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Actions of tramadol on micturition in awake, freely moving rats

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1 (±)-Tramadol, a widely used analgesic, is a racemate stimulating opioid receptors and inhibiting reuptake of noradrenaline and serotonin, that is, pharmacological principles previously shown to influence rat micturition.

2 We studied both (±)-tramadol and its enantiomers in conscious Sprague–Dawley rats undergoing continuous cystometry. The effects of these agents were compared to those of morphine (μ-opioid receptor agonist) and tested after pretreatment with naloxone (μ-opioid receptor antagonist). Cystometries were evaluated before and after intravenous (i.v.), intraperitoneal (i.p.) and intrathecal (i.t.) drug administrations.

3 The most conspicuous effects of i.v. (±)-tramadol (0.1–10 mg kg^{-1}) was an increase in threshold pressure and an increase in micturition volume.

4 These effects were mimicked by (+)-tramadol (0.1–5 mg kg^{-1} i.v.), whereas (−)-tramadol (5 mg kg^{-1} i.v.) did not influence threshold pressure and micturition volume.

5 The effects of (±)-tramadol 5 mg kg^{-1} on micturition volume were blocked by pretreatment with naloxone 0.3 mg kg^{-1}. Morphine (0.3–10 mg kg^{-1} i.p.) increased threshold pressure but did not significantly increase micturition volume in doses not resulting in overflow incontinence.

6 (±)-Tramadol 10 mg kg^{-1} increased urine production, an effect blocked by desmopressin 25 ng kg^{-1}.

7 (±)-Tramadol effectively inhibits micturition in conscious rats by stimulating μ-opioid receptors. A synergy between opioid receptor stimulation and monoamine reuptake inhibition may contribute to the micturition effects.

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Abbreviations: ANOVA, analysis of variance; MP, micturition pressure; MV-BC, estimated bladder capacity; MV, micturition volume; TP, threshhold pressure

Introduction

Overactive bladder is characterised by symptoms of urgency, with and without urge incontinence, usually with frequency and nocturia (Abrams *et al*., 2002). In Western Europe, the disorder was estimated to occur in nearly 17% of the population above 40 years of age (Milsom *et al*., 2001). Detrusor overactivity, the generic term for involuntary bladder contractions, is often the underlying condition. The most common drug treatment for overactive bladder is antimuscarinic drugs. However, side effects such as dry mouth and constipation remain a problem (Andersson *et al*., 2002), and alternative therapies are needed.

The micturition reflex can be influenced at spinal and supraspinal sites by interference with various neurotransmitters (de Groat & Yoshimura, 2001). Despite this, there are only few drugs with a defined action within the central nervous system, which have been used to treat disorders of micturition. These drugs include agonists at $GABA_B$ (γ-aminobutyric acid) receptors and antidepressants, which inhibit the reuptake of 5-hydroxytryptamine (5-HT) and noradrenaline (Andersson, 2002).

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Tramadol is widely used as an analgesic. For moderate pain, it is as effective as morphine, without causing respiratory depression that is associated with opioids (Lehmann, 1997). Tramadol combines weak effects on opioid receptors with reuptake inhibition of 5-HT and noradrenaline (Table 1). The latter effects contribute to the analgesic action of (±) tramadol (Raffa *et al*., 1992; Sevcik *et al*., 1993; Raffa & Friderichs, 1996). Tramadol is a racemate, and opioid receptor activity and 5-HT reuptake inhibition are mainly associated with the (+)-tramadol enantiomer, whereas (−)-tramadol is a reuptake inhibitor of noradrenaline (Driessen & Reimann, 1992; Driessen *et al*., 1993; Raffa *et al*., 1993). Morphine is effective in blocking micturition reflexes within the central nervous system (Dray & Nunan, 1987b), and reuptake inhibitors of noradrenaline and/or 5-HT can influence micturition (Andersson, 2002). A combination of these principles may have interesting effects on micturition.

The main aim of this study was to investigate the potential effect of (±)-tramadol on rat micturition. We studied the effects of intravenous (i.v.) (±)-tramadol in conscious rats who underwent continuous cystometry. In order to discriminate effects between the enantiomers, rats were also given (+)- and (−)-tramadol. In addition, (±)-tramadol was given intrathecally (i.t.), to investigate a potential spinal action

Table 1 Inhibition of opioid receptor binding and monoamine reuptake of morphine and tramadol, in assays using selective opioid receptor agonists and 5-HT and noradrenaline expressed as K_i (nM ± s.e.m.)

