Renal Excretory Responses Produced by the Delta Opioid Agonist, BW373U86, in Conscious Rats

SENA F. SEZEN, VELGA A. KENIGS and DANIEL R. KAPUSTA

Department of Pharmacology and Experimental Therapeutics, Louisiana State University Medical Center, New Orleans, Louisiana

Accepted for publication May 26, 1998

This paper is available online at http://www.jpet.org

ABSTRACT

Studies were performed in conscious Sprague-Dawley rats to characterize the changes in renal excretory function produced by activation of delta opioid systems. The intravenous infusion of 50 µg/kg/min, BW373U86 (BW), a nonpeptide delta opioid receptor agonist, produced a significant increase in urine flow rate and urinary sodium excretion. The infusion of BW at a dose of 30 µg/kg/min produced diuresis without affecting urinary sodium excretion. In contrast, BW did not alter either renal excretory parameter at a dose of 10 µg/kg/min. The renal responses produced by BW occurred without changes in heart rate or mean arterial blood pressure. The diuretic and natriuretic responses produced by the i.v. infusion of BW (50 µg/kg/min) were prevented by pretreatment of animals with the selective delta opioid receptor antagonist, naltrindole (1 mg/kg, i.v.). When administered alone, naltrindole (1 mg/kg, i.v.) failed to change any systemic cardiovascular or renal excretory parameter. In other groups of animals, the peripheral administration of the delta opioid receptor agonist, SNC80, also evoked a profound diuretic and natriuretic response (naltrindole sensitive) similar to that produced by BW. In contrast to these findings, the diuretic and natriuretic response produced by BW infusion (30 or 50 µg/kg/min, i.v.) was abolished in rats having undergone chronic bilateral renal denervation. Together, these results demonstrate that the peripheral administration of BW373U86 or SNC80 produce marked diuretic and natriuretic responses in conscious Sprague-Dawley rats via a delta opioid receptor pathway and that intact renal nerves are required for mediating these responses. Although endogenous delta opioid systems do not appear to exert a tonic influence under basal conditions, these findings suggest that delta opioid pathways may evoke significant changes in renal excretory function under conditions in which these systems are activated.

Administration of opioid agonists produce profound changes in renal excretory function in animals and man (for review see Kapusta, 1995). Moreover, under various conditions the administration of opioid receptor antagonists alter the renal excretion of water and sodium (Kapusta, 1995). These findings suggest that endogenous opioid systems may have an important role in the renal handling of water and sodium under different physiological conditions. However, to fully understand the functional impact of opioid systems on this regulatory process, it is essential to understand how each opioid system (e.g. mu, delta, or kappa) acts individually to modify the renal excretion of sodium and water. In this regard, the renal responses (and mechanisms) produced by the administration of highly selective mu or kappa opioid agonists have, at least in part, been characterized in conscious animals. Under different experimental conditions the administration of mu opioid agonists (e.g. morphine, dermorphin, etc.) produce either a diuretic or antidiuretic response (Marchand, 1970; Huidobro et al., 1981; Kapusta et al., 1993; Gutkowska and Schiller, 1996). In contrast to these variable effects, kappa opioid agonists characteristically produce an increase in urine flow rate (Leander, 1983; Slizgi et al., 1984; Rimoy et al., 1991; Kapusta and Obih, 1993). Concurrent with either change in urine flow rate, both mu and kappa opioid agonists elicit a decrease in urinary sodium excretion (Huidobro and Huidobro-Toro, 1979; Walker and Murphy, 1984; Kapusta et al., 1989a). Thus, while mu and kappa opioid systems produce similar effects on the renal handling of sodium, these opioid systems can produce opposing effects on the renal excretion of water.

