JPP Journal of Pharmacy and Pharmacology

JPP 2002, 54: 1645–1650

Received April 10, 2002
Accepted September 10, 2002
DOI 10.1211/002235702397
ISSN 0022-3573

# Inhibition of urinary bladder motility by a spinal action of U-50488H in rats

Akinobu Gotoh, Kazuhiro Goto, Atsushi Sengoku, Toshiro Shirakawa, Yoshinobu Akao, Masato Fujisawa, Hiroshi Okada, Soichi Arakawa, Hiroshi Sasaki and Sadao Kamidono

**Abstract**

We examined the effect of a kappa agonist, U-50488H, upon the bladder motility of anaesthetized rats. The frequency of distension-induced rhythmic bladder contractions was reduced by the intravenous (10 mg $kg^{-1}$) or intrathecal (10–100 $\mu$g) administration of U-50488H. The effect of intravenous U-50488H was inhibited by pre-treatment with nor-binaltorphimine (10 mg $kg^{-1}$, s.c.). The inhibition of bladder contractions by intrathecal U-50488H (30 $\mu$g) was eliminated with the concomitant use of nor-binaltorphimine (10 mg $kg^{-1}$, s.c.), and diminished by reserpine (4 mg $kg^{-1}$, i.p.), yohimbine (10 $\mu$g, i.t.) or methysergide (20 $\mu$g, i.t.). The amplitude of bladder contractions induced by an electrical stimulation of the pontine micturition centre was not inhibited by intrathecal U-50488H (30 and 100 $\mu$g). These results suggested that a kappa agonist could inhibit micturition reflex as well as other opioids, and at least part of the inhibition was due to the diminishment of bladder sensation based on the activation of the descending monoaminergic systems through the spinal kappa-opioid receptors.

## Introduction

Opioids are widely used for the relief of acute and chronic pain. However, the disturbances in micturition or urinary retention due to the use of opioids are considerable and persistent, especially in elderly men (Petros et al 1992; Malinovsky et al 1998). Over-distension of the bladder can lead to potential damage and dysfunction of the detrusor muscle. Further, catheterization for urinary retention itself is a factor, which is not only uncomfortable for the patient, but also increases the risk of infection.

Selective kappa agonists are opioids that do not result in psychological dependence (Di Chiara & Imperato 1988; Tsuji et al 2001), and their clinical application as analgesics is expected. Kappa agonists have a well-known diuretic effect (Vonvoigtlander et al 1983; Leander et al 1985; Soulard et al 1996). Meanwhile, previous studies described that urinary bladder motility was inhibited by the activation of spinal and supraspinal mu- or delta-opioid receptors, but was not influenced by the stimulation of kappa-opioid receptors (Dray & Metsch 1984a, b; Dray 1985). Since then, the possibility of modulating bladder function using kappa agonists has not been considered. However, in recent years it was found that the kappa agonist U-50488H, injected into the sacral level of cats, inhibited bladder contractions due to the stimulation to the sacral dorsal root (Abdelmagid & Gajewski 1997). In clinical examinations, nalbuphine, a putative mu antagonist and kappa agonist, administered intravenously, delayed full bladder sensation and an increase in bladder capacity was observed (Malinovsky et al 1998). In addition, dynorphin A facilitates the contraction of the detrusor muscle through peripheral kappa-opioid receptors (Berggren et al 1992). These reports made us even more determined to clarify the effect of kappa agonists upon the micturition reflex.

The opioid-mediated inhibition of bladder motility and antinociception involve common physiological mechanisms (Yaksh 1979; Kuraishi et al 1983). The inhibition of distension-induced rhythmic bladder contractions by intracerebroventricular morphine

Division of Urology, Department of Organs Therapeutics, Faculty of Medicine, Kobe University Graduate School of Medicine, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe, Hyogo 650-0017, Japan

Akinobu Gotoh, Atsushi Sengoku, Toshiro Shirakawa, Yoshinobu Akao, Masato Fujisawa, Hiroshi Okada, Soichi Arakawa, Sadao Kamidono

Kampo & Pharmacognosy Laboratories, Tsumura & Co., 3586 Yoshiwara, Ami-machi, Ibaraki 300-1192, Japan