	Opioid receptors			*Monoamine reuptake*	
	μ	δ	κ	*Noradrenaline*	*5-HT*
Morphine	6 ± 1	80 ± 1	170 ± 20	> 1,00,000	> 1,00,000
(±)-Tramadol	6700 ± 300	1,00,000 ± 10,000	81,000 ± 2000	1800 ± 600	1900 ± 200
(+)-Tramadol	4100 ± 100	54,000 ± 3000	83,000 ± 7000	6900 ± 600	870 ± 14
(−)-Tramadol	2,00,000 ± 70,000	4,90,000 ± 30,000	81,000 ± 9000	590 ± 10	4800 ± 300
(±)-M1	40 ± 6	1400 ± 100	3300 ± 400	2400 ± 300	11,000 ± 500
(+)-M1	22 ± 3	690 ± 30	1800 ± 100	42,000 ± 8000	7500 ± 900
(−)-M1	1900 ± 100	26,000 ± 1000	35,000 ± 4000	1800 ± 400	43,000 ± 8000

From Frink *et al.* (1996) M1 = *O*-desmethyltramadol, metabolite of tramadol.

of the unmetabolised compound. To evaluate the importance of μ-opioid receptor stimulation for the effects of (±)-tramadol, naloxone was given as a pretreatment to some rats. The effects of morphine were also studied, allowing a comparison of the cystometrical effects of (±)-tramadol and morphine. In some rats, desmopressin was given as a pretreatment to study a potential diuretic effect of (±)-tramadol.

Methods

Animals

Female Sprague–Dawley rats weighing 200–250 g were used in this study. The Animal Ethics Committee, University of Lund, approved the experimental protocol. The rats were kept at a 12 h light/12 h dark schedule and given food and water *ad libitum*. After surgical procedures, only one rat was kept per cage, to prevent chewing of catheters. Their well-being was supervised daily.

Procedures

Anaesthesia Rats were anaesthetised with ketamine (Ketalar®, Pfizer, Sweden, 75 mg kg^{-1} i.p.) and xylazine (Rompun®, Bayer, Sweden, 15 mg kg^{-1} i.p.).

Bladder catheter implantation After an abdominal incision, a polyethylene catheter (Clay-Adams PE-50, Parsipanny, NJ, U.S.A.) with a cuff was inserted into the dome of the bladder and held in place with a purse string suture. The catheter was tunnelled subcutaneously and anchored to the skin of the back with a silk ligature. The free end of the catheter was sealed.

Intravenous and intraperitoneal (i.p.) catheter implantation In the same session as the bladder catheter implantation, a catheter (Clay-Adams PE-10, Parsipanny, NJ, U.S.A.) was inserted into either the femoral vein or into the abdomen, and tunnelled subcutaneously to the skin of the back and anchored as above.

Intrathecal catheter A polyethylene catheter (Clay-Adams PE-10, Parsipanny, NJ, U.S.A.) was heated under warm water, elongated to about twice its original length and filled with saline. Insertion of the intrathecal catheter was done on the same occasion as bladder catheter implantation. Through an incision in the neck, the atlanto-occipital membrane was exposed and a small hole was made in the dura. The catheter was inserted into the subarachnoid space through the hole and advanced carefully until the tip reached the level of the L6-S1 spinal cord segments. A suture in the muscle layer fixed the catheter and the free end of the catheter was sealed. If signs of paralysis were seen when the rat awakened, the rat was killed.

Cystometric investigation

Cystometric investigations were performed 3 days after the bladder catheterisation. The conscious rat was placed in a metabolic cage without any restraint and the bladder catheter was connected via a T-tube to a pressure transducer (P23 DC, Statham Instruments Inc., Oxnard, CA, U.S.A.) and to a microinjection pump (CMA100, CMA, Stockholm, Sweden). Micturition volumes were recorded by means of a fluid collector connected to a force displacement transducer (FT03 D, Grass Instruments Co, Quincy, MA, U.S.A.). Room temperature saline was infused into the bladder continuously at a rate of 10 ml h^{-1}. Intravesical pressure and micturition volumes were continuously recorded on a Grass polygraph (Model 7E, Grass Instruments Co, recording speed 10 mm min^{-1}) or a data acquisition and analysis system (Polyview ver. 2.4., Grass Instruments Co). The following urodynamic parameters were recorded: (i) micturition pressure (maximal bladder pressure during micturition), (ii) threshold pressure (bladder pressure immediately prior to micturition), (iii) basal pressure, (iv) micturition volume and (v) intercontraction interval expressed as infused volume. Residual volume and bladder capacity (residual volume at the latest previous micturition plus volume of the infused saline at the micturition) were estimated.

