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European Journal of Pharmacology 412 (2001) 171–179

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Pharmacological actions of AH-9700 on micturition reflex in anesthetized rats

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Received 14 July 2000; received in revised form 5 December 2000; accepted 8 December 2000

Abstract

In radioligand binding assays, AH-9700 (1-[2-(3,4-dihydro-6,7-dimethyl-2-naphthalenyl)ethyl]pyrrolidine fumarate) had high affinity for σ receptors and moderate affinity for muscarinic receptors. The affinity of AH-9700 for σ_1 receptors was significantly reduced in the presence of 5′-guanylyl-imidodiphosphate (GppNHp). In isolated bladder strips of rats, AH-9700 inhibited carbachol-induced contractions. In anesthetized rats, i.v. administration of AH-9700 and typical σ receptor ligands, (+)-pentazocine and 1,3-di-*o*-tolylguanidine (DTG), but not oxybutynin, dose-dependently inhibited rhythmic isovolumetric reflex bladder contractions. AH-9700 and oxybutynin suppressed the amplitude of rhythmic bladder contractions. On the other hand, at doses lower than used i.v., the i.c.v. administration of AH-9700 or the σ receptor ligands inhibited rhythmic bladder contractions without suppressing the amplitude. This inhibitory effect of AH-9700 was markedly reduced by pretreatment with i.c.v. pertussis toxin. These results suggest that AH-9700 exerts a marked anti-micturition reflex effect through central σ receptors possibly related to pertussis toxin-sensitive Gi/o-proteins and a moderate spasmolytic effect based on its peripheral anti-muscarinic activity.

Keywords: AH-9700; σ receptor; Bladder contraction, rhythmic isovolumetric reflex; Muscarinic receptor; Urinary tract, rat

1. Introduction

The number of patients suffering from urinary incontinence has been increasing in aging societies. The International Continence Society (1998) has defined four types of urinary incontinence: urge, genuine stress, reflex and overflow. Among them, urge incontinence is frequently observed in elderly patients. Although urinary incontinence is not a mortal disease, patients live an inconvenient life.

All existing drugs for the treatment of urge incontinence and frequent urination are characterized by their common ability to relax detrusor smooth muscles, based on anti-muscarinic and/or Ca^{2+}-antagonistic properties. However, these drugs cause systemic adverse effects such as dry mouth, blurred vision, constipation and hypotension, which restrict their clinical use (Ferguson and Christopherm, 1996). For example, oxybutynin, a currently used drug for the treatment of urge incontinence and frequent urination, frequently causes (at least 50%) anti-muscarinic adverse effects (Moisey et al., 1980; Yarker et al., 1995). Therefore, a new drug, which can facilitate urine storage through a different mechanism, has long been desired.

A σ-receptor was initially proposed by Martin et al. (1976) to explain the psychotomimetic actions of *N*-allylnormetazocine. Following subsequent biochemical and pharmacological studies, the σ receptor has been categorized into at least two subtypes, termed σ_1 and σ_2 (Walker et al., 1990; Quirion et al., 1992). Earlier studies have demonstrated that σ receptors relate to diverse pharmacological effects, for example, antipsychotic, antidepressant, anxiolytic, neuroprotective, anti-amnesic, anti-inflammatory, anti-tussive, anti-ulcer, intestinal motility modulation and anti-ion transport effects (Su et al., 1988; Walker et al., 1990; Su, 1991; Junien et al., 1991; Kamei et al., 1992; Riviere et al., 1993; Maurice et al., 1998; Nakazawa et al., 1998). In addition, we have shown for the first time that i.v. or i.c.v. administration of typical σ receptor ligands such as (+)-pentazocine or 1,3-di-*o*-tolylguanidine (DTG)

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0014-2999/01/$ - see front matter
PII: S0014-2999(00)00937-7

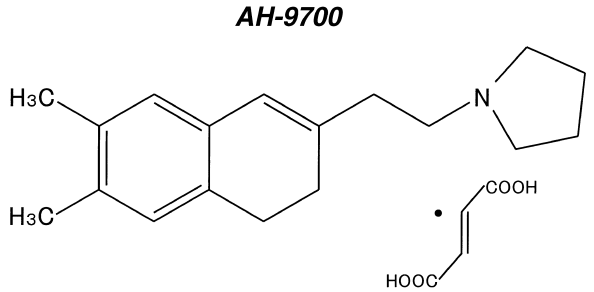

Fig. 1. Chemical structure of AH-9700.

increases bladder capacity on cystometrograms in rats, and central σ receptor(s) may play an important role in the micturition controlling system (Shimizu et al., 2000).

Recently, we have found that our newly synthesized compound, AH-9700 (1-[2-(3,4-dihydro-6,7-dimethyl-2-naphthalenyl)ethyl]pyrrolidine fumarate, Fig. 1), has a high affinity for σ receptors and facilitates urine storage in experimental animals. The present paper describes the pharmacological actions of AH-9700 in comparison to those of (+)-pentazocine, DTG and oxybutynin.

