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ORIGINAL ARTICLE

Comparison of the diuretic effects of chemically diverse kappa opioid agonists in rats: nalfurafine, U50,488H, and salvinorin A

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Abstract Kappa opioid receptor agonists induce water diuresis in animals and humans. We investigated the effects of s.c. nalfurafine, U50,488H, salvinorin A, and its longer-acting analog, 2-methoxymethyl-salvinorin B (MOM-sal B), on urinary output and sodium excretion over 5 h in euvolemic rats. Nalfurafine (0.005–0.02 mg/kg), U50,488H (0.1–10 mg/kg), and MOM-sal B (0.625–5 mg/kg) induced diuresis dose-dependently. Systemically (0.1–10 mg/kg) or centrally (50 μg, i.c.v.) administered salvinorin A was ineffective. 5′-Guanidinonaltrindole, a kappa receptor antagonist, inhibited nalfurafine- and MOM-sal B-induced diuresis. Nalfurafine and MOM-sal B had no effect on arginine vasopressin levels, measured at 2 h. Tolerance did not develop to the diuresis accompanying subchronic administration of nalfurafine (0.02 mg/kg). On the basis of our work, we (a) promote nalfurafine as a candidate diuretic to relieve water retention and (b) highlight salvinorin A as a kappa agonist that does not cause diuresis, probably because of its short duration of action.

Keywords Nalfurafine · U50,488H · Salvinorin A · 2-Methoxymethyl-salvinorin B · GNTI · Diuresis

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Introduction

Diuresis is a well-recognized effect of traditional kappa opioid receptor agonists, for example, in mice (Rathbun et al. 1983), rats (VonVoigtlander et al. 1983; Craft et al. 2000; Gottlieb et al. 2005), dogs (Brooks et al. 1997), monkeys (Ko et al. 2003), and humans (Bellissant et al. 1996; Gadano et al. 2000; Kramer et al. 2000). This diuresis is characterized by an increase in urine flow as well as a decrease in urine osmolality without an associated increase in Na^+ excretion (VonVoigtlander et al. 1983; Ashton et al. 1989; Yamada et al. 1989; Gadano et al. 2000; Gottlieb et al. 2005). Niravoline and (the peripherally restricted) asimadoline are examples of arylacetamide-type kappa agonists tested in humans (Bellissant et al. 1996; Kramer et al. 2000). Niravoline caused water diuresis and was effective in moderate doses to relieve water retention in cirrhotic individuals. However, high doses induced personality disorders and mild confusion in the patients (Gadano et al. 2000). Asimadoline also caused diuresis in healthy volunteers; headache and tiredness were reported as side effects only when it was given together with hypertonic saline infusion (Kramer et al. 2000).

The renin–angiotensin–aldosterone axis and vasopressin (AVP) are two of the important systems involved in regulating serum and urine electrolytes and osmolality. Angiotensin II stimulates aldosterone release from the adrenal cortex, and aldosterone stimulates sodium reabsorption through Na^+–H^+ and Na^+–K^+ pumps. AVP is a polypeptide hormone released from the posterior pituitary gland in response to changes in blood pressure and plasma osmolality. It regulates the permeability of the distal tubule and collecting ducts and affects urine osmolality. Kappa opioid agonist-induced suppression of AVP release was

established in several previous animal studies (Yamada et al. 1989; Craft et al. 2000).

In the present work, we investigated, for the first time, urine output and renal excretory response in conscious and euvolemic rats after administration of two non-arylacetamide kappa opioid receptor agonists, nalfurafine (Endoh et al. 1999; Togashi et al. 2002; Inan and Cowan 2004) and salvinorin A (Roth et al. 2002; Sheffler and Roth 2003; Wang et al. 2005), as well as 2-methoxymethyl-salvinorin B (MOM-sal B), a C-2 modified longer-acting analog of salvinorin A (Wang et al. 2008; Béguin et al. 2008). U50,488H (VonVoigtlander et al. 1983; Ko et al. 2003) served as the reference, arylacetamide kappa agonist.

Methods and materials

Animals

The study was conducted on 262 male, Sprague–Dawley rats (175–200 g; n=6–10; Ace Laboratories, Boyertown, PA, USA). The rats were housed three per cage with free access to food and water in the Animal Facility for at least 4 days. A standard light/dark cycle was maintained with a timer-regulated light period from 7:00 A.M. to 7:00 P.M. Experiments were carried out between 10:00 A.M and 5:00 P.M. Experimental procedures were approved by the Temple University Institutional Animal Care and Use Committee.

On the experimental day, animals were brought to the laboratory and acclimated for at least 1 h with free access to food and water before injections. At the end of the experiments, animals were euthanized using carbon dioxide gas.