Three to six reproducible micturition cycles, corresponding to a 20-min period, were analysed for base-line cystometrical parameters. Another 20-min period was chosen to evaluate drug effects on micturition, starting 10 min after i.v. and i.t., and 20 min after i.p. drug administration. For i.t. administrations, base-line parameters were obtained after saline injection, and these were compared to those after drug administration. Urodynamic parameters were compared also before and after i.v. injection of saline in a separate series of experiments.

Drug administration

For i.v., i.p. and s.c. administrations, drugs were given in a volume corresponding to 1 ml kg^{-1}, followed by a flush of 0.1 ml saline. The patency of i.v. catheters was assessed by an

i.v. injection of pentobarbital (avlivningsvätska, Apoteket, Stockholm, Sweden).

Intrathecal administrations were done in a volume of 10 μl, followed by a flush of 15 μl saline. Before drug administration, 25 μl saline was given i.t. and if there were behavioural or cystometric reactions, the animal was excluded. After the experiment, in order to verify the correct position of the catheter, 10 μl of dye (1% methylene blue) was given i.t. The position of the catheter and extent of dye distribution was examined. Dye distribution in the lumbar and sacral spinal cord and a catheter tip not penetrating into the spinal cord were the criteria for a successful intervention.

Drugs

(±)-Tramadol hydrochloride (Nobligan® 50 mg ml^{-1}, Searle, Sweden) was diluted in saline on the day of the experiment. The enantiomers (+)- and (−)-tramadol (Grünenthal, Aachen, Germany), as well as morphine sulphate (Gacell laboratories, Malmö, Sweden), were diluted in saline and stored as aliquots in −70°C. Desmopressin (Minirin® 4 μg ml^{-1}, Ferring, Malmö, Sweden) was diluted in distilled water at the day of experiment. Nobligan® and Minirin® contained, besides the active drug, sodium acetate and sodium chloride, respectively. Since the drugs were diluted 2.5–500 and 160 times, respectively, in saline, these additives were considered not to influence the experimental outcome.

Statistical analysis

To assess drug effects when several doses were given, the mean nominal change of cystometrical parameters for each drug and dose was analysed using one-way ANOVA with Bonferroni correction for multiple comparisons. For comparisons of drug effects at a specific dose, cystometrical values obtained before and after drug administration were analysed using Student's paired *t*-test. Analysis of drug effects in the absence or presence of a pretreatment with a second drug was done using Student's unpaired *t*-test on mean nominal changes in cystometrical parameters before and after drug administration. A probability of $<5\%$ was accepted as significant for all tests.

Results

Basal values for micturition volume as well as threshold and micturition pressure, for each tested drug and dose, are given in Table 2. Effects of drugs are presented as per cent change hereof in the figures. In the text below are given, for parameters with observed significant effects, the number of rats in whom the parameters increased or decreased after drug administration.

Intravenous administrations

Saline Saline injection did not cause any significant changes on the cystometrical parameters (Student's paired *t*-test; Table 3), except for a small decrease in basal pressure ($P<0.05$) and a small increase in estimated residual volume ($P<0.05$). Micturition volume exceeded estimated bladder capacity in two, but not in the remaining eight rats.

(±)-Tramadol With (±)-tramadol 1 mg kg^{-1} and above, micturition volume exceeded estimated bladder capacity in all rats, indicating a diuretic effect of the drug. We therefore inhibited endogenous urine production by desmopressin to be able to assess the effects of the drug on the urine storage capacity. In rats pretreated with desmopressin 25 ng kg^{-1} and given saline ($n=5$, data not shown), no significant changes (Student's paired *t*-test) in cystometrical parameters were observed. After (±)-tramadol 10 mg kg^{-1}, the mean difference between observed micturition volume and estimated bladder

Table 2 Baseline values (mean±s.e.m.)

