Effects of morphine metabolites on micturition in normal, unanaesthetized rats

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1 By means of continuous cystometry in normal, unanaesthetized rats, the effects on micturition of intrathecally (i.t.) administered morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G), the two main metabolites of morphine, were studied and compared with those of i.t. morphine.

2 Both M6G (0.01, 0.1, and 0.5 µg) and M3G (5 µg) were found to have significant effects on micturition. Like morphine (0.1, 0.5, and 10 µg), M6G was able to inhibit the micturition reflex, and produce urinary retention and dribbling incontinence in a dose-dependent manner. The potency of M6G for inhibiting micturition was approximately 10 times higher than that of morphine, and the duration of its effect was longer. All effects of M6G could be reversed by naloxone.

3 M3G (5 µg) facilitated the micturition reflex, resulting in decreases in bladder capacity and micturition volume, and an increase in spontaneous contractile activity. Pretreatment with naloxone (10 µg), which by itself had no effect on micturition, enhanced the facilitatory effects of M3G. In addition, M3G tended to counteract the inhibitory effects of both morphine and M6G on micturition. M3G (5 µg) also produced an excitatory behavioural syndrome.

4 It is concluded that in rats, i.t. M3G has excitatory effects on micturition and behaviour, probably not mediated via opioid receptors. I.t. M6G has a potent inhibitory effect on micturition mediated by stimulation of opioid receptors. It may have effects on somatosensory afferent input in lower doses than those required for effects on micturition.

Keywords: Morphine; morphine-3-glucuronide; morphine-6-glucuronide; naloxone; intrathecal administration; micturition; cystometry; unanaesthetized rat

Introduction

It is well known that urinary retention may occur following epidural administration of morphine and other opiates (Bromage *et al.*, 1982; Rawal *et al.*, 1983; Stenseth *et al.*, 1985). This is due to complex effects on central and may be also peripheral neurogenic mechanisms controlling the micturition reflex (Dray & Metsch, 1984a,b,c,d; Hisamitsu & de Groat, 1984; Dray *et al.*, 1985; Dray & Nunan, 1987; Sheldon *et al.*, 1987; 1988; Berggren *et al.*, 1992).

Morphine is metabolized predominantly to morphine-3-glucuronide (M3G) and to a minor extent to morphine-6-glucuronide (M6G; Boerner *et al.*, 1975). These metabolites are active and believed to contribute to the pharmacological effects of morphine. Higher concentrations of M6G than of morphine itself were found in plasma (Säwe *et al.*, 1983; Poulain *et al.*, 1990) and cerebro-spinal fluid (Poulain *et al.*, 1990) after oral administration of morphine in man. M6G was shown to have analgesic effects in mice (Shimomura *et al.*, 1971), and clinically, it produced long-lasting analgesia when given to cancer patients (Osborne *et al.*, 1992). M6G binds to μ and δ receptors with apparent affinities similar to those of morphine (Christensen & Jorgensen, 1987; Pasternak *et al.*, 1987; Frances *et al.*, 1990). After administration of M6G into the central nervous system, it has been reported to have a 9 to 650 fold greater analgesic effect than morphine administered via the same route (Pasternak *et al.*, 1987; Abbott & Palmour, 1988; Sullivan *et al.*, 1989; Paul *et al.*, 1989; Gong *et al.*, 1991; Frances *et al.*, 1990; 1992). In contrast, M3G does not bind to any opioid receptor subtype (Pasternak *et al.*, 1987), and is devoid of analgesic activity (Shimomura *et al.*, 1971; Yoshimura *et al.*, 1973; Pasternak *et al.*, 1987). However, the metabolite has been shown to produce hyperaesthesia/hyperalgesia via non-opioid related

mechanisms in rats when administered intrathecally (i.t.; Woolf, 1981; Yaksh *et al.*, 1986; Yaksh & Harty, 1988) and intracerebroventricularly (i.c.v.; Labella *et al.*, 1979). It may antagonize some pharmacological effects of morphine (Smith *et al.*, 1990) and M6G (Gong *et al.*, 1991; 1992).

The effects of morphine on micturition in unanaesthetized rats have been reported previously (Durant & Yaksh, 1988; Igawa *et al.*, 1992; Souldard *et al.*, 1992). However, the effects of M3G and M6G on micturition do not seem to have been established. Particularly, if there is a separation between its analgesic potency (high) and its potency for inhibiting the micturition reflex (low), M6G, which has a longer duration of analgesic action than morphine, would be an interesting alternative for i.t. opioid treatment of pain.