2. Methods

2.1. Animals

All experiments were carried out in accordance with the Guiding Principles for the Care and Use of Laboratory Animals written by the Japanese Pharmacological Society. Female Std–Wistar rats (Japan SLC, Shizuoka, Japan), weighing 150–230 g, and male Std–Hartley guinea pigs (Japan SLC), weighing 300–350 g, were used. They were housed in a room kept at 22–24°C under a 12-h light/dark cycle with free access to food and water.

2.2. In vitro experiments

2.2.1. σ Receptor binding assay

The affinities for σ receptors were determined using membranes from male guinea pig brains minus cerebellum as described by Mach et al. (1995) and Matsumoto et al. (1995). σ_1 and σ_2 receptors were labeled with 3 nM [^{3}H](+)-pentazocine (58.0 Ci/mmol) and 3 nM [^{3}H]DTG (35.0 Ci/mmol) in the presence of 100 nM (+)-pentazocine to mask the σ_1 sites, respectively. GTP shift studies were performed with 3 nM [^{3}H](+)-pentazocine in the absence and presence of 100 μM GppNHp. Non-specific binding was determined in the presence of 10 μM (+)-pentazocine and 5 μM DTG for σ_1 and σ_2 receptors, respectively.

2.2.2. Muscarinic receptor binding assay

The affinities for muscarinic receptors were determined using commercially sourced human recombinant muscarinic receptors (M_1, M_2 and M_3) according to the methods specified by the supplier. Briefly, the membranes from SF9 cells expressing human recombinant muscarinic receptors were diluted in an incubation buffer (50 mM Tris–HCl at pH 7.4, containing 10 mM $MgCl_2$ and 1 mM EDTA) and were incubated with drugs for 60 min at 27°C in the presence of 0.2 nM [^{3}H]*N*-methyl-scopolamine (82 Ci/mmol). Non-specific binding was determined in the presence of 1 μM atropine to define the specific binding. Binding reactions were stopped with ice-cold 50 mM Tris–HCl, pH 7.4, and bound and free ligands were separated by rapid filtration under vacuum through GF/C glass filters. The filters were washed four times with ice-cold 50 mM Tris–HCl, pH 7.4, and the radioactivity was measured.

2.2.3. Carbachol-induced contractions in isolated bladder detrusor strips of rats

This experiment was performed as described previously, with some modifications (Shimizu et al., 1999). The whole bladder was removed from female rats. Longitudinal detrusor strips, 1 cm long, were prepared and suspended under a resting tone of 1 g in a 10-ml organ bath maintained at 37°C and containing Krebs–Henseleit solution (118 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$ and 10 mM glucose) oxygenated with a gas mixture of 95% O_2–5% CO_2. Contractions were recorded isometrically with a Magnus automatic operation system (JT, IM-400, Tokyo, Japan). After the strips had been allowed to stand for at least 60 min, carbachol (1 μM) was applied for 5 min and washed out several times. The procedure was repeated at 15-min intervals. After the contractile responses had become stable, AH-9700 or oxybutynin was applied 10 min before the next application of carbachol.

2.3. In vivo experiments

2.3.1. Rhythmic isovolumetric reflex bladder contractions

This experiment was performed according to the method of Dray (1985) with some modifications. Female rats, but not male rats, were used in this experiment because a cannula could easily be inserted into the urinary bladder through the urethra without injuring the bladder. The rats were anesthetized with urethane (0.85 g/kg, s.c. and i.p.) and a 3-cm polyethylene cannula (PE 50) was inserted into the bladder. The urine in the bladder was removed through the cannula by gently pressing on the abdomen. Finally, the cannula was withdrawn. After laparotomy, both ureters were tied and cut at the side of the kidney. The abdomen was then sutured. The bladder was cannulated through the urethra using a polyethylene tube (PE 50) with a ligature around the urethra. The bladder cannula was connected to a pressure transducer (Nihon Kohden, TP-400T, Tokyo, Japan). At least 30 min after the operation, the bladder was filled through the bladder cannula with incremental 0.1 ml

volumes of room-temperature saline until spontaneous rhythmic bladder contractions occurred reproducibly, and the intravesical pressure was recorded continuously. After the rhythmic bladder contractions had become stable, drugs were administered intravenously through a cannula (PE 50) inserted into the right jugular vein or intracerebroventricularly through a cannula implanted as described below. The following parameters were evaluated: disappearance time and amplitude of rhythmic bladder contractions. For the amplitude, the average of pretreatment values for a period of 15 min was taken as 100%, and the average of post-treatment values for the same period after rhythmic bladder contractions had reappeared was calculated as percentage inhibition.

