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Effects of (+)-pentazocine and 1,3-di-*o*-tolylguanidine (DTG), sigma (σ) ligands, on micturition in anaesthetized rats

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1 The effects of two sigma (σ) binding site ligands, (+)-pentazocine and 1,3-di-*o*-tolylguanidine (DTG), on bladder functions were examined in rats.

2 Cystometry using urethane-anaesthetized rats showed that (+)-pentazocine (1–5 mg kg^{-1}, i.v.) and DTG (1–5 mg kg^{-1}, i.v.) prolonged micturition intervals, indicating increased bladder capacity and raised the threshold pressure.

3 The effects of (+)-pentazocine (2 mg kg^{-1}, i.v.) on micturition were not influenced by naloxone (0.5 mg kg^{-1}, i.v.), which antagonized similar effects of morphine (2 mg kg^{-1}, i.v.).

4 When administered intracerebroventricularly (i.c.v.), DTG (1 μg) and (+)-pentazocine (30 μg) prolonged micturition intervals with increased threshold pressure on the cystometrogram.

5 In isolated bladder detrusor strips of rats, (+)-pentazocine (3 μM) and DTG (1 μM) did not affect contractile responses to electrical field stimulation. A higher concentration of DTG (3 μM) slightly suppressed the response induced by 30 Hz stimulation.

6 The effects of (+)-pentazocine and DTG on micturition were abolished by pre-treatment with pertussis toxin (PTX, 1 μg, i.c.v.).

7 These results indicate that typical σ ligands, such as (+)-pentazocine and DTG, increase bladder capacity in anaesthetized rats. Moreover, the mechanism by which σ ligands change the urinary storage properties in rats may involve pathways in which the function of Gi/o proteins is necessary. *British Journal of Pharmacology* (2000) **131,** 610–616

Keywords: σ binding sites; (+)-pentazocine; DTG; micturition; cystometry; Gi/o proteins

Abbreviations: DTG, 1,3-di-*o*-tolylguanidine; PTX, pertussis toxin; σ, sigma

Introduction

Sigma (σ) binding sites (sometimes called σ receptors) were first proposed by Martin *et al*. (1976). This receptor has now been characterized as a non-opioid, non-dopamine D_2 and non-phencyclidine receptor (Gundlach *et al*., 1985; Largent *et al*., 1986; Walker *et al*., 1990). According to biochemical and radioactive ligand-binding experiments, σ sites have been classified into at least two subtypes, termed σ_1 and σ_2, (Quirion *et al*., 1992). It has also been shown that σ binding sites exist in the brain and peripheral organs such as the gastrointestinal tract, liver, testis, adrenal gland and ovary (Tam & Cook, 1984; Samovilova *et al*., 1985; Su *et al*., 1988; Wolfe *et al*., 1989; Walker *et al*., 1990). In 1996, Hanner *et al*. and Kekuda *et al*. first succeeded in cloning σ_1 from guinea-pig liver and human placental choriocarcinoma cells, respectively. Afterward, σ_1 was cloned from mouse kidney (Seth *et al*., 1997), mouse brain (Pan *et al*., 1998), human brain (Prasad *et al*., 1998) and rat brain (Seth *et al*., 1998). However, σ_2 have not been cloned yet. It has been demonstrated that σ sites are involved in a variety of physiological functions and that σ ligands have diversified pharmacological effects, e.g., antipsychotic effect, antidepressant effect, anxiolytic effect, neuroprotective effect, anti-amnesic effect, anti-inflammatory effect, antitussive effect, anti-ulcer effect, intestinal motility modulation effect, anti-ion transport effect and so forth (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). However, there are no reports on the effects of σ ligands on bladder functions.

The lower urinary tract stores and evacuates urine using a complex neural control system that coordinates two component activities; a reservoir (urinary bladder) and an outlet (bladder neck, urethra and urethral sphincter) (de Groat *et al*., 1993). In addition, several neurotransmitters, such as enkephalins, dopamine, norepinephrine, acetylcholine, gamma aminobutyric acid (GABA) and glutamic acid, and many peptides such as corticotrophin releasing factor (CRF), motilin, substance P and neuropeptide Y, have been demonstrated to play an important role in the central pathway controlling micturition (de Groat *et al*., 1993). However, the relationship between micturition mechanism and σ sites has not been investigated. The objective of this study is to determine the effects of (+)-pentazocine and 1,3-di-*o*-tolylguanidine (DTG), known σ ligands which have been described as an 'agonist' at this site (Walker *et al*., 1990; Quirion *et al*., 1992), on micturition induced by transvesical saline infusion in urethane-anaesthetized rats thereby shedding light on the relation between σ sites and functions of the lower urinary tract.