In the present study, we have by means of continuous cystometries in normal unanaesthetized rats, attempted to define the potency of i.t. M6G for inhibiting the micturition reflex, and compared it to that of i.t. morphine. We have also investigated how i.t. M3G by itself influences micturition, and whether the effects of i.t. morphine and M6G are antagonized by i.t. M3G.

Methods

Animals

Female Sprague-Dawley rats, weighing 200–240 g, were used in this study. The experimental protocol was approved by the Animals Ethics Committee, University of Lund.

Surgical procedures

The rats were anaesthetized with ketamine (75 mg kg⁻¹, i.m.) and xylazine (15 mg kg⁻¹, i.m.). A polyethylene catheter

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(Clay-Adams PE-10, NJ, U.S.A.) was implanted into the subarachnoid space at the level of L₆–S₁ spinal cord segments for i.t. administration of drugs as described in detail previously (Igawa *et al.*, 1993b). Thereafter, the abdomen was opened through a midline incision, and a polyethylene catheter (Clay-Adams PE-50, NJ, U.S.A.) was implanted into the bladder through the dome as described previously (Malmgren *et al.*, 1987). The catheters were tunnelled subcutaneously and orifices were made on the back of the animal. After implantation of the catheters, rats were housed individually in cages on a 12 h/12 h, light/dark photo cycle. The injection sites in the spinal cord and the extent of dye distribution were confirmed by injection of dye (methylene blue) in every animal at the end of the experiment.

Cystometric investigations

Cystometric investigations were performed without any anaesthesia three days after the surgical preparation. The bladder catheter was connected via a T-tube to a pressure transducer (P23 DC, Statham Instrument Inc., CA, U.S.A.) and a microinjection pump (CMA 100, Carnegie Medicine AB, Sweden). The conscious rat was placed, without any restraint, in a metabolic cage which also enabled measurements of micturition volumes by means of a fluid collector connected to a Grass force displacement transducer (FT 03 C, Grass Instrument Co., Quincy, Mass, U.S.A.). Room-temperature saline was infused into the bladder at a rate of 10 ml h⁻¹. Intravesical pressure and micturition volumes were recorded continuously on a Grass polygraph (Model 7E, Grass Instrument Co., Quincy, Mass, U.S.A.; recording speed: 10 mm min⁻¹). Three reproducible micturition cycles, corresponding to a 20 min period, were recorded before drug administration. After each drug administration, recording was continued for at least another 120 min. The following cystometric parameters were investigated (Malmgren *et al.*, 1987): basal pressure (the lowest bladder pressure during filling), threshold pressure (bladder pressure immediately prior to micturition), micturition pressure (the maximum bladder pressure during micturition), bladder capacity (residual volume at the latest previous micturition plus volume of infused saline at the micturition), micturition volume (volume of expelled urine), residual volume (bladder capacity minus micturition volume), and spontaneous contractile activity (mean amplitude and frequency of bladder pressure fluctuations during 2 min prior to micturition). Analysis was performed for a 20 min period before drug administration. Drug effects on cystometric parameters were assessed for 120 min, and the two most effective micturition cycles were subjected to analysis.

Administration of drugs

The following drugs were used for i.t. administration: morphine-3 β -glucuronide (M3G), morphine-6 β -glucuronide

(M6G), naloxone hydrochloride (Sigma Chemical Co., St. Louis, Mo, U.S.A.), and morphine sulphate (Gacell Laboratories, Malmö, Sweden). Drugs were dissolved in redistilled water, and then stored at -70°C. Subsequent dilutions of the drugs were made on the day of experiments with saline. Drugs were administered i.t. in 10 μ l of saline followed by a flush of 15 μ l of saline for 10 s; 10 μ l of saline was injected i.t. as control, prior to drug administration. Neither the placement of the i.t. catheter itself, nor i.t. injection of saline, affects the cystometric pattern (Igawa *et al.*, 1992; 1993b).

Statistical analysis

The results are given as mean values $\pm$ s.e.mean. Statistical comparisons for experiments were based on doses expressed as micrograms per rat. Student's paired two-tail *t* test was used for comparison between before and after treatments. Comparison between drugs was performed by using factorial analysis of variance, and followed by Scheffe's *F* test. To evaluate the antagonistic effects of M3G on the effects of morphine or M6G, the incidence of urinary retention produced by these drugs were compared in the presence and absence of M3G by using the Chi-square test. A probability level <0.05 was accepted as significant.