Diuresis study

Rats were given (s.c.) saline, nalfurafine (0.005–0.06 mg/kg), U50,488H (0.1–10 mg/kg), salvinorin A (0.1–10 mg/kg), MOM-sal B (0.625–5 mg/kg), or vehicle. The animals were then placed into individual metabolism cages with no access to food and water for urine collection over 5 h. Urine was collected in graduated cylinders, and total urine volume was recorded. Urine osmolality and Na^+ levels were measured. To follow the time course of nalfurafine-induced diuresis and compare with saline, U50,488H, and MOM-sal B, rats were given saline, nalfurafine (0.02 mg/kg), U50,488H (10 mg/kg), or MOM-sal B (2.5 mg/kg), placed in observation cages, and urine output noted every 30 min for 5 h.

Other groups of rats were injected with saline, nalfurafine (0.02 mg/kg), U50,488H (10 mg/kg, s.c.), or MOM-sal B (2.5 mg/kg) to measure AVP levels at 2 h. After the injections, rats were placed into metabolism cages, and 2 h later, heart blood was drawn with the animals anesthetized with isofluorane.

In order to establish if centrally administered salvinorin A induces diuresis, rats were anesthetized with ketamine (80 mg/kg, i.p.) in combination with xylazine (8 mg/kg, i.p.), and the right lateral cerebroventricle was cannulated. Cannulas, made from PE 10 tubing, were implanted (incision bar at 0 position, anterior-posterior 2 mm, lateral 2 mm, and vertical 2 mm from bregma) using stereotaxic equipment and fixed with dental cement. Either 3 or 4 days after surgery, vehicle or a fixed dose of salvinorin A (50 μg/5 μl) was infused over 30 s through the cannula into the lateral ventricle. The animals were then placed into individual metabolism cages for 5 h for cumulative urine collection. To select the dose of salvinorin A, we were guided by Ansonoff et al. (2006) who used 1–30 μg in mice. If we obtained diuresis with a high dose, we would develop a dose–response curve. At the end of the experiment, methylene blue (5 μl) was injected into the lateral cerebroventricle, the rat was euthanized, the brain removed, and the cannula placement confirmed.

Subchronic administration of nalfurafine

We conducted a multiple dose study with nalfurafine (0.02 mg/kg, s.c.) in rats to examine, first, whether tolerance develops to the diuretic effect and, second, to learn how serum and urine Na^+ levels may be affected. We injected nalfurafine, U50,488H (10 mg/kg, s.c.), or saline once a day for seven consecutive days. Total cumulative urine output in 5 h was recorded on both days 1 and 7. Urine samples from day 7 were also used to measure urine osmolality and Na^+ and K^+ levels. Also, at the end of urine collection on day 7, heart blood was drawn with animals under isofluorane anesthesia. Serum was obtained to measure Na^+ levels.

Experiments with 5′-guanidinonaltrindole, a kappa receptor antagonist

To potentially antagonize diuresis elicited by kappa opioid agonists, rats were pretreated s.c. with either saline or a fixed dose of 5′-guanidinonaltrindole (GNTI; 0.1 mg/kg) 30 min before the injection of either saline or a maximally effective dose of kappa agonist. Also, a dose–response curve for increasing doses of GNTI (0.1–3 mg/kg) against a maximally effective dose of nalfurafine was developed in rats. In order to establish if nalfurafine-induced diuresis is antagonized by centrally administered GNTI, the right lateral cerebroventricle of each animal was cannulated as mentioned previously. Either 3 or 4 days after surgery, saline or GNTI (0.005–25 μg/5 μl) was infused over 30 s through the cannula into the lateral ventricle. Nalfurafine (0.02 mg/kg) or saline was injected s.c. 15 min later, and rats were

placed in metabolism cages for 5 h. Total cumulative urine volume was then recorded. At the end of the experiment, the cannula placement was again confirmed.