2.3.2. I.c.v. cannulation for drug administration

Female rats were anesthetized with an i.p. injection of pentobarbital (45 mg/kg) and placed in a stereotaxic instrument. A stainless-steel guide cannula (22 gauge, Plastic Product, C313G, VA, USA) was introduced into the right lateral ventricle (A: −0.7 mm anterior to the bregma, L: +1.3 mm lateral to the midline, H: +2.2 mm below the surface of the dura mater) according to Paxinos and Watson's stereotaxic atlas (Paxinos and Watson, 1982), and fixed to the skull with stainless-steel screws and dental cement. A dummy cannula (Plastic Product, C313DC) was inserted into the guide cannula to seal its top and to keep tissue out of the tubing guide. The rhythmic bladder contractions test as described above was performed 4 days after implantation of the guide cannula. Vehicle (saline) and the drugs were infused into the right lateral ventricle (1 mm beyond guide cannula tip) in a volume of 5 μl for 2.5 min through an internal cannula (28 gauge, Plastic Product, C313I), which was inserted into the guide cannula instead of the dummy cannula.

2.3.3. I.c.v. injection of pertussis toxin

The treatment with i.c.v. pertussis toxin was performed according to the method of Oka et al. (1996) with some modifications. Female rats were anesthetized with an i.p. injection of pentobarbital (45 mg/kg) and placed in a stereotaxic instrument. An injection cannula (stainless steel, 27 gauge) was introduced into each of the lateral ventricles (A: −0.7 mm anterior to the bregma, L: ±1.3 mm lateral to the midline, H: +3.2 mm below the surface of the dura mater) according to Paxinos and Watson's stereotaxic atlas (Paxinos and Watson, 1982). Pertussis toxin was injected into both ventricles simultaneously at a dose of 0.5 μg (total: 1 μg) in a volume of 5 μl (total: 10 μl) for 2.5 min using a Harvard infusion pump. Sham-treated rats received the vehicle (10 mM sodium phosphate pH 7.0 containing 50 mM NaCl) instead of pertussis toxin. The rhythmic bladder contractions test as described above was performed 4 days after pertussis toxin injection.

Table 1
Specific binding of AH-9700, (+)-pentazocine or DTG to σ receptors in membrane from forebrain of guinea pig

	σ_1 Receptor[a]	σ_2 Receptor[b]
	IC_{50} (nM)	IC_{50} (nM)
AH-9700	4.3 ± 0.1	103.1 ± 6.8
(+)-Pentazocine	6.2 ± 0.1	652.0 ± 21.6
DTG	77.6 ± 2.7	27.1 ± 0.6

[a] Displacement of [^{3}H](+)-pentazocine.
[b] Displacement of [^{3}H]DTG binding in the presence of 100 nM (+)-pentazocine. Each value represents the mean ± S.E. of three determinations.

Table 2
Specific binding of AH-9700 to σ_1 receptors in the absence or presence of 100 μM GppNHp

	Specific binding of AH-9700 to σ_1 receptors[a]
	IC_{50} (nM)
Absence of GppNHp	5.2 ± 0.1
Presence of GppNHp	8.6 ± 0.6[b]
Ratio	1.7

[a] Displacement of [^{3}H](+)-pentazocine. Each value represents the mean ± S.E. of three determinations.
[b] $P < 0.01$: statistically significant differences from the value obtained in the absence of GppNHp (unpaired Student's *t*-test).

2.4. Materials

The radioactive drugs, [^{3}H](+)-pentazocine, [^{3}H]DTG and [^{3}H]*N*-methyl-scopolamine methylchloride, were purchased from NEN Life Science Product (Boston, MA, USA). AH-9700, oxybutynin hydrochloride, (+)-pentazocine and DTG acetate were synthesized in our laboratories. 5′-Guanylyl-imidodiphosphate (GppNHp), carbamylcholine chloride (carbachol) and atropine sulfate was purchased from Sigma (St. Louis, MO, USA). Pertussis toxin and human recombinant muscarinic M_1, M_2 and M_3 receptors were purchased from Research Biochemicals International (Natick, MA, USA).

For the binding assays, the drugs were first dissolved in dimethylsulfoxide (DMSO) and then diluted with deionized water. The final solvent concentration in the assay mixture did not exceed 0.1% DMSO. For rhythmic bladder contraction testing, pertussis toxin was dissolved in 10 mM sodium phosphate buffer (pH 7.0) containing 50 mM

Table 3
Specific binding of AH-9700 and oxybutynin to human recombinant muscarinic M_1, M_2 and M_3 receptors

	Human recombinant muscarinic receptors[a] IC_{50} (μM)		
	M_1	M_2	M_3
AH-9700	5.10 ± 0.40	3.39 ± 0.03	1.81 ± 0.08
Oxybutynin	0.0053 ± 0.0002	0.0426 ± 0.0040	0.0057 ± 0.0001

[a] Displacement of [^{3}H]*N*-methyl-scopolamine. Each value represents the mean ± S.E. of three determinations.