Methods

Animal

Male Std-Wistar rats (Japan SLC Inc., Shizuoka, Japan), weighing 250–340 g, were used. They were housed in a room

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kept at 22–24°C under a 12 h light/dark cycle with free access to food and water until use.

Cystometrography following i.v. administrations of drugs

The experiment was performed as described previously (Shimizu *et al*., 1999). Rats were anaesthetized with urethane (1.0 g kg^{-1}, s.c.) and the right jugular vein was cannulated for drug injection. After laparotomy, both ureters were tied and cut at the side of the kidney. A polyethylene tube (PE 60) was then inserted through a small incision at the apex of the bladder into the bladder lumen and ligated. The bladder cannula was connected to a pressure transducer (Nihon Kohden, TP-400T, Tokyo, Japan) and a Harvard infusion pump by means of polyethylene T-tube. Room-temperature saline was infused into the bladder at a rate of 3.3 ml h^{-1} and the intravesical pressure was recorded continuously. After the cystometrogram had become stable, the drugs were administered immediately after voiding. In the experiment of pretreatment with naloxone, (+)-pentazocine or morphine was administered immediately after the first voiding following administration of naloxone. The following urodynamic parameters were evaluated: micturition intervals (reflecting the bladder capacity), micturition pressure (maximum bladder pressure during micturition) and threshold pressure (bladder pressure immediately before a sharp bladder micturitional contraction). When a dribbling pattern, due to urinary incontinence, was observed after drug administration, the micturition interval was defined as the interval prior to a complete urination. The urodynamic parameters of one voiding cycle immediately after drug administration were compared to the average of two voiding cycles immediately before drug administration because the most pronounced change of micturition intervals was observed immediately after drug administration.

Cystometrography following i.c.v. administrations of drugs

Rats were anaesthetized by an i.p. injection of pentobarbitone (45 mg kg^{-1}) and placed in a stereotaxic instrument. A stainless steel guide cannula (22 gauge, Plastic Product Company, C313G, Virginia, U.S.A.) was introduced into the right lateral ventricle (A: −0.7 mm anterior to bregma, L: +1.5 mm lateral to the midline, H: 2.2 mm below the surface of the dura mater) according to Paxinos and Watson's stereotaxic atlas (1982), and fixed to the skull with stainless steel screws and dental cement. A dummy cannula (Plastic Product Company, C313DC, Virginia, U.S.A.) was then inserted into the guide cannula to seal its top and to keep tissue out of the tubing guide. Cystometry as described above was performed at least 4 days after implantation of the guide cannula. (+)-Pentazocine or DTG was infused into the right lateral ventricle (1.3 mm beyond guide cannula tip) in a volume of 5 μl for 1 min through an internal cannula (28 gauge, Plastic Product Company, C313I, Virginia, U.S.A.) which was inserted into the guide cannula instead of the dummy cannula.

I.c.v. injection of pertussis toxin (PTX)

The treatment with i.c.v. PTX in rats was performed according to the method of Oka *et al*. (1996) with some modifications. Rats were anaesthetized by an i.p. injection of pentobarbitone (45 mg kg^{-1}) and placed in a stereotaxic instrument. Two injection cannulas (stainless steel, 27 gauge) were introduced into both lateral ventricles (A: −0.7 mm anterior to bregma, L: ±1.5 mm lateral to the midline, H: 3.5 mm below the surface of the dura mater) according to Paxinos and Watson's stereotaxic atlas (1982). PTX 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 1 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 PTX. Cystometry as described above was performed 4 days after PTX-injection.