Measurement of adenylyl cyclase activity in hypothalamus of rats after subchronic injection of nalfurafine

Activation of kappa opioid receptors by an agonist inhibits adenylyl cyclase activity (Yasuda et al. 1993; Gharagozlou et al. 2006). Microinjection of U50,488H to the paraventricular area of the hypothalamus induces diuresis in rats (Gottlieb et al. 2005). To determine whether the hypothalamus is involved in nalfurafine-induced diuresis in rats, we measured adenylyl cyclase activity in this species. Rats, administered saline or nalfurafine (s.c., once a day for 7 days), were exposed to carbon dioxide gas for 15 s and subsequently killed by decapitation. Their brains were rapidly removed, and the hypothalamus was dissected on ice to measure adenylyl cyclase activity in crude membranes. These membranes were prepared by homogenizing the tissue in 5 ml of 20 mM Tris–HCl (pH 7.4), 2 mM ethylene glycol bis(2-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 1 mM $MgCl_2$, and 250 mM sucrose followed by centrifugation at 30,000×*g* for 15 min at 4°C. The pellets were resuspended in 5 ml of buffer and centrifuged again for 15 min. The supernatants were discarded and the pellets homogenized in 40 volumes of ice-cold buffer containing 2 mM Tris–HCl (pH 7.4) and 2 mM EGTA and stored at −80°C. Adenylyl cyclase activity was measured as described previously (Unterwald et al. 1993). Aliquots of tissue homogenate (10 μl) were incubated in 80 mM Tris–HCl (pH 7.4), 10 mM theophylline, 1 mM $MgSO_4$, 0.8 mM EGTA, 30 mM NaCl, 0.25 mM adenosine triphosphate (ATP), and 0.01 mM guanosine triphosphate (GTP) in triplicate for 5 min at 30°C. Adenylyl cyclase activity was terminated by placing the tubes into boiling water for 2 min. The amount of adenosine 3′,5′-cyclic phosphate (cAMP) formed was determined by a [^{3}H]cAMP protein binding assay (Brown et al. 1971). [^{3}H]cAMP (final concentration 4 nM) in citrate phosphate buffer (pH 4.0), followed by binding protein prepared from bovine adrenal glands, were added to each sample. Additional samples were prepared, without tissue, containing known amounts of cAMP and served as standards for quantification. The binding reaction was allowed to reach equilibrium by incubation for 90 min at 4°C. The assay was terminated by the addition of charcoal and centrifugation to separate free cAMP from that bound to the binding protein. Aliquots of the supernatants were assayed for radioactivity by liquid scintillation spectrometry using CytoScint Scintillation Fluid (ICN Biomedicals, CA, USA). Radioactivity was converted to picomole of cAMP by comparison with the curve derived from standards.

Measurements

Osmolality was measured by osmometric depression of the freezing point method (Advanced Instruments Osmometer 3300, MA, USA). Serum and urine Na^+, as well as urine K^+ levels, were measured using the connectivity method (Beckman LX20, Fullerton, CA, USA) in the clinical laboratory of Temple University Hospital, and urinary sodium excretion ($U_{Na}V$) and urinary potassium excretion (U_KV) were calculated. Serum AVP and angiotensin II levels were measured in duplicated samples using enzyme-linked immunosorbent assay kits described by the manufacturer (Phoenix Pharmaceuticals, CA, USA).

Compounds

U50,488H [(*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-benzene-acetamide)methane sulfonate] (Sigma, St. Louis, MO, USA), nalfurafine (a generous gift from Adolor Company, Exton, PA, USA), and 5′-guanidinonaltrindole (Tocris, Ellisville, MI, USA) were dissolved in saline. Salvinorin A and MOM-sal B were provided by Dr. D.Y.-W. Lee and dissolved in ethanol/Tween 80/water vehicle (1:1:8 proportion by volume).

[^{3}H]cAMP (adenosine 3′,5′-cyclic phosphate, ammonium salt; specific activity 31.0 Ci/mmol) was obtained from PerkinElmer Life Sciences (Waltham, CA, USA). ATP, GTP, cAMP, theophylline, and EGTA were purchased from Sigma.

Statistical analysis

Data are expressed as mean±standard deviation (SD) from mean and considered statistically significant at a p value of 0.05 or less. A one-way analysis of variance (ANOVA), followed by the Newman–Keuls test, was used to compare groups. The unpaired Student's t test was used to compare two groups.

Results

Kappa agonist-induced diuresis in rats

Nalfurafine (0.005–0.06 mg/kg), U50,488H (0.1–10 mg/kg), and MOM-sal B (0.625–5 mg/kg) increased urine output in rats. Salvinorin A given either systemically (0.1–10 mg/kg) or i.c.v. (50 μg/5 μl, data not shown) had no marked effect on urine output (Fig. 1). Urine output for rats injected with saline (s.c.) was 1.37±0.17 ml/5 h and, for rats injected with the vehicle (s.c.) for both salvinorin A and MOM-sal B, was 2.57±0.46 ml/5 h. Our results showed that nalfurafine is a more potent diuretic than U50,488H and MOM-sal B. A centrally administered high