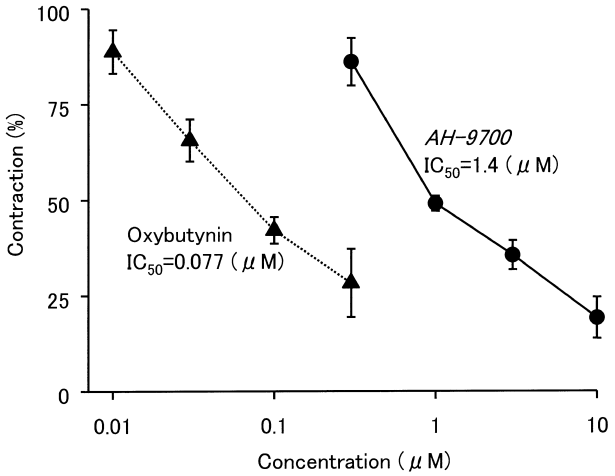

Fig. 2. Effect of AH-9700 (●) or oxybutynin (▲) on contractile responses to carbachol in isolated bladder strips of rats. Each point represents the mean ± S.E. for three rats. AH-9700 or oxybutynin was applied after the carbachol (1 μM)-induced contractions became stable.

NaCl. (+)-Pentazocine was first dissolved in saline containing 0.1 N HCl at 25 mg/ml and neutralized with 1 N NaOH. It was then diluted with saline. Other drugs were dissolved in saline. For the experiment using isolated bladder strips, AH-9700 and oxybutynin were dissolved in saline. These solvents did not affect the experimental results in this study (data not shown).

2.5. Statistical analysis

The results were expressed as the means ± S.E. Statistically significant differences were identified with the SAS system for Windows (version 6.12, SAS Institute). Dunnett's multiple range test was used for multiple comparisons. Unpaired Student's *t*-test was used for two comparisons. The significance level was set at $P < 0.05$. The IC_{50} (concentration required to produce 50% inhibition of specific binding of radioactive drugs or carbachol-induced contractions in isolated bladder strips), ED_{10min} (dose required to inhibit rhythmic bladder contractions for 10 min) and ID_{15} (dose required to produce 15% suppression in the amplitude of rhythmic bladder contractions) values were determined by logit analysis.

3. Results

3.1. Specific binding of AH-9700, (+)-pentazocine and 1,3-di-o-tolylguanidine (DTG) to σ receptors

The results of σ receptor binding assays are shown in Table 1. AH-9700 and the σ receptor ligands, (+)-penta-

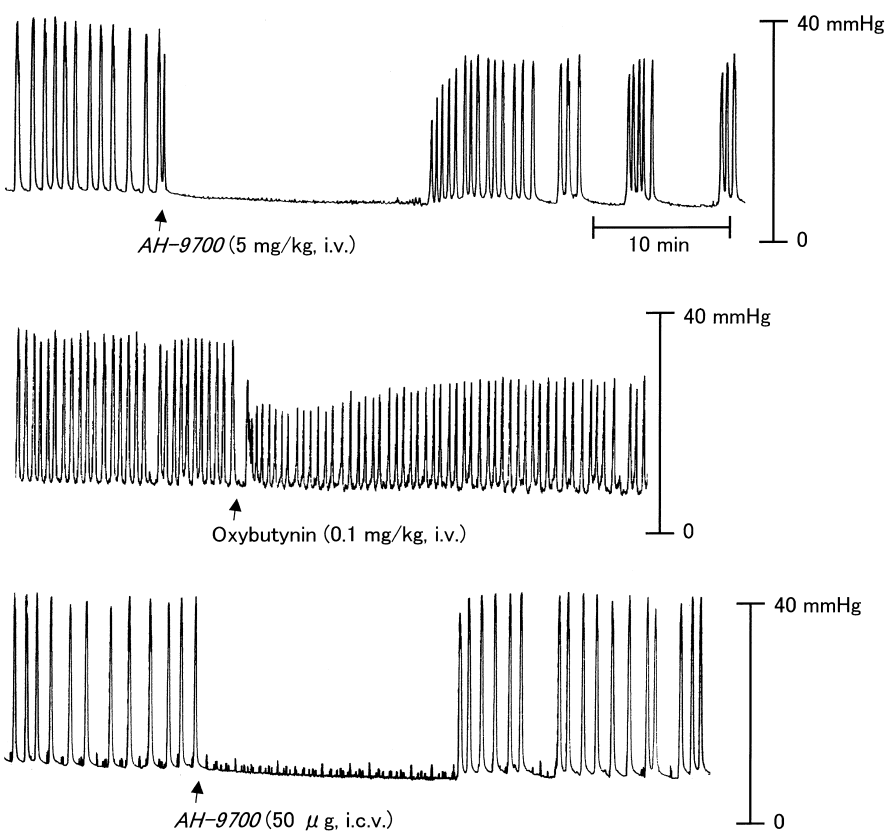

Fig. 3. Typical tracing showing the effects of AH-9700 or oxybutynin on rhythmic isovolumetric reflex bladder contractions in anesthetized rats. Arrows indicate i.v. or i.c.v. administration.

zocine and DTG, inhibited the binding of [^{3}H](+)-pentazocine to σ_1 receptors (IC_{50}; 4.3 ± 0.1, 6.2 ± 0.1 and 77.6 ± 2.7 nM, respectively). AH-9700, (+)-pentazocine and DTG also inhibited the binding of [^{3}H]DTG in the presence of 100 nM (+)-pentazocine to σ_2 receptors (IC_{50}; 103.1 ± 6.8, 652.0 ± 21.6 and 27.1 ± 0.6 nM, respectively). The binding affinity of AH-9700 for σ_1 receptors was greater than that for σ_2 receptors.