	MP (cm H_2O)	*TP* (cm H_2O)	*MV* (ml)
Intravenous			
Saline ($n=10$)	79±6	19±2	0.96±0.07
(±)-Tramadol 0.1 mg kg^{-1} ($n=5$)	69±11	8±2	0.75±0.12
(±)-Tramadol 1 mg kg^{-1} ($n=6$)	94±7	14±2	0.75±0.09
(±)-Tramadol 5 mg kg^{-1} ($n=10$)	103±10	12±1	0.76±0.06
(±)-Tramadol 5 mg kg^{-1}[a] ($n=7$)	78±11	22±3	1.03±0.04
(±)-Tramadol 10 mg kg^{-1} ($n=9$)	74±5	15±1	0.85±0.05
(±)-Tramadol 10 mg kg^{-1}[b] ($n=6$)	80±10	17±2	1.01±0.11
(+)-Tramadol 0.3 mg kg^{-1} ($n=8$)	71±4	8±0	0.86±0.07
(+)-Tramadol 0.5 mg kg^{-1} ($n=5$)	73±12	8±1	1.07±0.06
(+)-Tramadol 5 mg kg^{-1} ($n=9$)	60±4	8±1	0.81±0.07
(−)-Tramadol 5 mg kg^{-1} ($n=10$)	69±4	7±2	1.07±0.13
Naloxone 0.3 mg kg^{-1} ($n=6$)	91±12	24±4	1.15±0.06
Intraperitoneal			
(±)-Tramadol 5 mg kg^{-1} ($n=6$)	86±14	17±2	0.50±0.04
Morphine 0.3 mg kg^{-1} ($n=8$)	85±8	18±2	0.80±0.05
Morphine 1 mg kg^{-1} ($n=10$)	60±4	11±2	0.71±0.06
Intrathecal			
(±)-Tramadol 200 μg ($n=6$)	93±6	15±1	1.02±0.12

MP: micturition pressure, TP: threshold pressure, MV: micturition volume. Results are expressed as mean±the standard error of the mean. [a]Rats given naloxone 0.3 mg kg^{-1} s.c., 5 min before tramadol. [b]Rats pretreated with desmopressin 25 ng kg^{-1} s.c.

Table 3 Cystometrical parameters after intravenous saline ($n=10$)

	BP (cm H_2O)	*TP* (cm H_2O)	*MP* (cm H_2O)	*MV* (ml)	*BC* (ml)	*RV* (ml)
Baseline	9±2	19±2	79±6	0.96±0.07	0.97±0.07	0.01±0.00
After saline	7±2 *	17±2	77±8	0.97±0.06	0.99±0.05	0.05±0.02 *

BP: basal pressure, TP: threshold pressure, MP: micturition volume, MV: micturition volume, BC: estimated bladder capacity, RV: estimated residual volume. *$P<0.05$, Student's paired *t*-test.