Electrical field stimulation-induced contractions in isolated bladder detrusor strips

This experiment was performed as described previously (Shimizu *et al*., 1999). The whole bladder was removed from a rat. Longitudinal detrusor strips, 1 cm long, were prepared and suspended under a resting tone of 1 g in 10 ml organ bath maintained at 37°C containing Krebs-Henseleit solution (in mM: NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 10) oxygenated with a gas mixture of 95% O_2–5% CO_2. Contractions were recorded isometrically by a force-displacement transducer (Nihon Kohden, ST-1B, Tokyo, Japan). After the strips were allowed to stand for at least 60 min, they were field stimulated (supramaximal voltage (square plus), 10 s trains, 0.5 ms duration, various frequencies (0.3–30 Hz), every 3 min) by means of two parallel wire platinum electrodes connected to an electrical stimulator (Nihon Kohden, SEN-1101, Tokyo, Japan). After the field stimulation-responses had become stable, (+)-pentazocine or DTG was applied 10 min before starting the next stimulation.

Drugs

The following drugs were used: (+)-pentazocine and DTG acetate (synthesized by Discovery Research Laboratories I, Dainippon Pharmaceutical Co. Ltd); naloxone hydrochloride and atropine sulphate (Sigma Chemical Co., St. Louis, MO, U.S.A.); rimcazole dihydrochloride and PTX (Research Biochemicals International, Natick, MA, U.S.A.); morphine hydrochloride (Dainippon Pharmaceutical Co. Ltd., Osaka, Japan). Rimcazole was dissolved in deionized water. PTX was dissolved in 10 mM sodium phosphate buffer (pH 7.0) containing 50 mM NaCl. For cystometry, (+)-pentazocine was dissolved in deionized water containing less than 1% lactate acid, and for *in vitro* experiments, it was first dissolved in 0.1 N HCl at 100 mM and then diluted with saline (final HCl concentration in the organ bath was 3.3 μM). Other drugs were dissolved in saline. These solvents did not affect the experimental results in this study.

Statistical analysis

The results were expressed as mean ± s.e.mean. Statistically significant differences were identified by the MUSCOT statistical analysis program (Yukmus Co., Tokyo, Japan). Paired Student's *t*-test was used for comparison of urodynamic parameters between, before, and after drug administration. Unpaired Student's *t*-test or Dunnett's multiple range test was used for comparison between two groups or for multiple comparisons of *in vitro* experiments, respectively. The statistical significance level was set at $P<0.05$.

Results

Effects of i.v. administrations of (+)-pentazocine and DTG on micturition

When administered intravenously at doses of 1–5 mg kg^{-1}, (+)-pentazocine dose-dependently extended micturition intervals, indicating an increase in bladder capacity, and raised the threshold pressure in anaesthetized rats (Table 1 and Figure 1). At 5 mg kg^{-1}, (+)-pentazocine abolished the micturition reflex and urinary dribbling was observed in two out of four rats. DTG at 1–5 mg kg^{-1}, i.v. also dose-dependently extended micturition intervals and raised the threshold pressure (Table 1 and Figure 1). At 5 mg kg^{-1}, DTG abolished the micturition reflex and urinary dribbling was observed in four out of four rats. Rimcazole (5 mg kg^{-1}, i.v.), a σ ligand that has been described as an antagonist at this site, extended micturition intervals and raised the threshold pressure although its effects were less potent than those of (+)-pentazocine or DTG (Table 1).

Atropine (10 μg kg^{-1}, i.v.), a muscarinic antagonist that directly affects bladder smooth muscle, decreased the micturition pressure without changing micturition intervals and the threshold pressure (Table 1).

Morphine (2 mg kg^{-1}, i.v.), an opioid agonist, produced urinary dribbling in all rats and considerably extended micturition intervals (Table 1 and Figure 1). In addition, it increased the micturition and threshold pressure. Naloxone, an opioid antagonist, administered alone at 0.5 mg kg^{-1}, i.v., did not affect the cystometric parameters (micturition interval; pre 7.5±0.5, post 6.8±0.6 min: micturition pressure; pre 15.9±1.2, post 15.5±1.2 mmHg: threshold pressure; pre 3.3±0.3, post 2.8±0.3 mmHg: $n=8$). Although the effects of morphine (2 mg kg^{-1}, i.v.) were antagonized by naloxone (0.5 mg kg^{-1}, i.v.) ($P>0.05$; in comparison to each pre-value), those of (+)-pentazocine (2 mg kg^{-1}, i.v.) were not affected by this dose of naloxone (Table 1 and Figure 1).