3.2. Effect of 5′-guanylyl-imidodiphosphate (GppNHp) on AH-9700-induced inhibition of [^{3}H](+)-pentazocine binding

The binding affinity of AH-9700 for σ_1 receptors was determined in the absence or presence of 100 μM GppNHp (Table 2). The IC_{50} value increased from 5.2 ± 0.1 nM in the absence of GppNHp to 8.6 ± 0.6 nM in its presence ($P < 0.01$). This result indicates that AH-9700 has agonistic properties for σ_1 receptors.

3.3. Effects of AH-9700 and oxybutynin on muscarinic receptors and contractile responses induced by carbachol in bladder detrusor strips

AH-9700 had moderate affinity for muscarinic receptors, although less than that for σ receptors; the IC_{50} values for human recombinant M_1, M_2 and M_3 receptors were 5.10 ± 0.40, 3.39 ± 0.03 and 1.81 ± 0.08 μM, respectively (Table 3). The affinity of oxybutynin, an antimuscarinic drug, for these receptors was much greater than that of AH-9700 (Table 3).

AH-9700 and oxybutynin concentration-dependently inhibited contractile responses induced by carbachol (1 μM) in isolated bladder detrusor strips of rats; the IC_{50} values of AH-9700 and oxybutynin were 1.4 and 0.077 μM, respectively (Fig. 2).

3.4. Effects of AH-9700, (+)-pentazocine, DTG and oxybutynin on rhythmic isovolumetric reflex bladder contractions in anesthetized rats

AH-9700 at 1–5 mg/kg, i.v., as well as (+)-pentazocine and DTG at almost the same doses, dose-dependently inhibited rhythmic bladder contractions (Figs. 3 and 4). Furthermore, AH-9700 at 5 mg/kg, but not (+)-pentazocine or DTG, significantly suppressed the amplitude after rhythmic bladder contractions reappeared. On the other hand, oxybutynin at 0.02–0.1 mg/kg, i.v. dose-dependently suppressed the amplitude of rhythmic bladder contractions, although it did not inhibit the appearance of rhythmic bladder contractions at all (Figs. 3 and 4).

Figs. 3 and 5 show the effects of i.c.v. administration of AH-9700, (+)-pentazocine and DTG on rhythmic bladder contractions. AH-9700 (10–100 μg) markedly inhibited

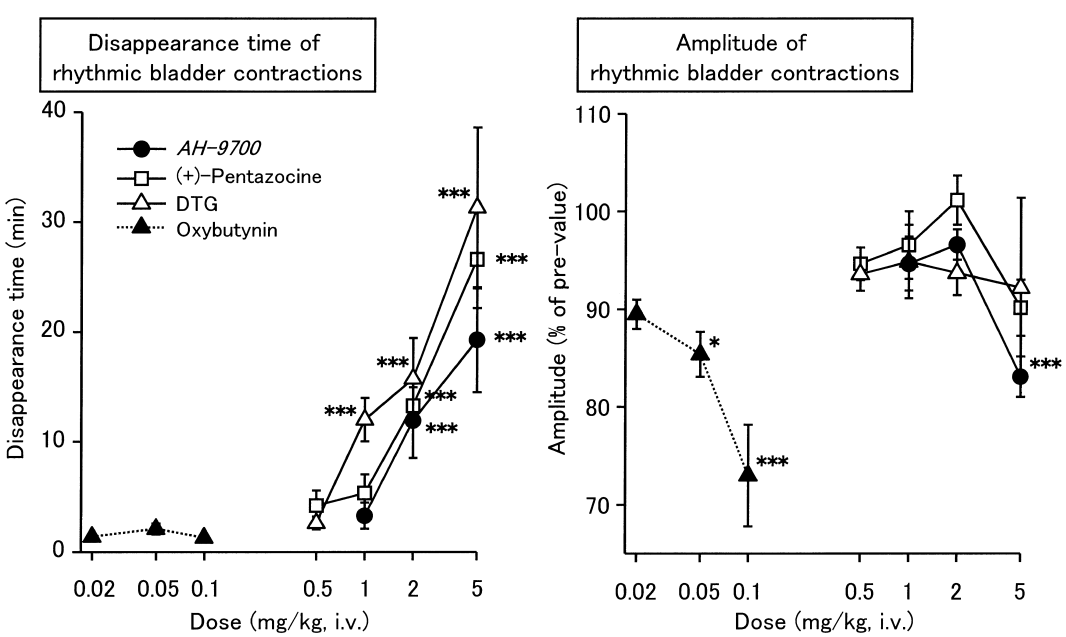

Fig. 4. Effects of AH-9700 (●), (+)-pentazocine (□), DTG (△) and oxybutynin (▲), administered i.v., on rhythmic bladder contractions in anesthetized rats. Each point represents the mean ± S.E. for four or five rats. The disappearance time and the amplitude of rhythmic bladder contractions following i.v. administration of vehicle (saline) were 1.8 ± 0.3 min and $98.5 \pm 1.0\%$ ($n = 9$), respectively. $^{**}P < 0.01$, $^{***}P < 0.001$: statistically significant differences from the corresponding value for vehicle administration (Dunnett's multiple range test).