Kazuhiro Goto, Hiroshi Sasaki

**Correspondence:** A. Gotoh, Division of Urology, Department of Organs Therapeutics, Faculty of Medicine, Kobe University Graduate School of Medicine, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe, Hyogo 650-0017, Japan. E-mail: gotoh@med.kobe-u.ac.jp

was abolished by reserpine, and was reduced by pretreatment with 6-hydroxydopamine or 5,7-dihydroxytryptamine as well as by yohimbine or methysergide (Dray & Nunan 1985, 1987). Taken together, these findings suggested that the activation of the descending monoaminergic systems after the administration of morphine inhibited not only pain sensation but also bladder activity. After stimulating spinal kappa-opioid receptors, the antinociception is closely related to the activation of the descending noradrenergic and serotonergic systems (Nakazawa et al 1991b; Omiya et al 1999). It was pointed out that in the antinociceptive effect of kappa agonists administered systemically, the kappa-opioid receptors in the spinal cord were more important than those located supraspinally (Takemori et al 1988; Nakazawa et al 1991a). Therefore, we examined the effect of intravenous or intrathecal U-50488H upon distension-induced bladder contractions of anaesthetized rats, with the purpose of clarifying whether spinal kappa-opioid receptors change the bladder activity through the descending inhibitory systems.

## Materials and Methods

### Drugs

The following drugs were obtained from the indicated sources: urethane (Sigma Chemical Co., St Louis, MO), α-chloralose (Wako Pure Chemical Industries Ltd, Osaka, Japan), U-50488H (Research Biochemicals International; RBI, Natick, MA), (+)-MK-801 (RBI), nor-binaltorphimine (nor-BNI; RBI), reserpine (Sigma Chemical Co.), yohimbine (RBI) and methysergide (RBI).

### Animal preparation

All animal experiments were performed in accordance with our institutional guidelines after obtaining the permission of the Laboratory Animal Committee. Male Wistar rats (210–290 g), dehydrated for more than 4 h, were anaesthetized with urethane (700 mg $kg^{-1}$, s.c.) and α-chloralose (35 mg $kg^{-1}$, s.c.), and a catheter filled with saline for drug administration was inserted into a femoral vein as required. Through a midline incision of the abdomen, bilateral ureters were cut on the rostral side after being ligatured, and the urethra was double ligatured. PE-50 polyethylene tubing filled with saline was catheterized from a small incision of the apex of the bladder dome, and secured in place by double ligatures around the incision. The intravesicular pressure was measured using a pressure transducer under constant volume and recorded continuously on a chart recorder. The temperature of the rectum of the rats was kept at 37.2–37.6°C during the experiment, using a feedback type of heating mat.

### Measurement of distension-induced bladder contractions

Rats were left to stabilize for at least 30 min before the start of the experiment. The bladder was slowly filled with saline warmed to 37°C through the catheter until the spontaneous bladder contractions appeared. Only the rats that developed bladder contractions at a stable frequency with an amplitude over 20 mmHg were selected when the baseline value of the intravesicular pressure was 8–13 mmHg. After confirmation of the appearance of rhythmic bladder contractions for at least 15 min, drug was administered. As intravenous U-50488H gradually increased the resting intravesicular pressure, only a transient rise in intravesicular pressure, with an amplitude of more than 10 mmHg, was regarded as a spontaneous bladder contraction after the injection of U-50488H. Based on the number of bladder contractions before the administration of drugs, the percentage change in the contraction frequency every 15 min was estimated.

### Measurement of pontine micturition centre stimulation-induced bladder contractions

After positioning the rat in a stereotaxic apparatus, a 1-mm hole was drilled in the cranium dorsal to the pontine micturition centre (Satoh et al 1978; Hida & Shimizu 1982). The coordinates for the placement of a bipolar stimulating electrode (tip diameter 0.2 mm), derived from the atlas of Paxinos & Watson (1986), were as follows: A −9.2 mm, L 1.0 mm, H −7.0 mm. The bladder was partially distended due to the infusion of saline and resting intravesicular pressure was kept at 3–6 mmHg. The dorsolateral pontine tegmentum was electrically (1 ms, 50 Hz, 75–175 μA) stimulated for 10 s every 5 min in the quiescent bladder. After confirmation that the increase in intravesicular pressure due to 3 continual stimuli remained relatively constant, each drug was administered. Based on the mean value of 3 stimuli before the administration of drug, the percentage change in the evoked contraction amplitude was estimated.