Despite extensive investigations that have been performed to examine the role of mu and kappa opioid systems in the regulation of renal function, it remains essentially unknown as to whether delta opioid systems also influence the renal handling of water and sodium. Several lines of evidence suggest that delta opioid systems may, in fact, have important influences on renal excretory function. First, delta opioid receptors are widely distributed in central nervous system...
regions involved in the regulation of cardiovascular function and fluid and electrolyte balance (Mansour et al., 1995; Jenab et al., 1995; George et al., 1994). Delta opioid receptors are located in peripheral tissues such as the kidneys and the adrenal glands (Wittert et al., 1996). Although not previously tested, stimulation of delta opioid receptors in these cardiovascular and renal regulatory centers may evoke significant changes in urine output or urinary sodium excretion. Indirect evidence to support this possibility comes from the observation that administration of methionine- or leucine-enkephalin produces changes in urine flow rate and urinary sodium excretion (Bisset et al., 1978; Grossman et al., 1980; Brownell et al., 1980; Zerbe et al., 1982). These findings are of importance since the enkephalins have a high affinity for delta opioid receptors and have been proposed to be the endogenous ligands for delta opioid receptors. Despite their high affinity for this receptor subtype, the role of delta opioid receptor mechanisms in mediating enkephalin-induced renal responses has not been specifically elucidated with the use of selective delta opioid receptor antagonists. These types of studies are critical since the enkephalins also have a high degree of affinity for the mu opioid receptor and therefore, could evoke changes in renal function by a mu opioid receptor pathway.

Thus, the present investigations were performed to critically examine the role of delta opioid systems in the regulation of renal function. For this purpose, changes in renal excretory function were examined in groups of conscious Sprague-Dawley rats during activation of delta opioid receptors produced by the peripheral administration of the selective delta opioid receptor agonist, BW373U86 (BW) or SN680. Both BW and SN680 are nonpeptide agonists with high selectivity for delta opioid receptors (Chang et al., 1993; Bilsky et al., 1995; Clark et al., 1997). In the present studies, the delta opioid receptor selectivity of BW and SN680 on changes in renal function were tested with the use of the selective delta opioid receptor agonist, naltrindole (Portoghese et al., 1988). Finally, to investigate the role of the renal nerves in mediating the renal responses produced by BW, certain studies were repeated in rats in which the influence of the renal nerves on kidney function was removed via chronic bilateral renal denervation.

**Materials and Methods**

**Subjects.** Male Sprague-Dawley rats (Harlan Inc., Indianapolis, IN) weighing between 275 and 335 g were used in these studies. Rats were housed in groups of five or less under 12-hr, light-dark cycle, until the day of the experiments. Rats that had undergone prior surgical procedures were housed in individual cages during the period of recovery. All rats were fed with standard rodent chow (Laboratory rodent diet #5001, PMI Feeds Inc., St. Louis, MO) and allowed tap water *ad libitum*. All experimental procedures were conducted in accordance with the Louisiana State University Medical Center and National Institutes of Health guidelines for the care and use of animals.

**Surgical procedures.** On the experimental day, rats were anesthetized with methohexital sodium (Brevital, Eli Lilly, Indianapolis, IN) 20 mg/kg i.p., suplemented with 10 mg/kg i.v. as needed. The polyethylene catheters (PE-10 tubing attached to PE-50, Becton Dickinson and Company, Sparks, MD) was implanted into the left femoral artery and vein for the recording of arterial pressure and administration of drugs, respectively. Through a suprapubic incision, a flanged polyethylene cannula (PE-240, Becton Dickinson and Company, Sparks, MD) was inserted into the urinary bladder. The bladder catheter was then exteriorized and secured by suturing to adjacent muscle, tissue and skin.

For certain experiments, the influence of the renal nerves on kidney function was removed. For these studies, rats were subjected to chronic bilateral renal denervation 7 to 10 days before experimentation. Bilateral renal denervation was performed in pentobarbital anesthetized rats (50 mg/kg i.p., supplemented with 10 mg/kg i.v. as needed, SoloPak Laboratories, Elk Grove Village, IL). The left kidney was exposed via a retroperitoneal flank incision. Under a dissecting microscope, the adventitial layer was stripped from the renal artery and vein, and the renal nerve bundles were cut. The renal artery and vein were then coated with a solution of 10% phenol in absolute ethanol as described previously (DiBona and Sawin, 1983; Kapusta and Obih, 1995). The flank incision was then closed by suturing the muscle layers and the skin. The same surgical procedure was then employed on the opposite side to denervate the right kidney. This renal denervation procedure prevents the renal vasoconstrictor response to supraprenal lumbar sympathetic nerve stimulation, prevents the antinatriuretic response to environmental stress and reduces renal tissue norepinephrine concentration to <5% of control for up to 15 days postdenervation (DiBona and Sawin, 1983). Our laboratory has previously and repeatedly verified that this renal denervation procedure completely removes the influence of the renal nerves on kidney function (Kapusta et al., 1989a, 1993).