Effects of i.c.v. administrations of (+)-pentazocine and DTG on micturition

Table 2 shows the effects of i.c.v. administrations of (+)-pentazocine and DTG on micturition. When injected at 30 μg, i.c.v., but not 10 μg, (+)-pentazocine extended micturition

Table 1 Effects of (+)-pentazocine, DTG and other drugs administered intravenously on micturition in anaesthetized rats

Dose (i.v.)		*Micturition interval* (min)	*Micturition pressure* (mmHg)	*Threshold pressure* (mmHg)
(+)-Pentazocine				
(1 mg kg^{-1})	pre	9.9±1.0	18.2±1.4	1.9±0.2
($n=4$)	post	10.9±1.6	17.2±1.5	2.3±0.3
(2 mg kg^{-1})	pre	9.3±1.1	17.8±1.4	2.3±0.2
($n=4$)	post	15.0±0.6*	18.9±1.6	6.7±0.8*
(5 mg kg^{-1})	pre	8.8±1.5	17.7±1.3	3.2±1.3
($n=4$)	post	21.1±1.1**	24.1±2.9**	13.7±2.6*
DTG				
(1 mg kg^{-1})	pre	11.2±2.8	17.5±0.6	3.4±0.4
($n=4$)	post	12.6±2.9	17.6±1.0	4.0±0.9
(2 mg kg^{-1})	pre	11.6±2.6	17.2±0.8	3.3±0.6
($n=4$)	post	17.1±3.0**	20.6±1.5*	9.9±2.0*
(5 mg kg^{-1})	pre	12.7±3.8	16.0±1.0	3.7±0.9
($n=4$)	post	30.3±3.8**	22.5±1.7	13.2±1.0**
Rimcazole				
(5 mg kg^{-1})	pre	7.0±0.6	15.4±1.3	2.2±0.6
($n=3$)	post	9.3±1.0*	17.4±1.0	4.1±0.7**
(+)-Pentazocine				
(2 mg kg^{-1})	pre	6.5±1.0	15.0±0.9	3.0±0.3
($n=4$)	post	11.5±1.2**	16.4±1.8	6.7±1.2*
(+)-Pentazocine (2 mg kg^{-1}) in the presence of Naloxone (0.5 mg kg^{-1})				
($n=4$)	pre	7.0±0.6	13.1±0.5	3.4±0.6
	post	11.1±0.8**	16.8±1.1	9.9±1.7*
Morphine				
(2 mg kg^{-1})	pre	9.4±0.6	17.2±2.1	2.8±0.9
($n=3$)	post	81.5±4.9**	25.0±1.1*	16.8±1.4**
Morphine (2 mg kg^{-1}) in the presence of Naloxane (0.5 mg kg^{-1})				
($n=4$)	pre	8.1±0.7	18.7±1.1	3.1±0.4
	post	11.2±1.9	20.5±3.0	6.3±1.9
Atropine				
(10 μg kg^{-1})	pre	10.0±2.0	17.9±0.7	2.3±0.2
($n=4$)	post	10.1±2.0	14.6±0.6**	2.6±0.6

Each value represents the mean±s.e.mean. *$P<0.05$, **$P<0.01$: statistically significant differences from each value before drug administration (paired Student's t-test). n: number of rats.