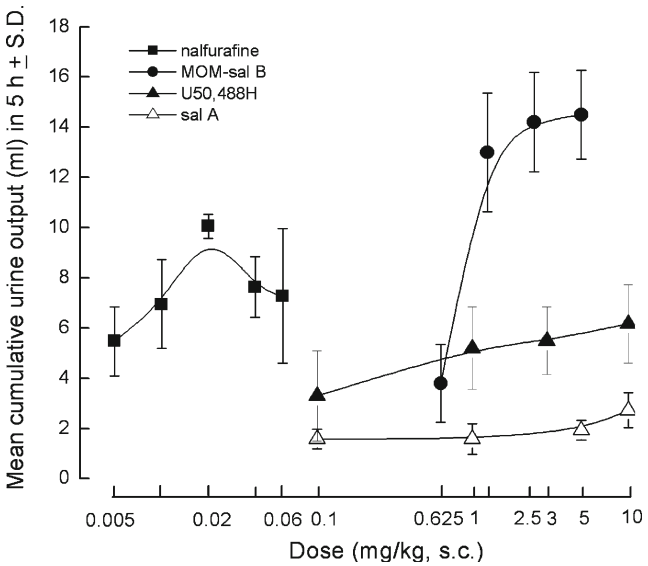

Fig. 1 Urine output in 5 h in rats treated s.c. with nalfurafine, U50,488H, salvinorin A, MOM-sal B, and vehicle (n=6–10). Nalfurafine, U50,488H, and MOM-sal B cause increases in urine output. Urine output for rats injected with saline (s.c.) was 1.37±0.17 ml/5 h and for rats injected with the vehicle (s.c.) for both salvinorin A and MOM-sal B was 2.57±0.46 ml/5 h

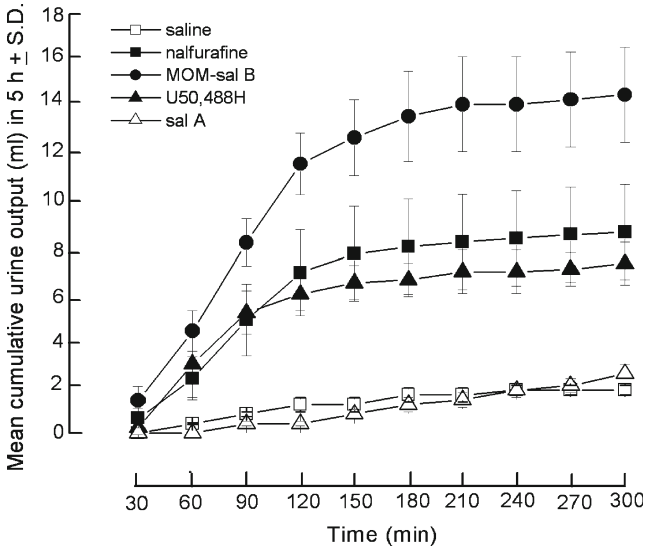

Fig. 2 Time-course of diuresis induced by nalfurafine, U50,488H, and MOM-sal B in rats (n=8). Animals were injected s.c. with saline, nalfurafine (0.02 mg/kg), U50,488H (10 mg/kg), MOM-sal B (2.5 mg/kg), or vehicle and urine output was measured every 30 min for 5 h

dose of salvinorin A failed to increase urine output (2.65±0.65 and 2.30±0.59 ml/5 h after injections of salvinorin A and vehicle, respectively). Lower urine osmolality and low $U_{Na}V$ levels were found in rats injected with nalfurafine (0.02 mg/kg), U50,488H (10 mg/kg), and MOM-sal B (2.5 mg/kg) compared with saline-injected animals, suggesting a water diuresis which is typical for kappa agonist-induced diuresis (Table 1). The time course of nalfurafine-, U50,488H-, and MOM-sal B-induced diuresis is shown in Fig. 2. All compounds elicited their maximum effect around 210 min. Serum angiotensin II levels were similar in rats injected with saline–saline, GNTI-saline, saline-nalfurafine, saline-U50,488H, GNTI-nalfurafine and GNTI-U50,488H (data not shown). While nalfurafine and MOM-sal B had no effect on serum AVP levels (at 2 h), an increase in serum AVP level was detected in rats injected with saline-U50,488H (10 mg/kg; Fig. 3).