Results

Effects of morphine

Morphine, administered i.t. in doses of 0.1 ($n=7$), 0.5 ($n=9$), 10 μ g ($n=8$), dose-dependently inhibited micturition, eventually ending with urinary retention and dribbling incontinence (Figure 1). After 0.1 μ g, threshold pressure, bladder capacity and micturition volume increased ($P<0.05$; Table 1), but dribbling incontinence did not occur. The onset time of the effects of morphine (0.1 μ g) was approximately 20 min. The maximum effect was seen 30 to 40 min after the injection. Dribbling incontinence was observed after 0.5 (6 out of 9 animals) and 10 μ g (8 out of 8; Figure 2). The onset times of dribbling incontinence after 0.5 ($n=6$) and 10 μ g ($n=8$) were 43 ± 7 and 19 ± 2 min, respectively. The duration of dribbling incontinence after 0.5 μ g was 51 ± 8 min ($n=5$).

Effects of morphine-6-glucuronide

M6G, administered i.t. in doses of 0.01 ($n=7$), 0.05 ($n=10$), and 0.5 μ g ($n=7$), inhibited micturition in a dose-dependent manner (Figure 3). Dribbling incontinence was observed after 0.01 (1 out of 7 animals), 0.05 (7 out of 10), and 0.5 μ g (7 out of 7; Figure 2). After 0.01 μ g, threshold pressure, bladder capacity, and micturition volume increased ($P<0.01$; Table 1). The onset time of the effects of M6G (0.01 μ g) was approximately 15 min. The maximum effect was seen within 30 min after the injection. The onset time of dribbling incontinence

Figure 1 Effects of morphine administered intrathecally (i.t.) on bladder pressure (BP cmH₂O) and micturition volume (MV ml) during cystometry in a normal rat. Morphine (Morph 0.5 μ g) inhibited micturition, and produced dribbling incontinence. The cystometric pattern was restored to the pre-administration state about 140 min after the administration. * and $\uparrow$ indicate adjustment to baseline position and initiation of dribbling incontinence, respectively.

tinence after M6G (0.05 μ g; $n = 7$) was 20 ± 2 min, which was significantly ($P < 0.01$) shorter than the corresponding value (43 ± 7 min) after morphine (0.5 μ g; $n = 6$). The duration of dribbling incontinence after M6G (0.05 μ g; $n = 5$) was 165 ± 29 min. This was significantly ($P < 0.01$) longer than that (51 ± 8 min) after morphine (0.5 μ g; $n = 5$).

Table 1 Effects of intrathecal administration of morphine (0.1 μ g; $n = 7$) and morphine-6-glucuronide (M6G 0.01 μ g; $n = 6$) on cystometric parameters in normal, unanaesthetized rats

	Morphine		M6G	
	before	after	before	after
Th P	10.7 ± 2.1	$19.2 \pm 3.6^*$	15.0 ± 2.5	$39.0 \pm 5.4^{**}$
M P	66.7 ± 15.9	40.5 ± 5.4	63.8 ± 15.7	61.5 ± 16.7
B C	1.15 ± 0.09	$1.51 \pm 0.13^*$	0.81 ± 0.09	$1.49 \pm 0.16^{**}$
M V	1.11 ± 0.06	$1.48 \pm 0.13^*$	0.75 ± 0.10	$1.29 \pm 0.15^{**}$
R V	0.03 ± 0.03	0.03 ± 0.02	0.06 ± 0.02	0.20 ± 0.08

Th P: threshold pressure (cmH₂O); M P: micturition pressure (cmH₂O); B C: bladder capacity (ml); M V: micturition volume (ml); R V: residual volume (ml). Results are expressed as mean $\pm$ s.e.mean. Before vs after: $^*P < 0.05$; $^{**}P < 0.01$.

Figure 2 Dose-response relations for the inhibition of micturition produced by morphine (O; $n = 7-10$) and morphine-6-glucuronide (M6G, ●, $n = 7-10$) administered intrathecally (i.t.). The effects of morphine-3-glucuronide (M3G, 0.5 μ g administered i.t. 30 min before morphine or M6G) on the effects of morphine (Δ ; $n = 6$) and M6G (Δ ; $n = 6$) are included.