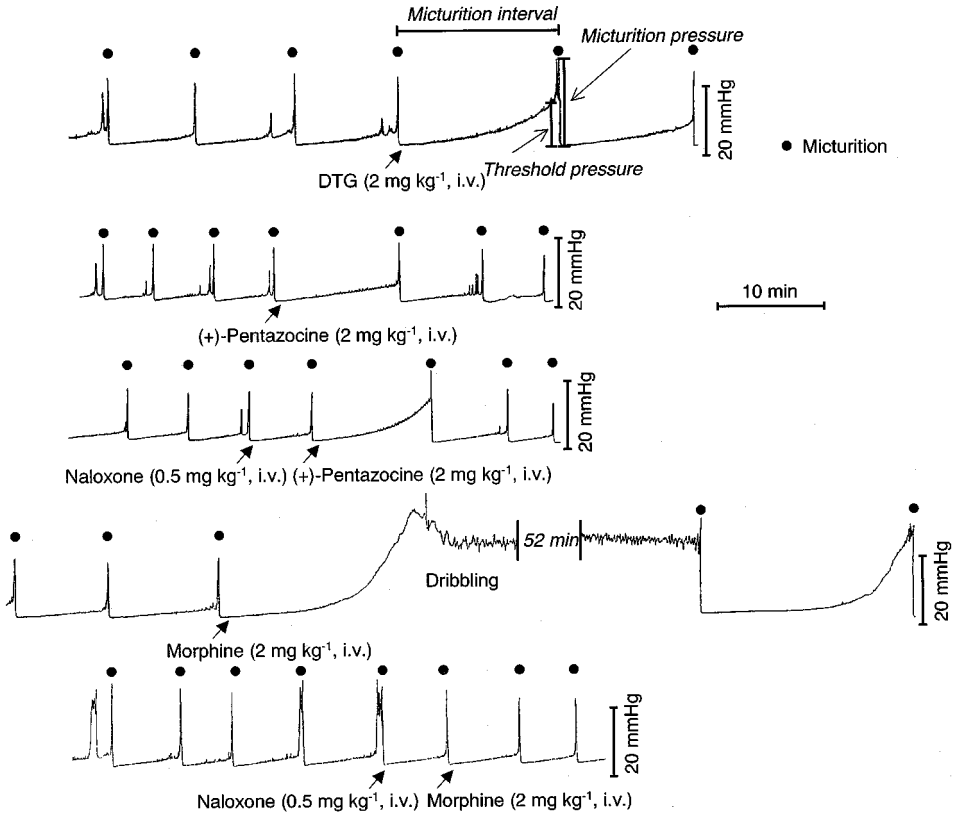

Figure 1 Typical tracing showing the effects of (+)-pentazocine, DTG and morphine on micturition in anaesthetized rats.

Table 2 Effects of (+)-pentazocine and DTG administered intracerebroventricularly on micturition in anaesthetized rats

Dose (i.c.v.)		*Micturition interval* (min)	*Micturition pressure* (mmHg)	*Threshold pressure* (mmHg)
(+)-Pentazocine				
(10 μg)	pre	9.6±2.0	12.3±1.5	4.6±7.2
(*n*=3)	post	11.9±1.5	14.5±1.6	7.2±0.9
(30 μg)	pre	8.7±0.7	10.9±0.8	3.4±0.9
(*n*=4)	post	16.4±1.0**	19.8±0.9**	15.6±1.2**
DTG				
(0.3 μg)	pre	8.9±0.4	12.6±1.4	4.0±0.3
(*n*=4)	post	9.2±1.6	12.9±2.5	7.6±3.1
(1 μg)	pre	10.0±1.0	13.6±1.6	4.1±1.0
(*n*=4)	post	15.5±0.7*	18.5±3.2	14.2±2.7**

Each value represents the mean±s.e.mean. *$P<0.05$, **$P<0.01$: statistically significant differences from each value before drug administration (paired Student's *t*-test). *n*: number of rats.

intervals and raised the threshold pressure. DTG at 1 μg, i.c.v., but not 0.3 μg, also extended micturition intervals and raised the threshold pressure.

Electrical field stimulation-induced contractions in isolated detrusor bladder strips

Electrical field stimulation (0.3–30 Hz) caused frequency-related contractions in isolated detrusor bladder strips of rats. (+)-Pentazocine at a concentration of 3 μM did not change the responses at any frequency in comparison to the control (Figure 2). DTG at 1 μM also failed to affect the responses although, at a higher concentration (3 μM), it slightly suppressed only the response induced by 30 Hz stimulation (Figure 2).

Effect of pre-treatment (4 days prior) with PTX i.c.v. administration on (+)-pentazocine- and DTG-induced increase in bladder capacity

In the i.c.v. vehicle-treated rats, (+)-pentazocine (2 mg kg^{-1}, i.v.) and DTG (2 mg kg^{-1}, i.v.) extended micturition intervals and raised the threshold pressure on the cystometrogram (Figure 3); these effects were almost equal to those observed in non-treated rats (Table 1). In PTX-treated rats, (+)-pentazocine changed none of the urodynamic parameters [micturition intervals; pre 12.0±1.0, post 12.4±1.1 min: micturition pressure; pre 13.9±1.5, post 13.6±1.7 mmHg: threshold pressure; pre 5.0±1.0, post 5.0±1.0 mmHg ($n=4$) ($P>0.05$; in comparison to each pre-value)] (Figure 3). DTG