### Drug administration

The intravenous injection of U-50488H dissolved in saline was carried out with a volume of 1 mL $kg^{-1}$ into a femoral vein as a bolus and followed by 0.1 mL of saline injection to flush the catheter. For drug administration into the subarachnoid space of the spinal cord, PE-10 polyethylene tubing with an inner volume of 5 μL filled with drug solution was inserted from a slit in the dura mater of the intervertebral foramen between L1 and L2, and passed cranially to the L5 or L6 spine level (Nadeson et al 1996). The intrathecal administration of U-50488H dissolved in 1–10 μL of saline was made at a rate of 2–3 μL $min^{-1}$, and was not followed by a subsequent injection of saline (Dray & Metsch 1984a; Dray 1985). U-50488H was given to some rats from a catheter, the end of which was positioned at segment T9. (+)-MK-801 was dissolved in 10 μL of saline and injected into the level of L5–6. Nor-BNI and reserpine were given 2–6 and 16–24 h before the experiment, respectively. Yohimbine was dissolved in 5 μL of saline and administered into the intrathecal space at the L5–6 level just before the intrathecal injection of 30 μg/5 μL of U-50488H. Due to the limit of dissolution, methysergide was mixed and injected with 30 μg/10 μL of U-50488H.

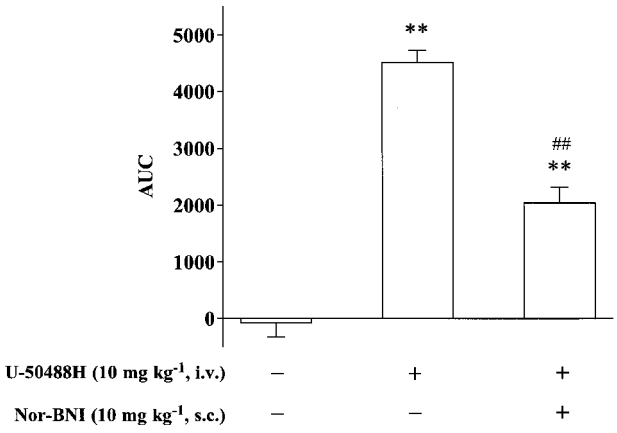


**Figure 1** Inhibitory effect of intravenous U-50488H on the frequency of rhythmic bladder contractions in rats. The percentage change in the contraction frequency was estimated every 15 min after intravenous administration of U-50488H for 60 min, and the inhibitory activity of U-50488H was expressed as the area under the time–response curve (AUC). Each column and vertical bar represents the mean ± s.e.m. of results from 6 rats. $^{**}P < 0.01$ compared with the saline-treated group (Tukey's test); $\#\#P < 0.01$ compared with results from rats treated with U-50488H alone (Tukey's test).

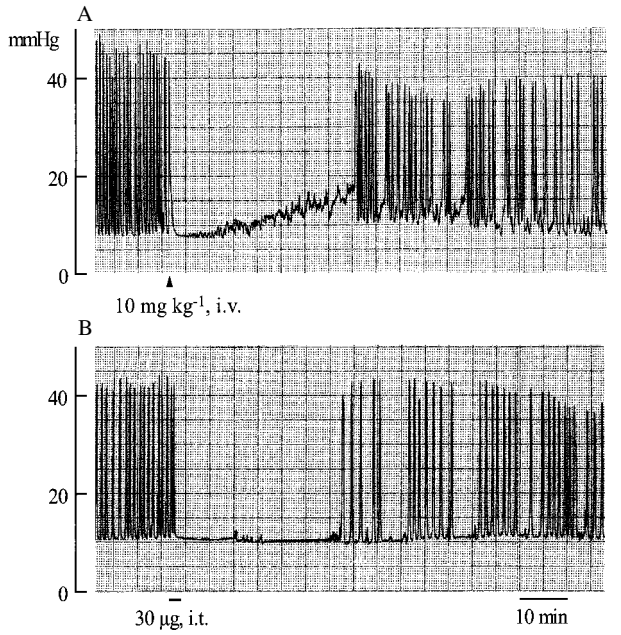


**Figure 2** Representative tracing showing suppression of rhythmic bladder contractions in rats by U-50488H under constant volume. U-50488H was administered intravenously (A) and intrathecally (B) (arrowhead and bar, respectively). Note the irregular agitation and gradual increase in intravesicular pressure after intravenous, but not intrathecal, injection of U-50488H.

The potential distribution of drug within the spinal cord was assessed by the intrathecal injection of Evans blue dye (60 $\mu$g) made in the same manner as the drug administrations. Ten minutes after the injection of 1 or 10 $\mu$L of dye solution, rats were killed by decapitation. The vertebral column was removed from the body and cut between L1 and L2, and the caudal side was partially frozen in dry ice. The stiffened column was then cut into 0.5-cm segments and the cord inside each segment removed by inserting an appropriately sized plunger (Yaksh & Rudy 1976). The dye in each sample was extracted by the method of Katayama et al (1978), and the content was calculated based on the absorbance of the supernatant measured at 620 nm.