Table 1 Mean±SD values of cumulative urine output, urine osmolality, free water clearance, and urinary excretion of sodium in rats (n=6) treated (s.c.) with saline, U50,488H, nalfurafine, and MOM-sal B

	Saline	U50,488H (10 mg/kg)	Nalfurafine (0.02 mg/kg)	MOM-sal B (2.5 mg/kg)
Urine output (ml/5 h)	2.1±0.9	6.2±0.9**	9.8±1.2**	14.2±1.99**
Uosm (mOsm/kg)	473±46.7	75.8±23.7**	106±32.1**	63.8±18.2**
C_{H2O} (ml/5 h)	−1.4±0.9	4.4±0.9**	6.2±1.1**	11.1±2.3**
$U_{Na}V$ (mEq/5 h)	46.3±21.2	2.7±0.8**	2.9±0.9**	1.3±0.8**

Uosm urine osmolality, C_{H20} free water clearance, $U_{Na}V$ urinary excretion of sodium
**p<0.01 (compared with saline)

Tolerance studies

Tolerance did not develop to the diuretic effect of nalfurafine (0.02 mg/kg, s.c.; Fig. 4). There was no

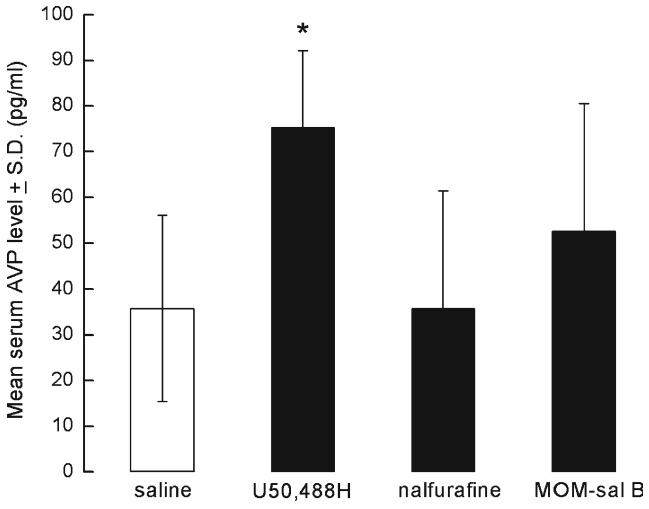

Fig. 3 Serum AVP levels in rats. An increase in serum arginine vasopressin level is obtained in rats (n=6) injected s.c. with U50,488H (10 mg/kg) but not with nalfurafine (0.02 mg/kg) or MOM-sal B (2.5 mg/kg; *p<0.05 one-way ANOVA and Newman–Keuls tests). Blood was drawn 2 h after the injection of compounds

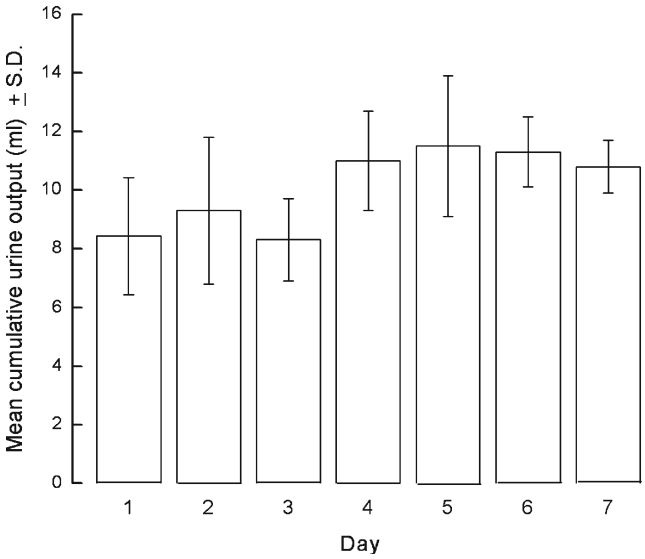

Fig. 4 Daily urine output in rats injected with nalfurafine. The daily urine output, over 5 h, is similar for 7 days. Tolerance to the diuretic effect of nalfurafine (0.02 mg/kg, s.c.) does not develop during once a day injection of nalfurafine to rats (n=6) for 7 days

statistically significant difference among urine outputs over the 7 days for nalfurafine (as well as for U50,488H). Day 1 urine volumes were 8.43±2.0 and 7.56±1.94 ml, whereas those on day 7 were 10.80±2.40 and 8.59±1.77 ml for nalfurafine and U50,488H, respectively. Urine osmolality was similar on days 1 and 7 for all three groups of rats (injected with saline, U50,488H, or nalfurafine) but was significantly lower in rats injected with U50,488H or nalfurafine compared with saline-injected rats both on days 1 and 7 (p<0.01). On day 7, significantly lower $U_{Na}V$ and U_KV levels were measured in rats injected with either U50,488H or nalfurafine compared with saline-injected rats. There was no difference among the three groups of rats for *serum* Na^+ levels (Table 2).