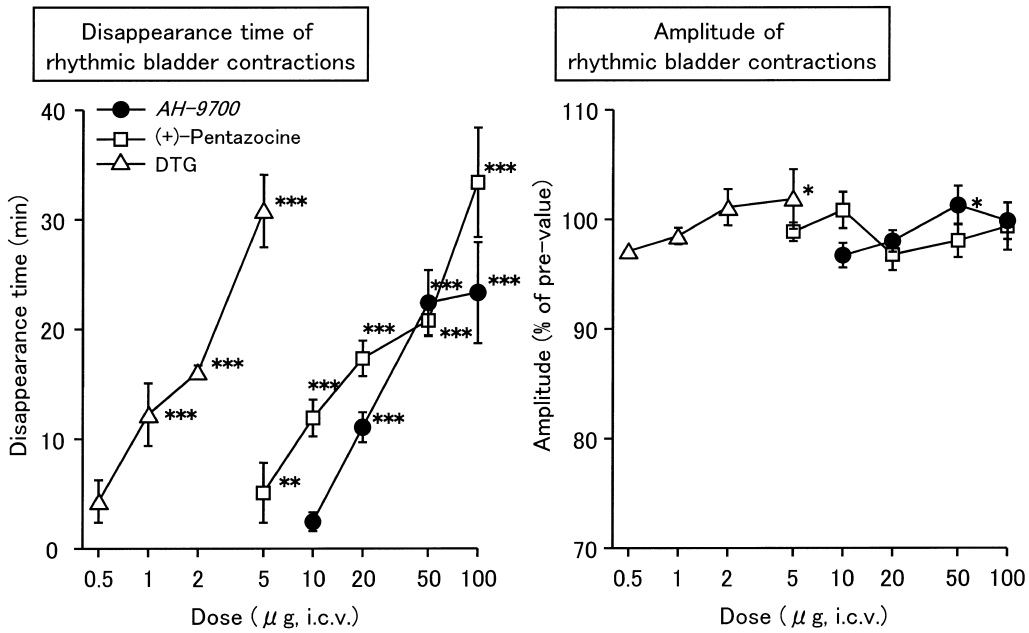

Fig. 5. Effects of AH-9700 (●), (+)-pentazocine (□) and DTG (△), administered i.c.v., on rhythmic bladder contractions in anesthetized rats. Each point represents the mean ± S.E. for four or five rats. The disappearance time and the amplitude of rhythmic bladder contractions following i.c.v. administration of vehicle (saline) were 1.2 ± 0.1 min and 95.8 ± 0.7 % ($n = 9$), respectively. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$: statistically significant differences from the corresponding value for vehicle administration (Dunnett's multiple range test).

rhythmic bladder contractions, but did not suppress the amplitude of rhythmic bladder contractions at any dose used. (+)-Pentazocine and DTG also inhibited rhythmic bladder contractions without suppressing the amplitude. The effective dose range of DTG was lower than that of the other drugs used. Incidentally, AH-9700 at 50 μg and DTG at 5 μg caused a significant increase in the amplitude of rhythmic bladder contractions, but the effects were very slight.

Table 4 shows ED_{10min} and ID_{15} values of the drugs used. The ED_{10min} values for i.c.v. administration of AH-9700, (+)-pentazocine and DTG were much lower than the corresponding values for i.v. administration. The ID_{15} values for i.v. administration of AH-9700 and oxybutynin were 4.6 and 0.04 mg/kg, respectively.

3.5. Effect of pretreatment with i.c.v. pertussis toxin (4 days prior) on i.v. AH-9700-induced inhibition of rhythmic bladder contractions

In the i.c.v. vehicle-treated rats, AH-9700 (5 mg/kg, i.v.) inhibited rhythmic bladder contractions and suppressed the amplitude (Fig. 6) with almost the same potency as in the non-treated normal rats (Fig. 4). In pertussis toxin (1 μg, i.c.v.)-treated rats, the AH-9700-induced inhibition of rhythmic bladder contractions was markedly reduced; there was a statistically significant difference be-

Table 4
ED_{10min} of disappearance time and ID_{15} of amplitude of rhythmic bladder contractions in anesthetized rats