### Statistical analysis

The inhibitory activity of drugs on the bladder contractions was expressed as the area under the time–response curve (AUC) calculated by plotting the increase in inhibitory rate (%) on the ordinate and time interval (min) on the abscissa. The results are expressed as the mean ± s.e.m. The significance of differences was determined using a one-way analysis of variance followed by Tukey's test. For all cases, differences of $P < 0.05$ were considered significant.

## Results

### Effect of intravenous U-50488H on distension-induced bladder contractions

When the resting intravesicular pressure was adjusted to 8–13 mmHg by infusion of an appropriate amount of saline, spontaneous bladder contractions were observed from 11 to 39 times per 15 min (20.5 ± 0.6, n = 112). There was no significant difference in the frequency of bladder contractions between the groups in the 15 min before the administration of drugs (data not shown).

The bladder motility was not affected by the saline (1 mL kg$^{-1}$, i.v.), but it was suppressed by U-50488H (10 mg kg$^{-1}$, i.v.) (Figure 1). The effect of U-50488H was attenuated by pre-treatment with nor-BNI (10 mg kg$^{-1}$, s.c.), a selective kappa antagonist. The cessation of bladder contractions after the intravenous administration of U-50488H always accompanied irregular agitation and a gradual rise of intravesicular pressure (Figure 2A). Nor-BNI also tended to inhibit the increase in resting intravesicular pressure after the intravenous injection of U-50488H.

### Effects of intrathecal U-50488H on distension-induced bladder contractions

U-50488H (10–100 $\mu$g) in a volume of 10 $\mu$L, which was injected into the level of L5–6, dose-dependently suppressed the rhythmic bladder contractions (Figure 3). The bladder-contraction responses of the rats to which U-50488H (10 and 30 $\mu$g, i.t.) was administered recovered within 20 min and 42 min, respectively. However, 4 out of 7 rats in which U-50488H (100 $\mu$g, i.t.) was injected did not show a re-appearance of bladder contractions during the observation period for 120 min after the administration. U-50488H (30 $\mu$g) administered to segment T9 had a similar inhibitory

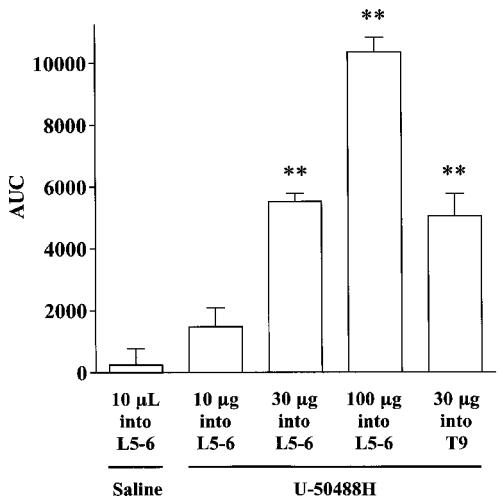


**Figure 3** Inhibitory effect of intrathecal U-50488H on the frequency of distension-induced bladder contractions in rats. The inhibitory activity was measured every 15 min after intrathecal injection of U-50488H for 120 min, and expressed as the area under the time–response curve (AUC). Each column and vertical bar represents the mean±s.e.m. of results from 6 or 7 rats. $^{**}P < 0.01$ compared with the saline-treated group (Tukey's test).

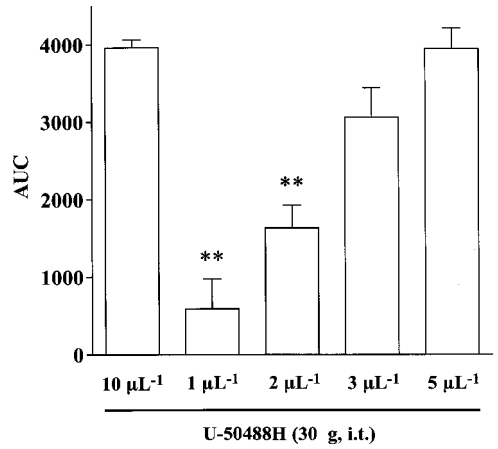


**Figure 4** Effect of injection volume on the inhibition of rhythmic bladder contractions in rats by intrathecal U-50488H. The percentage change in the contraction frequency was estimated every 15 min after intrathecal injection of U-50488H for 60 min, and the inhibitory activity of U-50488H was expressed as the area under the time–response curve (AUC). Each column and vertical bar represents the mean±s.e.m. of results from 6 or 7 rats. $^{**}P < 0.01$ compared with the group treated with 10 $\mu$L injection volume (containing 30 $\mu$g U-50488H) (Tukey's test).