Figure 3 Effects of morphine-6-glucuronide administered intrathecally (i.t.) on bladder pressure (BP cmH₂O) and micturition volume (MV ml) during cystometry in a normal rat. Morphine-6-glucuronide (M6G 0.05 μ g) inhibited micturition, and produced dribbling incontinence. Naloxone (Nalox 10 μ g, i.t.) reversed the effects of M6G. * and † indicate adjustment to baseline position and initiation of dribbling incontinence, respectively.

Figure 4 Effects of intrathecal (i.t.) administration of morphine-3-glucuronide (M3G 5 μ g) on bladder pressure (BP cmH₂O) and micturition volume (MV ml) during cystometry in a normal rat in the absence (a) and presence (b) of naloxone (Nalox; 10 μ g, i.t.). (a) In the absence of naloxone, M3G decreased bladder capacity and micturition volume, and facilitated the spontaneous bladder activity. (b) In the presence of naloxone (administered 10 min before M3G), the facilitatory effects of M3G were enhanced and dribbling incontinence was induced. * indicates adjustment to baseline position.

Table 2 Effects of intrathecal administration of morphine-3-glucuronide (5 µg) in the absence ($n = 7$) and presence ($n = 4$) of naloxone (10 µg i.t.) on cystometric parameters in normal, unanaesthetized rats

	Absence of naloxone		Presence of naloxone	
	before	after	before	after
ThP	16.3 ± 3.2	13.0 ± 2.0	13.8 ± 3.1	17.8 ± 2.7*
MP	65.0 ± 2.0	91.6 ± 20.6	61.8 ± 12.8	82.2 ± 12.5*
BC	0.94 ± 0.11	0.41 ± 0.04**	0.68 ± 0.09	0.16 ± 0.04*
MV	0.87 ± 0.08	0.39 ± 0.04***	0.61 ± 0.10	0.15 ± 0.04*
RV	0.07 ± 0.04	0.01 ± 0.01	0.06 ± 0.03	0.01 ± 0.01
ASA	1.7 ± 1.1	8.0 ± 1.4***	4.8 ± 2.1	13.2 ± 2.7*
FSA	0.4 ± 0.3	2.1 ± 0.2***	0.6 ± 0.3	3.6 ± 0.4**†

ThP: threshold pressure (cmH₂O); MP: micturition pressure (cmH₂O); BC: bladder capacity (ml); MV: micturition volume (ml); RV: residual volume (ml); ASA: amplitude of spontaneous activity (cmH₂O); FSA: frequency of spontaneous activity (min⁻¹). Results are expressed as mean ± s.e.mean. Before vs after * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Absence of naloxone vs presence of naloxone † $P < 0.05$.

Effects of morphine-3-glucuronide

M3G, administered i.t. in doses of 0.1 ($n = 5$) and 0.5 µg ($n = 5$), by itself had no consistent effects on the cystometric pattern (data not shown). After 5 µg, i.t. ($n = 7$), on the other hand, bladder capacity and micturition volume decreased. M3G also increased both the amplitude and frequency of the spontaneous contractile activity (Figure 4a; Table 2). The onset time of the effects of M3G was within 5 min. The maximum effect was seen approximately 5 to 10 min after the injection. The cystometric pattern was restored to the pre-administration state within 60 min after injection. After 5 µg, i.t. a behavioural syndrome, characterized by agitation, biting, vocalization and jumping, was also observed in all animals. In the presence of M3G (0.5 µg, i.t., 30 min before morphine or M6G), morphine (0.5 µg, i.t.) and M6G (0.05 µg, i.t.) produced dribbling incontinence in 1 out of 6 animals and 3 out of 6, respectively (Figure 2). The changes were not statistically significant. In these animals, the onset time of dribbling incontinence was 75 min (morphine) and 14 ± 3 min (M6G), and the duration 24 min (morphine) and 136 ± 28 min (M6G).

Effects of naloxone

Naloxone, administered i.t. in doses of 10 ($n = 7$) and 100 µg ($n = 6$), by itself had no effects on the cystometric pattern (Figure 4b). However, naloxone (10 µg, i.t.) immediately reversed the inhibitory effects of morphine (0.5 and 10 µg; $n = 12$) and M6G (0.05 and 0.5 µg; $n = 5$; Figure 3) on each occasion tested. In the presence of naloxone (10 µg, i.t., 10 min before M3G), the frequency of the spontaneous contractile activity induced by M3G (5 µg, i.t.; $n = 4$) was enhanced (Table 2). In three out of seven animals, M3G in the presence of naloxone, caused a pronounced bladder hyperactivity leading to dribbling incontinence (Figure 4b).