	Disappearance time of rhythmic bladder contractions		Amplitude of rhythmic bladder contractions	
	ED_{10min}		ID_{15}	
	(mg/kg, i.v.)	(μg, i.c.v.)	(mg/kg, i.v.)	(μg, i.c.v.)
AH-9700	1.8	18.9	4.6	> 100
(+)-Pentazocine	1.7	9.4	> 5.0	> 100
DTG	1.1	1.0	> 5.0	> 100
Oxybutynin	> 0.1	NT	0.04	NT

The values were calculated from the data shown in Figs. 4 and 5, using logit analysis. ED_{10min}, dose required to inhibit rhythmic bladder contractions for 10 min; ID_{15}, dose required to produce 15% suppression of the amplitude; NT, not tested. I.v. administration of (+)-pentazocine and DTG or i.c.v. administration of AH-9700, (+)-pentazocine and DTG did not produce more than 15% suppression of the amplitude at the doses used in this experiment.

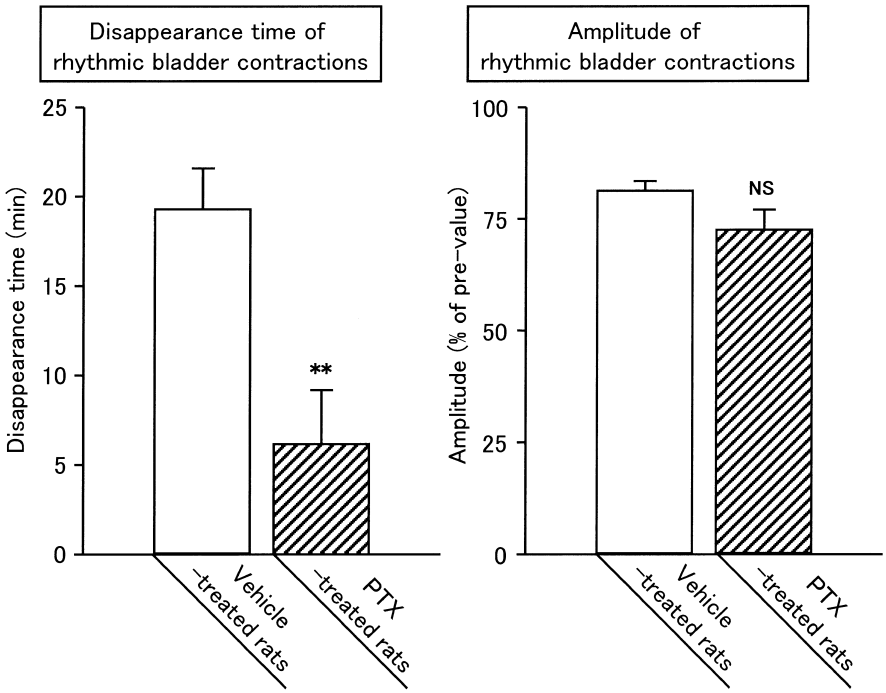

Fig. 6. Effect of pretreatment with i.c.v. pertussis toxin (4 days prior) on i.v. AH-9700-induced inhibition of rhythmic bladder contractions in anesthetized rats. Each bar represents the mean ± S.E. for four rats. Pertussis toxin (1 μg) or the vehicle (10 mM sodium phosphate pH 7.0 containing 50 mM NaCl) was injected i.c.v. in a volume of 10 μl 4 days before the experiment on rhythmic bladder contractions. AH-9700 was administered i.v. at a dose of 5 mg/kg. $^{**}P < 0.01$: statistically significant differences from the value for vehicle-treated rats (unpaired Student's *t*-test). NS: no statistically significant difference from the value for the vehicle-treated rats (unpaired Student's *t*-test).

tween vehicle- and pertussis toxin-treated rats ($P < 0.01$) (Fig. 6). However, the two groups did not differ for suppression of the amplitude ($P > 0.05$).

4. Discussion

The present radioligand binding studies indicated that AH-9700 has high affinity for σ receptors and moderate affinity for muscarinic receptors. In addition, AH-9700 had virtually no affinity for the other 25 receptors and ion channels tested (data not shown). The IC_{50} value of AH-9700 for σ_1 receptor binding was lower than that for σ_2 receptor binding, and its binding profile was similar to that of (+)-pentazocine, a known selective σ_1 receptor agonistic ligand (Walker et al., 1990; Quirion et al., 1992), but not that of DTG, a known non-selective σ receptor agonistic ligand (Walker et al., 1990; Quirion et al., 1992).

It was documented that σ receptors, particularly the σ_1 subtype, are coupled to Gi/o types of G proteins (Walker et al., 1990). In addition, Gilman (1987) demonstrated that agonists for some receptors coupled to G proteins decreased their receptor-binding affinities in the presence of GTP or GTP analogs such as GppNHp and guanosine 5′-(-thio)-triphosphate (GTPγS). As for σ receptors, Matsuno et al. (1996) reported that the affinity of (+)-pentazocine for σ_1 receptors in the presence of GTPγS was 2.3-fold weaker than that in the absence of GTPγS. In the present study, 100 μM GppNHp significantly reduced the affinity of AH-9700 for the σ_1 receptor; the IC_{50} value in the presence of GppNHp was 1.7-fold that in the absence of GppNHp. These results indicate that AH-9700 has agonistic properties for σ_1 receptors.