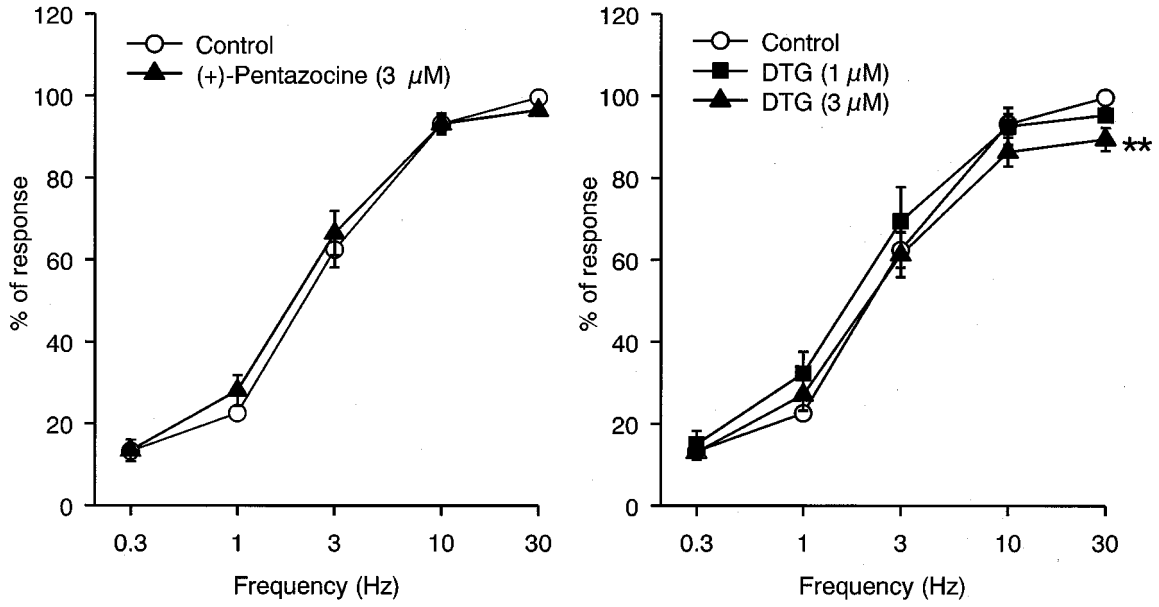

Figure 2 Effects of (+)-pentazocine and DTG on contractile responses to electrical field stimulation in isolated bladder strips. Each point represents the mean ± s.e.mean of four rats. (+)-Pentazocine; No statistically different values were found between the (+)-pentazocine-applied group and the control group (unpaired Student's *t*-test). DTG; $^{**}P<0.01$: statistically significant differences from the corresponding value in the control group (Dunnett's multiple range test).

also failed to significantly affect the urodynamic parameters in PTX-treated rats [micturition intervals; pre 10.1 ± 0.5, post 12.3 ± 2.2 min: micturition pressure; pre 13.6 ± 1.4, post 15.5 ± 1.4 mmHg: threshold pressure; pre 3.8 ± 0.4, post 7.2 ± 2.7 mmHg ($n=4$) ($P>0.05$; in comparison to each pre-value)] (Figure 3).

Discussion

This is the first report showing that (+)-pentazocine and DTG, two σ binding site ligands, increase bladder capacity in anaesthetized rats. Among many σ ligands, (+)-pentazocine is considered a selective σ_1 site 'agonist' with high affinity (nanomolar) for σ_1 sites and low affinity (micromolar) for σ_2 sites (Walker *et al*., 1990; Quirion *et al*., 1992). On the other hand, DTG is a non-selective σ site 'agonist' with high affinity (double digit nanomolar) for both σ_1 and σ_2 sites (Walker *et al*., 1990; Quirion *et al*., 1992). When administered intravenously, (+)-pentazocine and DTG prolonged micturition intervals and raised the threshold pressure in a dose-dependent manner (Table 1 and Figure 1). These changes in the urodynamic parameters are similar to those induced by centrally acting drugs, such as morphine and baclofen (Morikawa *et al*., 1989; Shimizu *et al*., 1999). There has been no report showing that σ sites exist in the bladder, although these sites were shown to be present in the brain and peripheral organs such as gastrointestinal tract, liver, testis, adrenal gland and ovary (Tam & Cook, 1984; Samovilova *et al*., 1985; Su *et al*., 1988; Wolfe *et al*., 1989; Walker *et al*., 1990). Therefore, it is unlikely that the effects of (+)-pentazocine and DTG on micturition are caused by their direct action on bladder detrusor muscle. Actually, (+)-pentazocine and DTG even at extremely higher concentrations than those required to produce 50% inhibition in σ_1 and/or σ_2 binding test (Walker *et al*., 1990; Quirion *et al*., 1992) scarcely affected the contractions induced by electrical field stimulation in isolated bladder strips of rats (Figure 2). Moreover, i.c.v. administration of (+)-pentazocine or DTG extended micturition intervals and raised the threshold pressure, and the effective doses of i.c.v. administrations were much lower than those of i.v. administrations (Tables 1 and 2). Combining these data, it can be assumed that the effects of (+)-pentazocine and DTG on micturition are mediated through central σ sites.