Discussion

The present study shows that M6G, and M3G, the two main metabolites of morphine, when given i.t., have significant effects on micturition in normal, unanaesthetized rats. Like morphine, M6G was able to inhibit the micturition reflex, and produce urinary retention and dribbling incontinence in a dose-dependent manner, whereas M3G facilitated the micturition reflex, resulting in decreases in bladder capacity and micturition volume. M3G also induced an increase in spontaneous contractile activity and counteracted the inhibitory

effects of both morphine and M6G on micturition. An inhibitory effect of i.t. morphine on micturition in unanaesthetized rats has been reported previously (Durant & Yaksh 1988; Igawa *et al.*, 1992; Soulard *et al.*, 1992), but to the best of our knowledge the micturition effects of M6G have not been described. The effects of M6G on micturition, like those of morphine, may be mediated via opioid receptors, since the opioid antagonist naloxone reversed its effects. Supporting this view, M6G has been found to bind to μ - and δ -sites with apparent affinities similar to those of morphine (Christensen & Jørgensen, 1987; Pasternak *et al.*, 1987; Frances *et al.*, 1990).

In the present study, the potency of i.t. M6G for inhibition of micturition was approximately 10 times higher than that of i.t. morphine (see Figure 2). With regard to antinociception, a higher potency of M6G than of morphine has been established in different tests including tail-flick, hot-plate, and writhing tests performed in rats and mice (Pasternak *et al.*, 1987; Abbott & Palmour, 1988; Paul *et al.*, 1989; Gong *et al.*, 1991; Frances *et al.*, 1990; 1992). However, the degree of relative antinociceptive potency of M6G is dependent on the pharmacological test chosen and the method of administration. The writhing test is thought to represent the response to noxious visceral stimulation (Schmauss & Yaksh, 1984; Porreca *et al.*, 1987), while both tail-flick and hot-plate tests may reflect the responses to somatosensory cutaneous pain (Groosman *et al.*, 1973; Holmgren *et al.*, 1986). Interestingly, the relative potency of i.t. M6G for inhibition of micturition observed in the present study, is almost the same as that of i.c.v. M6G for visceral antinociception, as judged by writhing test (9 times; Gong *et al.*, 1991) and for depression of ventilation (10 times; Gong *et al.*, 1991). This value is considerably lower than that for somatosensory antinociception as judged by tail-flick and/or hot-plate tests via the i.c.v. route (20–360 times; Pasternak *et al.*, 1987; Paul *et al.*, 1989; Gong *et al.*, 1991; Frances *et al.*, 1992) and via the i.t. route (650 times; Paul *et al.*, 1989). Thus, i.t. M6G may have a more selective effect on somatosensory antinociception than on micturition and ventilation. If this is the case also in man, it may be of clinical interest, since M6G would then be an interesting alternative for i.t. opioid treatment of certain types of pain.

In the rat, distinct μ and δ , but not κ , receptors are known to be involved in central opioid modulation of bladder motility, both at supraspinal (Dray & Metsch, 1984a,b; Dray *et al.*, 1985; Dray & Nunan, 1987; Sheldon *et al.*, 1987) and spinal (Dray & Metsch, 1984c,d; Dray *et al.*, 1985; Dray & Nunan, 1987; Sheldon *et al.*, 1988) sites. However, Sheldon *et al.* (1987; 1988) demonstrated in the rat that the identical inhibitory effects of several μ -receptor agonists on bladder motility could be antagonized differently. This suggested the presence of μ receptor subtypes (μ -isoreceptors) in the rat spinal cord and brain. Such receptors may be involved in the regulation of bladder function, and it may be speculated that the subtype of μ receptor involved in spinal and/or supraspinal modulation of bladder motility is different from that involved in somatosensory antinociception. Supporting such a view, Hucks *et al.* (1992) found that M6G had a 4 to 5 fold lower binding affinity for the μ_2 receptor subtype than morphine. The μ_2 receptor is believed to mediate, e.g., respiratory depression (Ling *et al.*, 1985). In line with this, spinal and/or supraspinal μ_2 receptors may be involved also in the inhibitory action of M6G on micturition.