Table 2 Mean±SD values of urine output, urine osmolality, free water clearance, urinary excretion of sodium, and serum Na^+ levels on day 7 in rats treated (s.c.) once a day for 7 days with saline, U50,488H, and nalfurafine

	Saline	U50,488H (10 mg/kg)	Nalfurafine (0.02 mg/kg)
Urine output (ml/5 h)	2.2±0.34	8.0±1.91**	10.8±0.94**
Uosm (mosm/kg)	290±42.8	122±63.9**	75±25.9**
C_{H2O} (ml/5 h)	−0.06±0.31	4.7±2.48**	7.9±1.56**
$U_{Na}V$ (mEq/5 h)	56.3±21.7	7.7±4.27**	4.2±2.0**
U_KV (mEq/5 h)	67.7±22.4	13.7±8.0**	6.1±2.87**
Serum Na^+ (mmol/L)	134±3.21	134±7.1	132±7.41

Uosm urine osmolality, C_{H20} free water clearance, $U_{Na}V$ urinary excretion of sodium, U_KV urinary excretion of potassium
**p<0.01 (compared to saline)

Antagonizing kappa agonist-induced diuresis with GNTI

Pretreatment of rats with GNTI (0.1 mg/kg, s.c., at −30 min) significantly decreased U50,488H-induced (10 mg/kg, s.c.) and MOM-sal B-induced (2.5 mg/kg, s.c.) diuresis (Fig. 5). GNTI (0.1–3 mg/kg, s.c. at −30 min) inhibited nalfurafine-induced (0.02 mg/kg, s.c.) diuresis in a dose-dependent manner (Fig. 6). Similarly, i.c.v. administration of GNTI (0.005–25 μg) antagonized this effect of nalfurafine in a dose-dependent manner (Fig. 7). A 25-μg dose of GNTI reduced urine output to control levels. Rats appeared behaviorally normal during systemic and i.c.v. injections of high doses of GNTI.

Nalfurafine's effect on basal cAMP level in hypothalamus

Nalfurafine caused a significant decrease, compared to saline, in basal cAMP level in the hypothalamus of rats (142.16±21.40 and 259.10±31.12 pmol/mg protein, respectively, p<0.01).

Discussion

We have studied diuretic effects of the chemically diverse kappa opioid agonists nalfurafine (an epoxymorphinan), U50,488H (an arylacetamide), salvinorin A (a neoclerodane diterpene), and MOM-sal B (the C-2 derivative of salvinorin A) in rats. Our results show that nalfurafine,

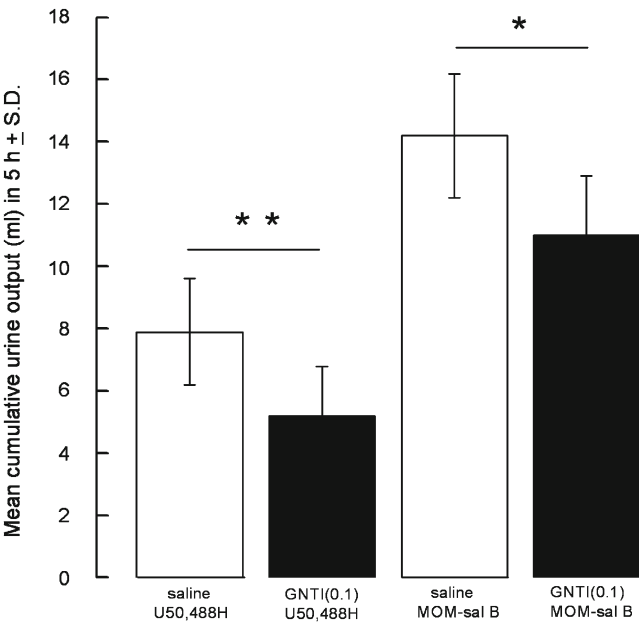

Fig. 5 GNTI (0.1 mg/kg, ?s.c.) antagonizes U50,488H (10 mg/kg, s.c.) as well as MOM-sal B-induced (2.5 mg/kg) diuresis (*p<0.05, **p<0.01, n=6)