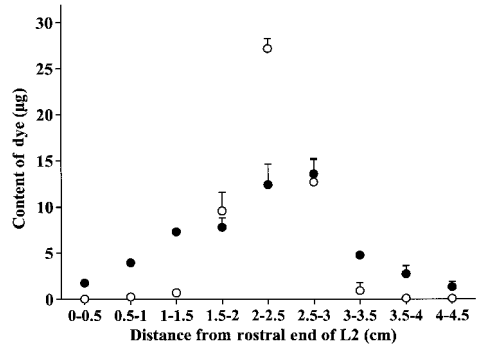


**Figure 5** Distribution of dye recovered in the spinal cord segments (0.5-cm lengths) of rats after intrathecal injection of Evans blue dye. ○, 60 $\mu$g/1 $\mu$L; ●, 60 $\mu$g/10 $\mu$L. Each point and vertical bar represents the mean±s.e.m. of results from 4 rats.

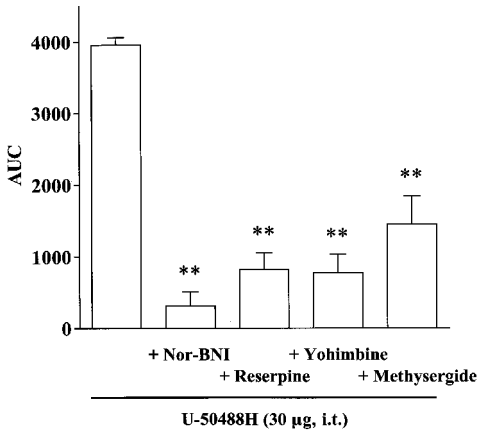


**Figure 6** Effect of antagonists on the inhibition of distension-induced bladder contractions in rats by U-50488H. Nor-binaltorphimine (Nor-BNI; 10 mg kg$^{-1}$, s.c.) and reserpine (4 mg kg$^{-1}$, i.p.) were given 2–6 and 16–24 h before the experiment, respectively. Yohimbine (10 $\mu$g, i.t.) was administered just before the injection of U-50488H. Methysergide (20 $\mu$g, i.t.) was mixed and injected with U-50488H. The inhibitory activity was measured every 15 min after intrathecal administration of U-50488H for 60 min, and expressed as the area under the time–response curve (AUC). Each column and vertical bar represents the mean±s.e.m. of results from 7 rats. $^{**}P < 0.01$ compared with rats treated with U-50488H alone (Tukey's test).

effect on bladder motility (Figure 3). In contrast with intravenous U-50488H, intrathecal U-50488H did not have an effect upon resting intravesicular pressure (Figure 2B).

There was no difference in the inhibition of distension-induced bladder contractions after the intrathecal injection of U-50488H (30 $\mu$g) dissolved in 5 or 10 $\mu$L of saline. However, the activity of less than 3 $\mu$L of U-50488H was reduced until the volume decreased even though the dose was the same (Figure 4). The distribution of dye after the intrathecal administration of Evans blue dye into the L5–6 level is shown in Figure 5. The recovery rate of dye from the spinal cord was 89–94%. After injecting 10 $\mu$L of dye solution, the presence of dye was noted in a broad area from L2 to 4.5 cm towards the caudal side. However, 1 $\mu$L of dye only spread within a distance of approximately 1.5 cm.

The inhibitory effect of intrathecal U-50488H (30 $\mu$g) on bladder contractions was abolished by nor-BNI (10 mg kg$^{-1}$, s.c.), and diminished with the concomitant use of reserpine (4 mg kg$^{-1}$, i.p.), a noradrenaline (norepine-

phrine) $\alpha_2$-antagonist yohimbine (10 $\mu$g, i.t.) or a serotonin antagonist methysergide (20 $\mu$g, i.t.) (Figure 6). In another experiment, it was confirmed that yohimbine or methysergide did not affect the frequency of spontaneous bladder contractions, which were observed for 90 min (data not shown).