(+)-Pentazocine has a very poor affinity for opioid receptors (Su, 1985) in addition to a high affinity for σ_1 sites. The opioid agonist morphine, as well as (+)-pentazocine, extended micturition intervals and increased the threshold pressure (Figure 1 and Table 1). However, the effects of (+)-pentazocine on micturition were not antagonized by naloxone, and opioid antagonist, while those of morphine were completely antagonized (Table 1 and Figure 1). Therefore, the effects of (+)-pentazocine on micturition are not apparently mediated through opioid receptors.

Haloperidol (a potent dopamine antagonist) was reported to show possibly antagonistic properties for σ sites at high doses (Walker *et al*., 1990; Su *et al*., 1991) and has generally been used as a tool for examining whether σ sites are involved in the effects of some drugs. Moreover, it has been reported that dopamine D_2 antagonists increase bladder capacity through central and/or spinal mechanisms (de Groat *et al*., 1993). In fact, haloperidol considerably extended micturition intervals on the cystometrogram in anaesthetized rats (data not shown). Instead of haloperidol, we used rimcazole, also reported to display so-called antagonistic properties at σ sties (Ferris *et al*., 1982; Walker *et al*., 1990). However, rimcazole unexpectedly increased bladder capacity and the threshold pressure although its effects were less potent than those of (+)-pentazocine and DTG (Table 1). Physiological and functional roles of σ sites are not fully understood and the distinction between σ 'agonists' and 'antagonists' is still unclear (Walker *et al*., 1990). For example, Campbell *et al*. (1989) and Hara *et al*. (1994) showed that what is called 'σ antagonistic ligands' (rimcazole and BMY-14802), could act as 'σ agonistic ligands' in their experiments. Namely, the effects of rimcazole on micturition in the present study may be the same as the cases mentioned above, although the phenomenon is not yet defined.