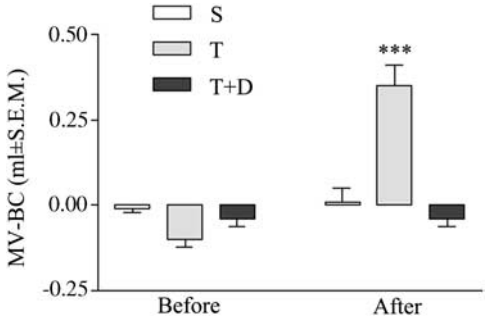

Figure 1

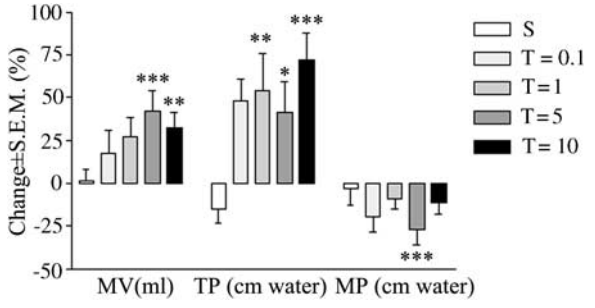

Figure 2

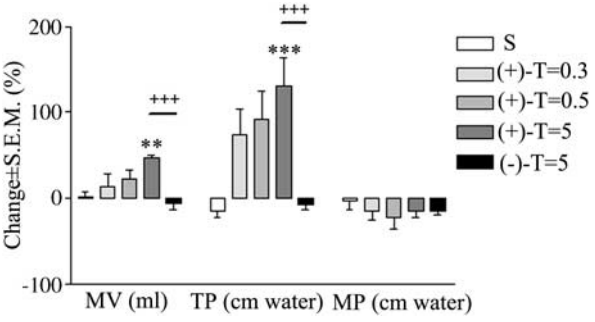

Figure 3

capacity (MV-BC) was significantly increased (Student's paired *t*-test, Figure 1). In desmopressin pretreated rats, no significant change in MV-BC (Figure 1) or estimated residual volume (Student's paired *t*-test) after (±)-tramadol 10 mg kg^{-1} was observed. Neither was micturition volume larger than estimated bladder capacity in any rat. However, both bladder capacity and micturition volume were increased (Student's paired *t*-test). Given this, micturition volume was considered a more appropriate parameter for bladder storage capacity than estimated bladder capacity, since true bladder capacity cannot be lower than micturition volume. Hence, to evaluate bladder storage capacity, micturition volume has been used.

Micturition volume was increased by (±)-tramadol 5 and 10 mg kg^{-1}, compared to baseline values in all rats (one-way ANOVA with Bonferroni correction for multiple comparisons; Figure 2).

Threshold pressure was increased by (±)-tramadol 1, 5 and 10 mg kg^{-1}, compared to baseline values in all rats. Micturition pressure was significantly higher after (±)-tramadol 5 mg kg^{-1} compared to baseline values, but not after the remaining doses (Figure 2). Basal pressures were not significantly changed (one-way ANOVA with Bonferroni correction for multiple comparisons).

(+)- and (−)-Tramadol (+)-Tramadol 5 mg kg^{-1} increased threshold pressure and micturition volume compared to baseline values in all rats (one-way ANOVA with Bonferroni correction for multiple comparisons; Figure 3). At this dose, micturition volume was larger than estimated bladder capacity in all rats and one rat developed overflow incontinence 15 min after drug injection. No changes were seen for mean change in micturition and basal pressures.

(−)-Tramadol 5 mg kg^{-1} i.v. did not significantly change micturition parameters before and after drug administration (Student's paired *t*-test). Mean nominal changes for threshold pressure and micturition volume were significantly different ($P<0.001$ and <0.001, respectively) compared to (+)-tramadol at 5 mg kg^{-1} each (Student's unpaired *t*-test; Figure 3). Basal pressure was not significantly changed.

(±)-Tramadol, intrathecal administration Compared to cystometrical parameters after saline administration in the same rat, there were no observed effects on the cystometrical pattern after (±)-tramadol 10 μg ($n=3$) and 50 μg ($n=5$). After (±)-tramadol 200 μg threshold pressure increased (27±14%; $P<0.01$) in all rats, micturition volume increased (30±15%; $P<0.001$) in all rats and micturition pressure decreased (13±7%; $P<0.05$; Student's paired *t*-test) in eight out of nine rats, compared to baseline values.

Intraperitoneal administrations Morphine 1 mg kg^{-1} increased threshold pressure in all rats, compared to baseline values. No significant changes were seen for micturition volume and pressure (one-way ANOVA with Bonferroni corrections for multiple comparisons; Figure 4). Micturition volume was larger than estimated bladder capacity in all rats, and MV-BC changed from −0.04±0.03 to 0.17±0.04 ml before and after morphine administration, respectively (compare with Figure 1). With 1 mg kg^{-1}, one rat developed dribbling incontinence 15 min after receiving the drug. Morphine 3 mg kg^{-1} i.p. ($n=3$) and 10 mg kg^{-1} ($n=3$) caused dribbling incontinence in all rats within 30 min.