To investigate the in vivo effects of AH-9700 on bladder functions in comparison to those of (+)-pentazocine, DTG and oxybutynin, we performed the rhythmic isovolumetric reflex bladder contraction test using anesthetized rats. In the test, the rhythmical and reproducible bladder contractions (which are induced by excessive micturition reflexes) occur because it is impossible to evacuate urine (saline). Therefore, it may be possible to consider that the bladder is overactive although the contractions are not caused by pathological disturbances. In such a test, both intravenous and intracerebroventricular administration of AH-9700, (+)-pentazocine and DTG dose-dependently inhibited rhythmic reflex bladder contractions. However, the doses for i.c.v. administration were much lower than those for i.v. administration. Furthermore, we have previously shown that the bladder capacity-increasing effect of (+)-pentazocine (which has very poor affinity for opioid receptors in addition to high affinity for σ_1 receptors (Su, 1985)) is apparently not mediated through opioid receptors because it was not antagonized by naloxone, an opioid receptor antagonist (Shimizu et al., 2000). In addition, (+)-pentazocine and DTG even at a high concentration (3 μM) scarcely inhibited the contractile responses to electri-

cal field stimulation in isolated bladder strips of rats (Shimizu et al., 2000). We have confirmed that AH-9700 easily crosses the blood–brain barrier after systemic administration, with a brain/plasma ratio of approximately 100 in pharmacokinetic studies using rats (unpublished data). Taken together, these results indicate that the inhibitory effect (i.e., anti-micturition reflex action) of AH-9700 as well as of (+)-pentazocine and DTG (two σ receptor ligands) on the appearance of rhythmic bladder contractions may be mediated through central σ receptors.

Pertussis toxin is known to enzymatically ADP-ribosylate the α-subunit of Gi/o-proteins and to inactivate the receptors coupled to Gi/o-proteins (Katada and Ui, 1982). In the present study, the inhibitory effect of AH-9700 on the appearance of rhythmic bladder contractions was markedly reduced by pretreatment with pertussis toxin (1 μg, i.c.v.), indicating that Gi/o-proteins are involved in the pathways through which AH-9700 mediates this effect. This result is consistent with that of a previous study showing that the effects of σ receptor ligands ((+)-pentazocine and DTG) on cystometrograms were also inhibited by pretreatment with pertussis toxin (1 μg, i.c.v.) (Shimizu et al., 2000).

Although the affinity of DTG for σ_1 receptors was less than that of AH-9700 or (+)-pentazocine, the inhibitory effect of DTG, especially when administered i.c.v., on the appearance of rhythmic bladder contractions was the most potent. The affinity of DTG for σ_2 receptors was greater than that of (+)-pentazocine or AH-9700. Moreover, we have shown that, in i.c.v. pertussis toxin-treated rats, DTG had slight effects on cystometrograms while the effects of (+)-pentazocine (a selective σ_1 receptor agonistic ligand) were completely abolished (Shimizu et al., 2000). Accordingly, these data suggest that σ_2 receptors, which are considered not to be related to Gi/o proteins (Walker et al., 1990), may be partly involved in the effects of DTG on cystometrograms and rhythmic bladder contractions. Therefore, the potent inhibitory effect of DTG on the appearance of rhythmic bladder contractions may be due to its high affinity for σ_2 receptors, although further study is needed in this respect.

Oxybutynin, which has potent anti-muscarinic activity (Yarker et al., 1995), suppressed the amplitude of rhythmic bladder contractions, although it lacked the effect on the appearance of contractions. The phenomenon could be explained by the fact that large parts of the excitatory bladder contractions in rats, but not in humans, are atropine-resistant (Maggi et al., 1984; Eglen et al., 1996). Intravenous administration of AH-9700 suppressed the amplitude of rhythmic bladder contractions, while i.c.v. administration did not. In addition, the suppressive effect of AH-9700 (i.v.) on the amplitude was not affected by pretreatment with i.c.v. pertussis toxin. AH-9700 also had affinity for muscarinic receptors in a radioligand binding assay at concentrations that inhibited the contractile responses induced by carbachol (1 μM). Accordingly, it is clear that the spasmolytic effect of systemically administered AH-9700 on the bladder detrusor is based on its peripheral anti-muscarinic activity.

In conclusion, AH-9700 possesses not only a marked anti-micturition reflex effect through σ receptors in the central nervous system but also a moderate spasmolytic effect based on its peripheral anti-muscarinic activity. The former effect of AH-9700 may be mediated through a central, pertussis toxin-sensitive mechanism, consistent with a role for central pertussis toxin-sensitive Gi/o-proteins coupled to σ receptors.

Acknowledgements

We thank Dr. T. Morie and Ms. A. Kanehira for synthesizing AH-9700, oxybutynin, (+)-pentazocine and DTG.

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