### Effect of intrathecal U-50488H on pontine micturition centre stimulation-induced bladder contractions

Electrical stimulation of the dorsolateral pontine tegmentum produced an increase in intravesicular pressure from 19 to 40 mmHg (28.2±1.1 mmHg, n = 22). The amplitude of evoked bladder contractions was gradually decreased by an *N*-methyl-D-aspartate (NMDA) antagonist (+)-MK-801 (16 $\mu$g, i.t.), although it was not influenced by U-50488H (30 and 100 $\mu$g, i.t.) during the observation period for 90 min after administration. AUC value of the rats treated with saline (10 $\mu$L, i.t.), U-50488H (30 $\mu$g), U-50488H (100 $\mu$g) and (+)-MK-801 was −86±687 (n = 5), 93±452 (n = 5; $P > 0.05$ vs saline-treated group), 123±770 (n = 6; $P > 0.05$) and 8495±1244 (n = 6; $P < 0.01$), respectively.

## Discussion

The present study using U-50488H indicated the feasibility of inhibiting the micturition reflex by stimulating kappa-opioid receptors. Berggren et al (1992) suggested that substance P was involved in the facilitation of bladder contraction due to the activation of peripheral kappa-opioid receptors. Neuropeptides such as substance P released from capsaicin-sensitive primary sensory nerve endings in the bladder wall produce various biological effects, including contraction of smooth muscle, potentiation of efferent neurotransmission and activation of micturition reflex (Maggi & Meli 1986; Maggi 1991; Ishizuka et al 1994). Thus, the irregular agitation and gradual rise in resting intravesicular pressure induced by intravenous, but not intrathecal, U-50488H may be due to the release of substance P after the activation of peripheral kappa-opioid receptors (Berggren et al 1992). If the resting intravesicular pressure had not risen, the inhibitory effect of intravenous U-50488H on the rhythmic bladder contractions may have been even stronger.

In this study, we have obtained evidence that spinal kappa-opioid receptors play an important role in the suppression of bladder motility by systemic U-50488H. In addition, we demonstrated that the effect of intrathecal U-50488H depends on not only the drug dose but also the volume; more than 5 $\mu$L is required to produce full activity. The fact that there was no difference between the activity of U-50488H administered into lumbo-sacral and thoracic regions suggested that all spinal kappa-opioid receptors could suppress the bladder activity. It is likely that the effect of U-50488H on bladder motility is produced indirectly due to activation of the descending monoaminergic systems (see below). The activation of the inhibitory mechanisms may require stimulation in not only a local, but also a broad area, in other words, an ample number of kappa-opioid receptors in the spinal cord. Our experiment that injected dye into the intrathecal space revealed that the spread of compound was in proportion to the volume of the solution. A similar result was shown in the study of Yaksh & Rudy (1976). Dray (1985) and Sheldon et al (1988), who reported that U-50488H administered into the lumbo-sacral regions using a hand-held syringe did not cause loss of bladder activity, selected an injection volume of 1 $\mu$L and 1–2 $\mu$L, respectively. The small volume of intrathecal U-50488H in previous studies may have caused an apparent difference in the spinal effect of U-50488H compared with our study.

The activation of descending monoaminergic systems accompanies the suppression of bladder motility (Dray & Nunan 1985, 1987). The results that the activity of U-50488H was decreased by reserpine, yohimbine or methysergide, at similar doses which prevented the inhibitory effect of morphine on the micturition reflex (Dray & Nunan 1985, 1987), support our hypothesis that both noradrenergic and serotonergic mechanisms play an important role in the inhibitory effect of U-50488H on bladder activity. Abdelmagid & Gajewski (1997) suggested that the effect of intrathecal U-50488H on the sacral dorsal root stimulation-induced bladder contractions may be the result of diffusion to the supraspinal level, because the inhibition of bladder contractions after the administration of U-50488H was not observed in chronic spinally transected cats. However, their results may be explained by the disconnection of afferent input required for the activation of descending inhibitory systems due to the spinal transection between spinal kappa-opioid receptors and the supraspinal central nervous system and the disappearance of the descending monoaminergic systems.