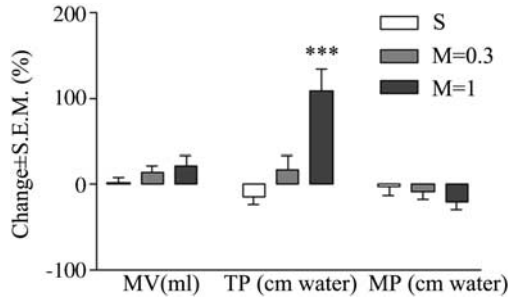

Figure 4

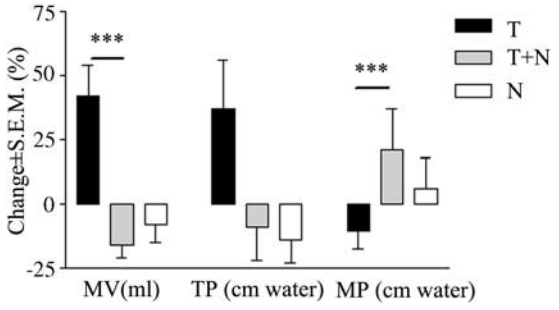

Figure 5

Mean nominal changes in threshold and micturition pressure, as well as micturition volume, after (±)-tramadol 5 mg kg^{-1} given i.p. were not significantly different from those after i.v. administration (Student's unpaired *t*-test).

Naloxone and (±)-tramadol, i.v. administrations Naloxone 0.3 mg kg^{-1} i.v. *per se* was without significant effects (Student's paired *t*-test). The mean nominal change in micturition volume and micturition pressure, induced by (±)-tramadol 5 mg kg^{-1} in the absence of naloxone 0.3 mg kg^{-1}, was significantly different from that in the presence of naloxone 0.3 mg kg^{-1} i.v. The mean nominal change in threshold pressure tended ($P<0.06$) to be different between the groups (Student's unpaired *t*-test; Figure 5).

Discussion

With (±)-tramadol given i.v. at 1, 5 and 10 mg kg^{-1}, micturition volume exceeded estimated bladder capacity in all rats, compared to two out of 10 after saline. Then, estimation of residual volume and bladder capacity cannot be made. This phenomenon was abolished after pretreatment with desmopressin (Figure 1), as tramadol 10 mg kg^{-1} in these rats increased both estimated bladder capacity as well as micturition volume without a significant change in residual volume. Thus, (±)-tramadol does not impair efficiency of bladder emptying up to 10 mg kg^{-1}. For the discussion below on experiments without desmopressin pretreatment, micturition volume is used to evaluate changes in bladder storage capacity. Thus, given that (±)-tramadol significantly increased micturition volume, it is concluded that (±)-tramadol increases rat bladder storage capacity.

(±)-Tramadol increased threshold pressure. Given the data shown in Figures 2–4, threshold pressure can be increased by ≈100% by (±)-, (+)-tramadol and morphine, before overflow incontinence is observed. Changes in threshold pressure, that is, pressure immediately before micturition, reflect changes in the urethra and/or bladder. During the continent storage phase, maximal urethral pressure exceeds bladder pressure. Since there was no significant change in basal bladder pressure in the presence of (±)-tramadol, the increase in threshold pressure could imply a change in sensitivity to, or reaction of, bladder wall tension receptors. Neither morphine nor (±)-, (+)- or (−)-tramadol decreased micturition pressure (except for (±)-tramadol 5 mg kg^{-1}). For morphine, this is consistent with results after i.t. administration, on micturition pressure in conscious rats (Igawa *et al*., 1993) and urethral resistance in anaesthetised dogs (Drenger *et al*., 1986). Hence, (±)-tramadol probably does not impair urethral closure. As shown in Figure 2, relative increases in micturition volume and threshold pressure seem to reach a plateau at 5 and 1 mg kg^{-1} (±)-tramadol, respectively. These doses are analogous with those resulting in analgesia (Raffa & Friderichs, 1996).

(±)-Tramadol stimulates opioid receptors and inhibits reuptake of 5-HT and noradrenaline. These are all pharmacological principles for micturition control (Andersson, 1993; de Groat & Yoshimura, 2001; de Groat, 2002).

