptor antagonist, TAK-637, inhibited the micturition reflex induced by bladder distension and increased the bladder capacity in guinea pigs (Doi et al., 1999, 2000). The micturition reflex could be caused by highly coordinated or harmonized co-operations of sacral and pontine micturition centers (Blok and Holstege, 1998). In view of the spinobulbospinal micturition reflex pathway and the distribution of tachykinin NK_1 receptors (Otsuka and Yoshioka, 1993), the supraspinal structures, the spinal cord and the bladder are thought to be candidates for the sites of action of TAK-637. As there was no marked difference in the effective doses of TAK-637 on the micturition reflex between spinal animals and intact ones (Doi et al., 2000), the spinal cord or the bladder seemed to be more important than supraspinal structures. Although the possibility that TAK-637 acts on the supraspinal site cannot be completely excluded, we focused on the spinal cord and the bladder as possible sites of action of TAK-637.

The direct evidence for a spinal site of action could have been arrived at using intrathecal injection of TAK-637, but, actually the vehicle (DMSO) itself had a significant effect on the micturition reflex, which ruled out experiments using intrathecal injection. The effects of systemic administration of TAK-637 on the bladder contractions induced by electrical stimulation of the central and the peripheral cut ends of the pelvic nerve were then compared. In the present study, TAK-637 inhibited the spinal vesico-vesical reflex induced by electrical stimulation of the central cut end of the pelvic nerve in spinal guinea pigs, but did not reduce the bladder contractions induced by electrical stimulation of the peripheral cut end of the pelvic nerve at all. These results showed that the site of action of TAK-637 is not in the peripheral efferent arc of the micturition reflex, but in the spinal cord.

The distribution of tachykinin NK_1 receptors in the urinary bladder is also well known, but their physiological role is not clear. TAK-637 influenced neither the contractile pressure of the distension-induced rhythmic bladder contractions in urethane-anesthetized guinea pigs (Doi et al., 2000) nor the bladder contractile pressure induced by electrical stimulation in vivo in the present experiments. Therefore, peripheral tachykinin NK_1 receptors may not be involved in the motor function of the bladder as had been reported (Lecci et al., 1993b, 1997).

There is much evidence that the tachykinin NK_1 receptors in the spinal cord play an important role in the micturition reflex. Capsaicin-sensitive primary afferent neurons, most of which are believed to be substance P-containing neurons, are present in the pelvic nerve innervating the mammalian urinary bladder (Sharkey et al., 1983) and their terminals, as well as substance P binding sites, are found in the areas of the spinal cord where the primary afferent of the pelvic nerve terminates (De Groat et al., 1983; Yashpal et al., 1990; Charlton and Helke, 1985). Intrathecal administration of non-peptide tachykinin NK_1 receptor antagonists is reported to inhibit the micturition reflex in rats (Lecci et al., 1993a). These findings suggest that substance P and its receptors in the spinal cord are involved in transmission of the afferent input from the bladder (Maggi, 1991). Therefore, TAK-637 seems to act primarily on the spinal cord and inhibit the afferent inputs from the bladder at the secondary neurons in the spinal cord.

Although the etiology of the overactive bladder is not clearly understood, the sensory pathway is reported to play an important role in the cause of detrusor overactivity (Klein, 1988). In fact, intravesical administration of capsaicin or resiniferatoxin, which inactivates the substance P-containing primary afferent neurons, is known to be highly effective on detrusor overactivity in humans (Fowler et al., 1994; Wiart et al., 1998; Lazzeri et al., 1997). In view of the above findings, TAK-637 may be a useful new drug for the treatment of abnormally frequent micturition by inhibiting the sensory system of the lower urinary tract.

Antimuscarinics or spasmolytics such as oxybutynin, tolterodine and propiverine (Anderson and Fredericks, 1977; Haruno, 1992; Kachur et al., 1988; Nilvebrant et al., 1997; Peterson and Noronha-Blob, 1989; Peterson et al., 1990; Tokuno et al., 1993) are used for overactive bladder and a centrally acting muscle relaxant, inaperisone, is expected to be the new drug for incontinence (Morikawa et al., 1992). The effects of TAK-637 and these drugs were studied in vivo, and also in vitro using bladder muscle strips.

Oxybutynin, tolterodine and propiverine showed an inhibitory effect in the in vivo experiments with peripheral stimulation of pelvic nerve-induced bladder contractions. They clearly showed an antimuscarinic effect in the in vitro experiment using carbachol as the stimulant. The bladder muscle contractions induced by electrical field stimulation were also inhibited at the higher concentrations. Inaperisone is reported to inhibit the micturition reflex in rats. As the effective dose of this drug is 100-fold smaller after i.c.v. than after systemic injection, the supraspinal structures are considered to be important as its site of action on bladder functions (Morikawa et al., 1992). However, in the present experiments, inaperisone slightly but significantly inhibited the bladder contractions induced by electrical stimulation of the peripheral cut end of the pelvic nerve, suggesting that its site of action is not only on the central nervous system but also on the bladder

itself. This is consistent with the report that the topical application of inaperisone to the bladder dome inhibits the bladder contractions induced by distension (Morikawa et al., 1988).

In contrast, TAK-637 showed neither an antimuscarinic nor an antispasmolytic effect in the in vitro experiments. Therefore, the mode of action of TAK-637 on the micturition reflex is clearly different from those of drugs used clinically for abnormally frequent micturition, which sometimes cause problems with incomplete bladder emptying because of the decreased detrusor contractile force.

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