The present study demonstrated that M3G by itself facilitated the micturition reflex, and functionally tended to antagonize the effects of both morphine and M6G on micturition. After a high dose of i.t. M3G, an excitatory behavioural syndrome was observed. The excitatory effects of M3G, and its antagonistic effect on the opioid-induced inhibition of micturition may not be related to an opioid-mediated mechanism, since M3G has been found not to bind to any type of opioid receptor (Pasternak *et al.*, 1987). This is in line with the view that M3G may induce hyperalgesia,

respiratory stimulation and behavioural excitation by non-opioid mechanisms (Yaksh *et al.*, 1986; Yaksh & Harty, 1988; Pelligrino *et al.*, 1989; Gong *et al.*, 1991). The finding that M3G-induced contractile activity in the bladder was enhanced by naloxone does not contradict this view. Even if naloxone by itself had no stimulatory effects on bladder activity, the drug can be expected to counteract any existing endogenous opioid inhibitory tone, and thus enhance the stimulatory actions of M3G. The excitatory actions of M3G have been postulated to be due to a block of γ -aminobutyric acid (GABA)-mediated inhibition (Robinson & Deadwyler, 1980), or to interference with glycine (Woolf, 1981). We have

found that bicuculline, a GABA_A antagonist given i.t. to normal, unanaesthetized rats, produced an excitatory effect on micturition (Igawa *et al.*, 1993a), which resembled the effect of M3G found in the present study. It may therefore be speculated that M3G facilitates the micturition reflex due to inhibition of central GABA-ergic transmission. Whether or not this is the case is currently under investigation.