Opioid receptor-mediated inhibition of micturition can be caused by stimulation of μ- and δ-opioid receptors (Dray & Metsch, 1984a; Hisamitsu & de Groat, 1984; Kontani & Kawabata, 1988; Shimizu *et al*., 2000). Thus, administration of opioid receptor active drugs (i) systemically (Sillen & Rubenson, 1986; Kontani & Kawabata, 1988), (ii) intrathecally (Dray & Metsch, 1984a; Hisamitsu & de Groat, 1984; Durant & Yaksh, 1988; Igawa *et al*., 1993) and (iii) intracerebroventricularly (Hisamitsu & de Groat, 1984; Sillen & Rubenson, 1986; Dray & Nunan, 1987b; Kontani *et al*., 1989) inhibits micturition. The main site for inhibitory effects *via* opioid receptor stimulation is likely to be within the central nervous system (Dray & Metsch, 1984b). However, the peripherally active opioid receptor agonist, loperamide, exerted a dose-dependent inhibitory action on induced bladder contractions (Berggren *et al*., 1992). Furthermore, naloxone may *in vitro* facilitate electrically induced contractile activity of rat bladder strips (Berggren *et al*., 1991). Thus, it cannot be completely excluded that peripheral opioid receptor stimulation influences micturition.

Pretreatment with naloxone, μ-opioid receptor antagonist, abolished the effects of (±)-tramadol on micturition volume and attenuated the effects on threshold pressure. This suggests that μ-opioid receptor activation plays a major role for (±)-tramadol. The spinal cord is probably essential for the effects of μ-opioid receptor stimulation on micturition, as inhibition of isovolumetric rat bladder contractions via systemic morphine, was abolished by i.t. naloxone (Dray & Metsch, 1984a) and isovolumetric rat bladder contractions were more effectively attenuated by morphine given i.t. vs i.c.v. at equal doses (Dray & Nunan, 1987b). In the rat spinal cord, opioid receptors are concentrated in the superficial dorsal horn (Coggeshall & Carlton, 1997), where bladder afferents merge into the spinal cord (Yoshimura & de Groat, 1997). In this region, μ-opioid receptors have a predominant distribution, but δ- and κ-opioid receptors are also present, and κ-opioid receptors may prevail in the lumbosacral region (Coggeshall & Carlton, 1997) where they may control urethral sphincter activity (de Groat & Yoshimura, 2001). The major importance of spinal μ-opioid receptor stimulation was demonstrated as (i)

the minute amount of 0.5 μg morphine given i.t. resulted in overflow incontinence in conscious rats (Igawa *et al*., 1993), and (ii) a μ-opioid receptor agonist given i.t. was more potent than a δ-opioid receptor agonist given i.t. to inhibit isovolumetric bladder contractions in anaesthetised rats. Stimulation of spinal κ-opioid receptors was ineffective (Dray & Metsch, 1984a; Dray & Nunan, 1987a).

(±)-Tramadol given i.t. (10–50 μg) was ineffective for micturition control, probably because of the low opioid receptor activity of unmetabolised (±)-tramadol (Frink *et al*., 1996). The effects of 200 μg, corresponding to ≈1 mg kg^{-1} may be because of systemic actions. Metabolites of (±)-tramadol–(±)-M1 and also the metabolite (±)-M5–have higher μ-opioid receptor affinity, but also higher δ- and κ-opioid receptor affinity, compared to unmetabolised (±)-tramadol (Table 1). These metabolites are probably responsible for the μ-opioid receptor-mediated actions of (±)-tramadol.

There are differences between the enantiomers of the metabolites in affinity for opioid receptors, for example, (+)-M1 has an affinity approximately 1 : 2–10 and (−)-M1 has an affinity approximately 1 : 200–300, for μ-, δ- and κ-opioid receptors, compared to morphine (Table 1). Nevertheless, (−)-M1 has a μ-opioid receptor affinity roughly equal to that of unmetabolised (+)-tramadol (Frink *et al*., 1996). Yet, (−)-tramadol was ineffective for micturition control. Thus, either metabolites of (−)-tramadol were not generated in sufficient amounts at a time when drug effects were evaluated, or (−)-tramadol metabolites were insufficiently active on opioid receptors to influence rat micturition. In support of the latter alternative, (±)-tramadol is rapidly metabolised (Lintz *et al*., 1981). As an inhibitor of noradrenaline reuptake, (−)-tramadol has an inhibitory potency similar to that of fluoxetine, a selective inhibitor of noradrenaline reuptake (Frink *et al*., 1996). This action may be of importance for the analgesic effect of (±)-tramadol (Raffa & Friderichs, 1996). It also represents a potential mechanism for micturition control, since a descending noradrenergic pathway from locus coeruleus has a facilitating effect on micturition (de Groat & Yoshimura, 2001), and in the bladder both α- and β-adrenoceptor may influence contractility (Andersson, 1993). However, noradrenaline reuptake inhibition was ineffective for cat micturition (Katofiasc *et al*., 2002) and (−)-tramadol was ineffective for rat micturition. Taken together, these results suggest that (+)-tramadol is the effective enantiomer.