Our finding that the pontine micturition centre stimulation-induced bladder contractions were inhibited by intrathecal (+)-MK-801 is consistent with the findings of a previous study (Matsumoto et al 1995). Based on the result that intrathecal U-50488H did not affect the amplitude of evoked bladder contractions, the possibility that the spinal effect of U-50488H may be involved in the efferent pathway of micturition reflex was excluded. The primary afferent fibres from the urinary bladder use various neuropeptides (substance P, somatostatin, vasoactive intestinal polypeptide, etc.) as sensory neurotransmitters or neuromodulators in the dorsal horn (Maggi & Meli 1986; Maggi 1991; Kawatani et al 1993; Igawa et al 1993). The activated descending inhibitory systems may have made the bladder sensation diminish by inhibiting the release of these neuropeptides through noradrenaline $\alpha_2$- and methysergide-sensitive serotonin receptors as well as analgesia-producing mechanisms (Kuraishi et al 1985, 1991). It was considered that the inhibitory effect of the kappa agonist on the spinal sensory neurotransmission explains why nalbuphine administered to patients induces a delayed full bladder sensation and an increase in bladder capacity (Malinovsky et al 1998).

## Conclusions

Our findings indicate that the stimulated kappa-opioid receptors in the spinal cord can inhibit bladder motility via the activation of descending monoaminergic systems. In addition, systemic U-50488H induces hyperactivity of the detrusor muscle, which may lead to the symptoms of urge. When using kappa agonists as analgesics clinically, it may be necessary to consider the changes in micturition as well as other opioids.

## References


Abdelmagid, M. E., Gajewski, J. B. (1997) Modulation of feline bladder and distal urethral responses to dorsal sacral root stimulation by intrathecal administration of a kappa$_1$-opioid agonist. *Urology* **49**: 802–807

Berggren, A., Dahlström, A., Rubenson, A., Sillén, U. (1992) Possible regulatory role of dynorphin A in the urinary bladder. *J. Neural. Transm.* **90**: 33–44

Di Chiara, G., Imperato, A. (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc. Natl Acad. Sci. USA* **85**: 5274–5278

Dray, A. (1985) The rat urinary bladder: a novel preparation for the investigation of central opioid activity in vivo. *J. Pharmacol. Methods* **13**: 157–165

Dray, A., Metsch, R. (1984a) 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. (1984b) Opioid receptor subtypes involved in the central inhibition of urinary bladder motility. *Eur. J. Pharmacol.* **104**: 47–53

Dray, A., Nunan, L. (1985) Opioid inhibition of reflex urinary bladder contractions: dissociation of supraspinal and spinal mechanisms. *Brain Res.* **337**: 142–145

Dray, A., Nunan, L. (1987) Supraspinal and spinal mechanisms in morphine-induced inhibition of reflex urinary bladder contractions in the rat. *Neuroscience* **22**: 281–287

Hida, T., Shimizu, N. (1982) The interrelation between the laterodorsal tegmental area and lumbosacral segments of rats as studied by HRP method. *Arch. Histol. Jap.* **45**: 495–504

Igawa, Y., Persson, K., Andersson, K.-E., Uvelius, B., Mattiasson, A. (1993) Facilitatory effect of vasoactive intestinal polypeptide on spinal and peripheral micturition reflex pathway in conscious rats with and without detrusor instability. *J. Urol.* **149**: 884–889

Ishizuka, O., Igawa, Y., Mattiasson, A., Andersson, K.-E. (1994) Capsaicin-induced bladder hyperactivity in normal conscious rats. *J. Urol.* **152**: 525–530

Katayama, S., Shionoya, H., Ohtake, S. (1978) A new method for extraction of extravasated dye in the skin and the influence of fasting stress on passive cutaneous anaphylaxis in guinea pigs and rats. *Microbiol. Immunol.* **22**: 89–101

Kawatani, M., Matsumoto, G., Birder, L. A., Fukuoka, H., Yoshiyama, M., Misaki, A., Suzuki, S. (1993) Intrathecal administration of NK1 receptor antagonist, CP96345, inhibits the micturition reflex in the rat. *Regul. Pept.* **46**: 392–395

Kuraishi, Y., Harada, Y., Aratani, S., Satoh, M., Takagi, H. (1983) Separate involvement of the spinal noradrenergic and serotonergic systems in morphine analgesia: the differences in mechanical and thermal algesic tests. *Brain Res.* **273**: 245–252

Kuraishi, Y., Hirota, N., Sato, Y., Kaneko, S., Satoh, M., Takagi, H. (1985) Noradrenergic inhibition of the release of substance P from the primary afferents in the rabbit spinal dorsal horn. *Brain Res.* **359**: 177–182

Kuraishi, Y., Minami, M., Satoh, M. (1991) Serotonin, but neither noradrenaline nor GABA, inhibits capsaicin-evoked release of immunoreactive somatostatin from slices of rat spinal cord. *Neurosci. Res.* **9**: 238–245