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References

- ABBOTT, F.V. & PALMOUR, R.M. (1988). Morphine-6-glucuronide: analgesic effects and receptor binding profile in rats. *Life Sci.*, **43**, 1685–1695.
- BERGGREN, A., RUBENSSON, A. & SILLEN, U. (1992). Involvement of opioid mechanisms in peripheral motor control of detrusor muscle. *Pharmacol. Toxicol.*, **71**, 179–184.
- BOERNER, U., ABBOTT, S. & ROE, R.L. (1975). The metabolism of morphine and heroin in man. *Drug Metab. Rev.*, **4**, 39–73.
- BROMAGE, P.R., CAMPORESI, E.M., DURANT, P.A.C. & NIELSEN, C.H. (1982). Nonrespiratory side effects of epidural morphine. *Anesth. Analg.*, **61**, 490–495.
- CHRISTENSEN, C.B. & JORGENSEN, L.N. (1987). Morphine-6-glucuronide has high affinity for the opioid receptor. *Pharmacol. Toxicol.*, **60**, 75–76.
- DRAY, A. & METSCH, R. (1984a). Morphine and the centrally-mediated inhibition of urinary bladder motility in the rat. *Brain Res.*, **297**, 191–195.
- DRAY, A. & METSCH, R. (1984b). Opioid receptor subtypes involved in the central inhibition of urinary bladder motility. *Eur. J. Pharmacol.*, **104**, 47–53.
- DRAY, A. & METSCH, R. (1984c). Inhibition of urinary bladder contractions by a spinal action of morphine and other opioids. *J. Pharmacol. Exp. Ther.*, **231**, 254–260.
- DRAY, A. & METSCH, R. (1984d). Spinal opioid receptors and inhibition of urinary bladder motility in vivo. *Neurosci. Lett.*, **47**, 81–84.
- DRAY, A. & NUNAN, L. (1987). Supraspinal and spinal mechanisms in morphine-induced inhibition of reflex urinary bladder contractions in the rat. *Neurosci.*, **22**, 281–287.
- DRAY, A., NUNAN, L. & WIRE, W. (1985). Central δ -opioid receptor interactions and the inhibition of reflex urinary bladder contractions in the rat. *Br. J. Pharmacol.*, **85**, 717–726.
- DURANT, P.A.C. & YAKSH, T.L. (1988). Drug effects on urinary bladder tone during spinal morphine-induced inhibition of the micturition reflex in unanesthetized rats. *Anesthesiology*, **68**, 325–334.
- FRANCES, B., GOUT, R., CAMPISTRON, G., PANCONI, E. & CROS, J. (1990). Morphine-6-glucuronide is more μ -selective and potent in analgesic tests than morphine. *Prog. Clin. Biol. Res.*, **328**, 477–480.
- FRANCES, B., GOUT, R., MONSARRAT, B., CROS, J. & ZAJAC, J.-M. (1992). Further evidence that morphine-6 β -glucuronide is a more potent opioid agonist than morphine. *J. Pharmacol. Exp. Ther.*, **262**, 25–31.
- GONG, Q.-L., HEDNER, J., BJÖRKMAN, R. & HEDNER, T. (1992). Morphine-3-glucuronide may functionally antagonize morphine-6-glucuronide induced antinociception and ventilatory depression in the rat. *Pain*, **48**, 249–255.
- GONG, Q.-L., HEDNER, T., HEDNER, J., BJÖRKMAN, R. & NORDBERG, G. (1991). Antinociceptive and ventilatory effects of the morphine metabolites: morphine-6-glucuronide and morphine-3-glucuronide. *Eur. J. Pharmacol.*, **193**, 47–56.
- GROSSMAN, W., JURNA, I., NELL, T. & THERES, C. (1973). The dependence of the anti-nociceptive effect of morphine and other analgesic agents on spinal motor activity after central monoamine depletion. *Eur. J. Pharmacol.*, **24**, 67–77.
- HISAMITSU, T. & DE GROAT, W.C. (1984). The inhibitory effect of opioid peptides and morphine applied intrathecally and intracerebroventricularly on the micturition reflex in the cat. *Brain Res.*, **298**, 51–65.
- HOLMGREN, M., HEDNER, T., MELLSTRAND, G., NORDBERG, G. & HEDNER, TH. (1986). Characterization of the antinociceptive effects of some adenosine analogues in the rat. *Naunyn-Schmied. Arch. Pharmacol.*, **334**, 290–293.
- HUCKS, D., THOMPSON, P.I., MCLOUGHLIN, L., JOEL, S.P., PATEL, N., GROSSMAN, A., REES, L.H. & SLEVIN, M.L. (1992). Explanation at the opioid receptor level for differing toxicity of morphine and morphine 6-glucuronide. *Br. J. Cancer*, **65**, 122–126.
- IGAWA, Y., ANDERSSON, K.-E., POST, C., UVELIUS, B. & MATTIAS-SON, A. (1992). A rat model for investigation of spinal mechanisms in detrusor instability associated with infravesical outflow obstruction. *J. Urol.*, **147**, 349A (abstract no. 546).
- IGAWA, Y., MATTIAS-SON, A. & ANDERSSON, K.-E. (1993a). Effects of GABA-receptor stimulation and blockade on micturition in normal rats and rats with bladder outflow obstruction. *J. Urol.*, (in press).
- IGAWA, Y., PERSSON, K., ANDERSSON, K.-E., UVELIUS, B. & MATTIAS-SON, A. (1993b). Facilitatory effect of vasoactive intestinal polypeptide on spinal and peripheral micturition reflex pathways in conscious rats with and without detrusor instability. *J. Urol.*, **149**, 884–889.
- LABELLA, F.S., PINSKY, C. & HAVILICEK, V. (1979). Morphine derivatives with diminished opiate receptor potency show enhanced central excitatory activity. *Brain Res.*, **174**, 263–271.
- LING, G.S., SPIEGEL, K., LOCKHART, S.H. & PASTERNAK, G.W. (1985). Separation of opioid analgesia from respiratory depression: evidence for different receptor mechanisms. *J. Pharmacol. Exp. Ther.*, **232**, 149–155.
- MALMGREN, A., SJÖGREN, C., UVELIUS, B., MATTIAS-SON, A., ANDERSSON, K.-E. & ANDERSSON, P.O. (1987). Cystometrical evaluation of bladder instability in rats with infravesical outflow obstruction. *J. Urol.*, **137**, 1291–1294.
- OSBORNE, R., THOMPSON, P., JOEL, S., TREW, D., PATEL, N. & SLEVIN, M. (1992). The analgesic activity of morphine-6-glucuronide. *Br. J. Clin. Pharmacol.*, **34**, 130–138.
- PASTERNAK, G.W., BODNAR, R.J., CLARK, J.A. & INTURRISI, C.E. (1987). Morphine-6-glucuronide: a potent μ agonist. *Life Sci.*, **41**, 2845–2849.
- PAUL, D., STANDIFER, K.M., INTURRISI, C.E. & PASTERNAK, G.W. (1989). Pharmacological characterization of morphine-6 β -glucuronide, a very potent morphine metabolite. *J. Pharmacol. Exp. Ther.*, **251**, 477–483.
- PELLIGRINO, D.A., RIEGLER, F.X. & ALBRECHT, R.F. (1989). Ventilatory effects of fourth cerebroventricular infusions of morphine-6- or morphine-3-glucuronide in the awake dog. *Anesthesiology*, **71**, 936–940.
- PORRECA, F., MOSBERG, H.I., OMNAAS, J.R., BURKS, T.F. & COWAN, A. (1987). Supraspinal and spinal potency of selective opioid agonists in the mouse writhing test. *J. Pharmacol. Exp. Ther.*, **240**, 890–894.
- POULAIN, P., RIBON, A.M., HANKS, G.W., HOSKIN, P.J., AHERNE, G.W. & CHAPMAN, D.J. (1990). CSF concentrations of morphine-6-glucuronide after oral administration of morphine. *Pain*, **41**, 115–116.
- RAWAL, N., MÖLLEFORS, K., AXELSSON, K., LINGÅRDH, G. & WIDMAN, B. (1983). An experimental study of urodynamic effects of epidural morphine and of naloxone reversal. *Anesth. Analg.*, **62**, 641–647.