The importance of μ-opioid receptors for the effects of (±)-tramadol on micturition is consistent with the naloxone-induced blockade of the (±)-tramadol-mediated increase in micturition volume. Furthermore, both (±)-tramadol and morphine increased threshold pressure. Yet, in contrast to the significant increase in bladder storage capacity after (±)-tramadol 5–10 mg kg^{-1} i.v., without signs of overflow incontinence, morphine did not significantly increase bladder storage capacity in doses not resulting in overflow incontinence. In anaesthetised rats, morphine i.v. at 0.1 mg kg^{-1} doubled the bladder capacity (Kontani & Kawabata, 1988). Also in anaesthetised rats, morphine at 1–2 mg kg^{-1} i.v. abolished micturition (Kontani & Kawabata, 1988; Shimizu *et al*., 2000). Thus, the effects of (±)-tramadol, and (+)-tramadol, cannot solely be explained by interaction with μ-opioid receptors. Within the brain, both μ- and δ-opioid receptors may influence micturition control (Hisamitsu & de Groat, 1984; Dray *et al*., 1985; de Groat, 1990). Despite that (+)-M1 is approximately 30 times more potent at μ- than δ-opioid receptors (Frink *et al*., 1996), it cannot be excluded that δ-opioid receptor activation in the brain may influence the outcome.

5-HT receptor modulation is important for micturition control (de Groat, 2002). Reuptake inhibition of 5-HT due to (+)-tramadol, which is approximately 40 times less potent than the selective 5-HT reuptake inhibitor fluoxetine (Frink *et al*., 1996), may therefore be of importance. However, the selective 5-HT reuptake inhibitor citalopram was ineffective in isovolumetric bladder contractions (Testa *et al*., 1999). Still, activation of supraspinal opioid receptors influences micturition via mechanisms modulated through a descending 5-HT containing system. Thus, the inhibitory action of morphine given i.c.v., but not i.t., was attenuated by (i) 5-HT depletion in the CNS and (ii) i.t. methysergide (5-HT receptor antagonist) (Dray & Nunan, 1987b). Speculatively, effects of (±)-tramadol on micturition, *via* supraspinal μ-opioid receptor activation, may be in synergy with (+)-tramadol-mediated 5-HT reuptake inhibition.

Effects on urine production

That micturition volume was larger than estimated residual volume in all rats given high doses of (±)- and (+)-tramadol, an effect not observed in saline controls with and without desmopressin pretreatment, is considered a diuretic effect. Since the same effect was observed with (±)- and (+)-tramadol as well as morphine, but not (−)-tramadol, involvement of opioid receptors is suggested. Desmopressin, a vasopressin analogue that reduces urine production in rats (Vavra *et al*., 1974), abolished the diuretic effect of (±)-tramadol, for example, mean MV-BV was below zero and no rats had a larger micturition volume than estimated bladder capacity.

Stimulation of κ-opioid receptors in the rat hypothalamus is known to stimulate diuresis by suppressing vasopressin release (Leander *et al*., 1987; Brooks *et al*., 1993). The (+)-M1 metabolite of (±)-tramadol, has affinity for the κ-opioid receptor (Frink *et al*., 1996; Lai *et al*., 1996), which may explain the diuretic effect. Though stimulation of κ-opioid receptors in the kidney may attenuate the response to vasopressin (Slizgi & Ludens, 1982), experiments with Brattleboro rats discourage a renal site of action (Abrahams *et al*., 1986). To our knowledge, (±)-tramadol does not have a diuretic effect in humans. This might be because of a difference between primates and rodents, for example, observed on brain κ- and μ-opioid receptor distribution in rats and monkeys (Mansour *et al*., 1988).

Conclusions

(±)-Tramadol causes an increase in both threshold pressure and bladder storage capacity, without impairing bladder emptying. These effects of (±)-tramadol on rat micturition at doses similar to those giving analgesia in rats (Apaydin *et al*., 2000) and humans (Lehmann, 1997), together with the clinical experience of (±)-tramadol as a safe drug in the management of pain, render the drug potentially interesting in the treatment of detrusor overactivity and or nocturia.

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