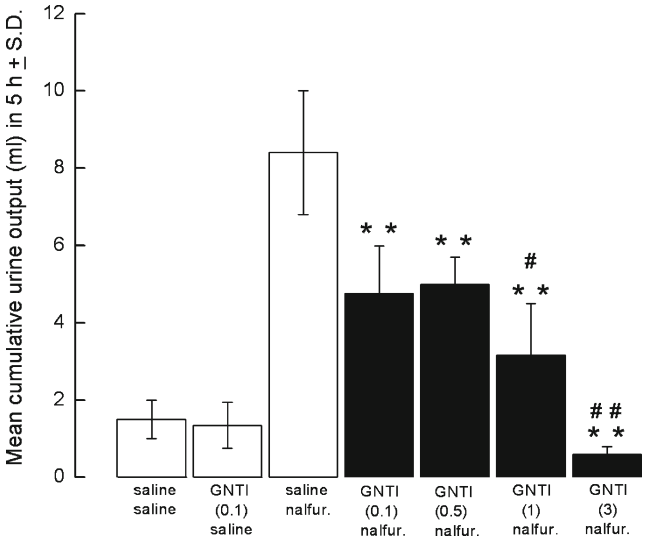

Fig. 6 GNTI (0.1–3 mg/kg, s.c. at −30 min) inhibits nalfurafine-induced (0.02 mg/kg, s.c.) diuresis in a dose-dependent manner. $^{**}p<0.01$ compared with saline-nalfurafine; *single pound sign* $p<0.05$ and *double pound sign* $p<0.01$ compared with different doses of GNTI pretreatment, $n=6–8$

U50,488H, and MOM-sal B induce water diuresis, whereas salvinorin A is ineffective. Nalfurafine-induced diuresis was antagonized dose-dependently by both systemic and central administration of the kappa opioid receptor antagonist, GNTI, suggesting that the diuresis is mediated by kappa opioid receptors. Additionally, a fixed dose of GNTI inhibited fixed doses of both U50,488H- and MOM-sal B-induced diuresis in rats. A decrease in the basal cAMP level after subchronic injection of nalfurafine indicates that the hypothalamus is involved in nalfurafine-induced diuresis in rats.

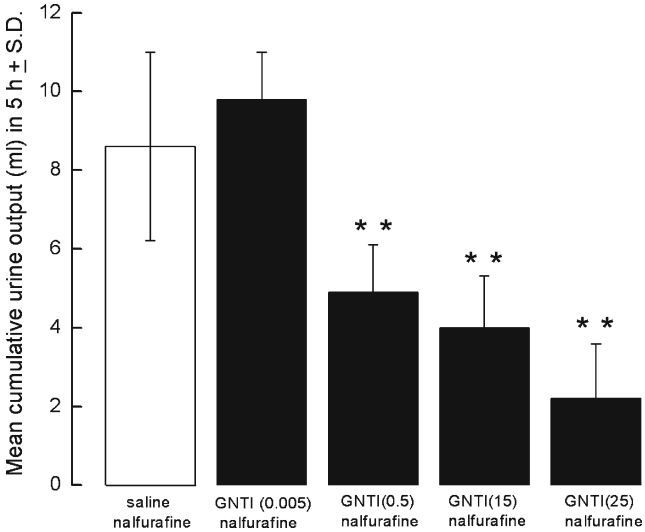

Fig. 7 Intracerebroventricular injection of GNTI (0.005–25 μg/5 μl at −15 min) inhibits nalfurafine-induced (0.02 mg/kg, s.c.) diuresis in rats ($^{**}p<0.01$; $n=6$)

Salvinorin A is an active (hallucinatory) ingredient of the psychoactive plant *Salvia divinorum* that has been used by the Mazatec people in Mexico for traditional spiritual practices. The leaves of the plant and various extracts are available over the internet. Salvinorin A is the first non-nitrogenous, naturally occurring, highly selective kappa opioid receptor agonist (Roth et al. 2002; Sheffler and Roth 2003; Wang et al. 2005; Prisinzano and Rothman 2008). It has hallucinogenic effects which last approximately 1 h in doses between 200 and 500 μg after smoking by humans (Prisinzano 2005). Carlezon et al. (2006) described a depressive-like effect in rats with 0.125–2 mg/kg, i.p. doses of salvinorin A using the forced swim test. Butelman et al. (2004) reported that salvinorin A elicits kappa opioid agonist-like discriminative effects in rhesus monkeys. Salvinorin A (s.c.) was inactive in mice as an antipruritic against compound 48/80-induced scratching behavior and showed only brief antinociception in this species (Wang et al. 2005; Ansonoff et al. 2006; McCurdy et al. 2006).

Salvinorin A was not a diuretic in rats. Furthermore, salvinorin A (1–4 mg/kg, both i.p. and s.c.) did not increase urine output in mice (unpublished data). Schmidt et al. (2005) determined salvinorin A and its inactive metabolite, salvinorin B, in body fluids (from monkeys and humans) using high-performance liquid chromatography-atmospheric pressure chemical ionization. From their work, the duration of action of salvinorin A seems to be short. This brief duration of action is probably responsible for the ineffectiveness of salvinorin A. It has been suggested that the site of interaction of salvinorin A to kappa opioid receptors differs from that of its analogs (Prisinzano and Rothman 2008). This might provide another reason for the lack of diuresis associated with salvinorin A. MOM-sal B, the longer acting derivative of salvinorin A, shows antinociceptive and hypothermic effects in rats (Wang et al. 2008). Furthermore, we report here that MOM-sal B increases urine output in this species.