- ROBINSON, J.H. & DEADWYLER, S.A. (1980). Morphine excitation: effects on field potentials recorded in the in vitro hippocampal slice. *Neuropharmacol.*, **19**, 507–514.
- SCHMAUSS, C. & YAKSH, T.L. (1984). In vivo studies on spinal opiate receptor systems mediating antinociception. II. Pharmacological profiles suggesting a differential association of mu, delta and kappa receptors with visceral chemical and cutaneous thermal stimuli in the rat. *J. Pharmacol. Exp. Ther.*, **228**, 1–12.
- SHELDON, R.J., NUNAN, L. & PORRECA, F. (1987). Mu antagonist properties of kappa agonists in a model of rat urinary bladder motility in vivo. *J. Pharmacol. Exp. Ther.*, **243**, 234–240.
- SHELDON, R.J., NUNAN, L. & PORRECA, F. (1988). U50,488H differentially antagonizes the bladder effects of μ agonists at spinal sites. *Eur. J. Pharmacol.*, **146**, 229–235.
- SHIMOMURA, K., KAMATA, O., UEKI, S., IDA, S., OGURI, K., YOSHIMURA, H. & TSUKAMOTO, H. (1971). Analgesic effect of morphine glucuronides. *Tohoku J. Exp. Med.*, **105**, 45–52.
- SMITH, M.T., WATT, J.A. & CRAMOND, T. (1990). Morphine-3-glucuronide – a potent antagonist of morphine analgesia. *Life Sci.*, **47**, 579–585.
- SOULARD, C., PASCAUD, F., ROMAN, F.J., GROUHEL, A. & JUNIEN, J.L. (1992). Pharmacological evaluation of JO 1870: relation to the potential treatment of urinary bladder incontinence. *J. Pharmacol. Exp. Ther.*, **260**, 1152–1158.
- STENSETH, R., SELLEVOLD, O. & BREIVIK, H. (1985). Epidural morphine for postoperative pain: Experience with 1085 patients. *Acta Anaesthesiol. Scand.*, **29**, 148–156.
- SULLIVAN, A.F., MCQUAY, H.J., BAILEY, D. & DICKENSON, A.H. (1989). The spinal antinociceptive actions of morphine metabolites, morphine-6-glucuronide and normorphine, in the rat. *Brain Res.*, **482**, 219–224.
- SÄWE, J., SVENSSON, J.O. & RANE, A. (1983). Morphine metabolism in cancer patients on increasing doses – no evidence for autoinduction or dose-dependence. *Br. J. Clin. Pharmacol.*, **16**, 85–93.
- WOOLF, C.J. (1981). Intrathecal high dose morphine produces hyperalgesia in the rat. *Brain Res.*, **209**, 491–495.
- YAKSH, T.L., HARTY, G.J. & ONOFRIO, B.M. (1986). High doses of spinal morphine produce a nonopiate receptor-mediated hyperesthesia: clinical and theoretic implications. *Anesthesiology*, **64**, 590–597.
- YAKSH, T.L. & HARTY, G.J. (1988). Pharmacology of the allodynia in rats evoked by high dose intrathecal morphine. *J. Pharmacol. Exp. Ther.*, **244**, 501–508.
- YOSHIMURA, H., IDA, S., OGURI, K. & TSUKAMOTO, H. (1973). Biochemical basis for analgesic activity of morphine-6-glucuronide. I. penetration of morphine-6-glucuronide in the brain of rats. *Biochem. Pharmacol.*, **22**, 1423–1430.

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