Leander, J. D., Zerbe, R. L., Hart, J. C. (1985) Diuresis and suppression of vasopressin by *kappa* opioids: comparison with *mu* and *delta* opioids and clonidine. *J. Pharmacol. Exp. Ther.* **234**: 463–469

Maggi, C. A. (1991) The role of peptides in the regulation of the micturition reflex: an update. *Gen. Pharmacol.* **22**: 1–24

Maggi, C. A., Meli, A. (1986) The role of neuropeptides in the regulation of the micturition reflex. *J. Auton. Pharmacol.* **6**: 133–162

Malinovsky, J.-M., Le Normand, L., Lepage, J.-Y., Malinge, M., Cozian, A., Pinaud, M., Buzelin, J.-M. (1998) The urodynamic effects of intravenous opioids and ketoprofen in humans. *Anesth. Analg.* **87**: 456–461

Matsumoto, G., Hisamitsu, T., de Groat, W. C. (1995) Role of glutamate and NMDA receptors in the descending limb of the spinobulbospinal micturition reflex pathway of the rat. *Neurosci. Lett.* **183**: 58–61

Nadeson, R., Guo, Z., Porter, V., Gent, J. P., Goodchild, C. S. (1996) $\gamma$-Aminobutyric acid$_A$ receptors and spinally mediated antinociception in rats. *J. Pharmacol. Exp. Ther.* **278**: 620–626

Nakazawa, T., Furuya, Y., Kaneko, T., Yamatsu, K. (1991a) Spinal *kappa* receptor-mediated analgesia of E-2078, a systemically active dynorphin analog, in mice. *J. Pharmacol. Exp. Ther.* **256**: 76–81

Nakazawa, T., Yamanishi, Y., Kaneko, T. (1991b) A comparative study of monoaminergic involvement in the antinociceptive action of E-2078, morphine and U-50,488E. *J. Pharmacol. Exp. Ther.* **257**: 748–753

Omiya, Y., Goto, K., Suzuki, Y., Ishige, A., Komatsu, Y. (1999) Analgesia-producing mechanism of processed *Aconiti* tuber: role of dynorphin, an endogenous $\kappa$-opioid ligand, in the rodent spinal cord. *Jpn. J. Pharmacol.* **79**: 295–301

Paxinos, G., Watson, C. (1986) *The Rat Brain in Stereotaxic Coordinates*, 2nd edn. Academic Press, San Diego

Petros, J. G., Rimm, E. B., Robillard, R. J. (1992) Factors influencing urinary tract retention after elective open cholecystectomy. *Surgery* **174**: 497–500

Satoh, K., Shimizu, N., Tohyama, M., Maeda, T. (1978) Localization of the micturition reflex center at dorsolateral pontine tegmentum of the rat. *Neurosci. Lett.* **8**: 27–33

Sheldon, R. J., Nunan, L., Porreca, F. (1988) U50,488H differentially antagonizes the bladder effects of $\mu$ agonists at spinal sites. *Eur. J. Pharmacol.* **146**: 229–235

Soulard, C. D., Guerif, S., Payne, A., Dahl, S. G. (1996) Differential effects of fedotozine compared to other $\kappa$ agonists on diuresis in rats. *J. Pharmacol. Exp. Ther.* **279**: 1379–1385

Takemori, A. E., Ho, B. Y., Naeseth, J. S., Portoghese, P. S. (1988) Nor-binaltorphimine, a highly selective *kappa*-opioid antagonist in analgesic and receptor binding assays. *J. Pharmacol. Exp. Ther.* **246**: 255–258

Tsuji, M., Takeda, H., Matsumiya, T., Nagase, H., Narita, M., Suzuki, T. (2001) The novel kappa-opioid receptor agonist TRK-820 suppresses the rewarding and locomotor-enhancing effects of morphine in mice. *Life Sci.* **68**: 1717–1725

Vonvoigtlander, P. F., Lahti, R. A., Ludens, J. H. (1983) U-50,488: a selective and structurally novel non-*mu* (*kappa*) opioid agonist. *J. Pharmacol. Exp. Ther.* **224**: 7–12

Yaksh, T. L. (1979) Direct evidence that spinal serotonin and noradrenaline terminals mediate the spinal antinociceptive effects of morphine in the periaqueductal gray. *Brain Res.* **160**: 180–185

Yaksh, T. L., Rudy, T. A. (1976) Chronic catheterization of the spinal subarachnoid space. *Physiol. Behav.* **17**: 1031–1036