Nalfurafine possesses antinociceptive (Endoh et al. 1999, 2000, 2001; Suzuki et al. 2004) and antipruritic (Togashi et al. 2002; Inan and Cowan 2004, 2006a, b) effects in animals. Critically, for drug development in the field of kappa opioid research, nalfurafine has been found effective in patients suffering from uremic pruritus (Wikström et al. 2005). Side effects traditionally associated with kappa agonists (e.g., paresthesia, abnormal thinking, dysphoria) were absent. Diuresis was not mentioned. Our results indicate that nalfurafine may be a candidate diuretic for treating water retention. First of all, this kappa agonist induces diuresis without electrolyte imbalance in rats and perhaps in other species. During acute injection of nalfurafine, antinatriuresis was noted. In our multiple-dose study, serum Na^+ levels remained within normal physiological limits. In clinical practice, maintenance

of electrolyte levels during diuretic treatment is desirable. Furosemide and other loop diuretics cause diuresis as well as natriuresis and kaliuresis. Acute tolerance develops to the diuresis (Hammarlund et al. 1985; Wakelkamp et al. 1996) and natriuresis (Wakelkamp et al. 1996) after multiple doses of furosemide in healthy volunteers. In our study, after once a day injection of nalfurafine to rats for 7 days, daily urine volumes were comparable, suggesting that tolerance does not develop. Similarly, tolerance to the diuretic effect of U50,488H does not develop during chronic injections in rats (VonVoigtlander et al. 1983) and in monkeys (Ko et al. 2003).

The mechanisms underlying the diuresis induced by kappa agonists are not clear. For example, kappa agonists have been shown to increase, decrease, or cause no change in serum AVP levels. Gottlieb et al. (2005) reported that microinjection of U50,488H to the magnocellular area of the hypothalamic paraventricular nucleus (PVN), but not the parvocellular area of the PVN, produces diuresis by inhibiting AVP secretion. Their data suggested that an increase in renal sympathetic outflow is responsible for the antinatriuretic effect of U50,488H. Decreased AVP levels in rats (Yamada et al. 1989; Craft et al. 2000) and the lack of diuresis in Brattleboro rats (which do not have endogenous AVP; Slizgi and Ludens 1986; Yamada et al. 1989) following systemically given kappa agonists have been reported. However, Ashton et al. (1989) demonstrated that U50,488H does not alter plasma AVP levels in rats. Kramer et al. (2000) investigated plasma AVP, cyclic adenosine monophosphate, and atrial natriuretic peptide levels as well as urine endothelin levels during administration of asimadoline, the peripherally directed kappa agonist. Under basal conditions, asimadoline did not change these parameters. Only plasma AVP levels were suppressed by high doses of asimadoline after stimulation with 2.5% saline infusion. Similarly, Rimoy et al. (1991) reported that spiradoline, an arylacetamide kappa agonist, causes water diuresis in human volunteers with no change in plasma AVP levels. After bremazocine (a benzomorphan kappa agonist) injection in rats, Huidobro-Toro and Parada (1985) reported an increase in plasma AVP, whereas Craft et al. (2000) found a decrease in plasma AVP. In the present study, we found an increase in serum AVP in rats injected with U50,488H but no change in rats injected with nalfurafine or MOM-sal B. We drew blood at the end of the 2-h experimental period. In previous studies, AVP levels were measured 20 min to 2 h after kappa agonist injection (Ashton et al. 1989; Craft et al. 2000). Also, most of the studies measured AVP after stimulated AVP secretion (induced by either hyperosmolar fluid infusion or 24 h water depletion). Craft et al. (2000) detected a decrease in AVP levels in 24 h water-deprived rats but not in normally hydrated rats. In our experiments, we used normally hydrated rats. Given these diverse results, it is not possible to conclude that kappa opioid agonists induce diuresis solely by inhibiting AVP secretion.

In summary, we investigated the effects of nalfurafine, salvinorin A, and MOM-sal B on urine output and renal excretory response in rats. Our results show that (1) nalfurafine induces water diuresis and may be a candidate diuretic for relieving water retention, and (2) salvinorin A, a recognized kappa opioid receptor agonist, does not cause diuresis, whereas its longer-acting C-2 derivative, MOM-sal B, increases urine output. The lack of diuresis associated with salvinorin A is probably due to its short duration of action.

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