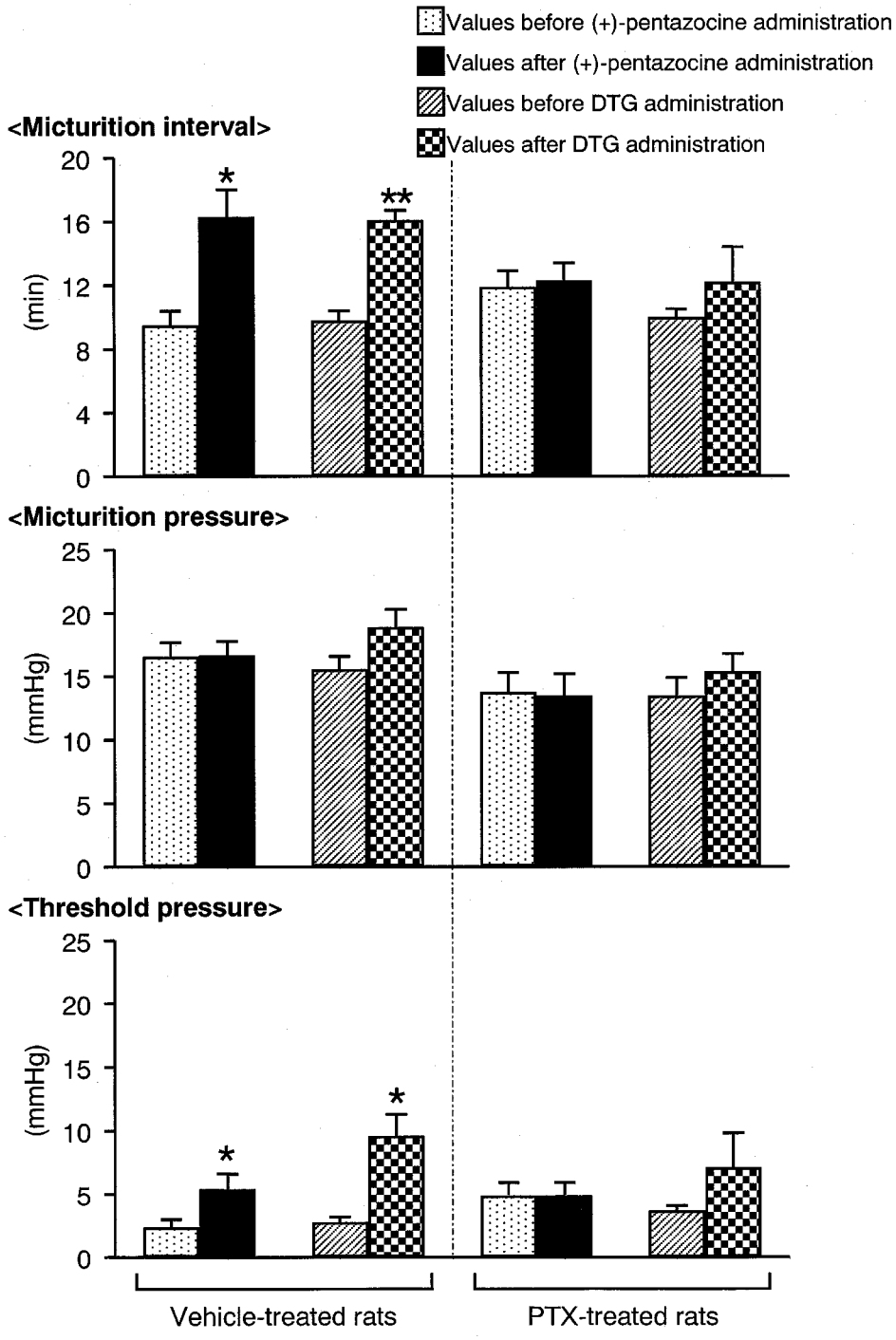

Figure 3 Effect of pre-treatment with PTX on (+)-pentazocine- and DTG-induced changes in urodynamic parameters in anaesthetized rats. Each bar represents the mean ± s.e.mean of four rats. PTX (1 μg) or the vehicle (10 mM sodium phosphate pH 7.0 containing 50 mM NaCl) was injected i.c.v. in a volume of 10 μl 4 days before the cystometry. (+)-Pentazocine or DTG was administered i.v. at a dose of 2 mg kg^{-1} on cystometrography. *$P<0.05$, **$P<0.01$: statistically significant differences from each value before (+)-pentazocine or DTG administration (paired Student's t-test).

The present study showed that the effects of (+)-pentazocine and DTG on micturition were abolished by pre-treatment with i.c.v. administration of PTX, an irreversible inhibitor of Gi/o types of G proteins (Figure 3). This result is consistent with the assumption – in some reports – that σ sites, particularly σ_1 sites, may be associated with Gi/o proteins (Walker *et al*., 1990; Junien *et al*., 1991; Quirion *et al*., 1992). Namely, this result indicates that Gi/o proteins are also involved in the pathway through which σ ligands mediate their affect. However, it is unclear whether σ_2 sites are related to the effects of (+)-pentazocine and DTG on micturition. When DTG (a non-selective σ_1 and σ_2 site 'agonist') was administered i.c.v., its effects on micturition were more potent than those of (+)-pentazocine (a selective σ_1 site 'agonist') (Table 2), although the affinity of DTG for σ_1 binding sites is approximately 9 fold weaker than that of (+)-pentazocine (Walker *et al*., 1990). Moreover, in PTX-treated rats, DTG was observed to have slight effects on micturition as compared to those of (+)-pentazocine (Figure 3). These results suggest that σ_2 sites, 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 micturition. In this respect, a more detailed analysis is needed.

In conclusion, the present study indicates that typical σ ligands, such as (+)-pentazocine and DTG, increase bladder capacity in anaesthetized rats. Moreover, the mechanism by which σ ligands change the urinary storage properties in rats may involve pathways in which the function of Gi/o proteins is necessary.

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