**Experimental protocol.** In the morning of the experimental day, rats were instrumented with femoral arterial and venous catheters and a bladder cannula. The rats were then placed in rat holders to permit steady-state urine collection, allowed to recover from anesthesia and studied in the conscious state. The arterial cannula was flushed and connected to a pressure transducer (Statham P23Db, Grass Instruments, Quincy, MA) for measurement of arterial pressure. Heart rate was derived from the pulse pressure by a tachograph (Grass model 7 P4H; Grass Instruments, Quincy, MA). Arterial pressure and heart rate were recorded on a Grass model 7 polygraph (Grass Instruments, Quincy, MA). The venous cannula was connected to an infusion pump (Harvard Apparatus model 11, N. Satick, MA) for administration of isotonic saline. An i.v. infusion of isotonic saline (0.9% NaCl, w/v) (55 μl/min) was started and continued for the duration of the experiment.

After attaining stable levels of urine flow rate and urinary sodium excretion (4–6 hr) the experimental protocol commenced. Urine samples were collected in preweighed vials during two control periods (C, 10 min each). The infusate was then switched to a solution of isotonic saline vehicle that contained the delta opioid receptor agonist, BW (10 μg/kg/min, n = 5; 30 μg/kg/min, n = 7; 50 μg/kg/min, n = 5). Five min after the start of BW infusion, experimental urine samples were collected for six consecutive periods (10 min each; denoted B1 to B6).

In additional studies, the cardiovascular and renal responses produced by BW were examined in rats (n = 7) pretreated with the selective delta opioid receptor antagonist, naltrindole (Portoghese et al., 1988). Following isotonic saline control urine collections (2 consecutive 10 min periods), naltrindole (1 mg/kg, i.v.) was administered and allowed to distribute for 20 min. Two consecutive experimental naltrindole urine samples were then collected (10 min each; 40 min total pretreatment time). Previous studies have demonstrated that 40 min pretreatment time is required for naltrindole to produce maximal antagonism of delta opioid receptors (Ayres et al., 1990; Comer et al., 1993). The infusate was then switched to a solution of isotonic saline containing BW (50 μg/kg/min, i.v.) and infused for the duration of the experiment. Five mins after the start of BW infusion, experimental BW urine samples were collected for 1 hr (six consecutive periods of 10 min each, B1 to B6).

For comparative purposes, the cardiovascular and renal responses produced by the nonpeptide delta opioid receptor agonist, SN680 (Bilsky et al., 1995; Clark et al., 1997), were also examined. For these studies, the above mentioned protocols for BW were repeated in
separate groups of rats with the exception that SNC80 (5 mg/kg, i.v. bolus) was administered in place of BW. Immediately after SNC80 injection, consecutive 10 min experimental urine samples were collected for 1 hr, denoted S1 to S6.

Further studies were performed to examine the delta receptor selectivity of the dose of naltrindole (1 mg/kg, i.v.) used in the antagonism studies described above. For these studies, the cardiovascular and renal excretory responses produced by the selective mu opioid receptor agonist, dermorphin (0.3 mg/kg, i.v.) or the kappa opioid receptor agonist, U50,488H (20 μg/kg/min, i.v.), were examined in naive (e.g. nonpretreated) rats and in rats pretreated with naltrindole (1 mg/kg, i.v.; 40 min pretreatment time). The experimental protocol for these experiments were similar to those previously described for BW infusion.

Studies were performed to determine whether the renal nerves are required for mediating the changes in renal excretory function produced by delta opioid agonists. For this purpose the cardiovascular and renal excretory responses produced by BW infusion (50 μg/kg/min, n = 11; 30 μg/kg/min, n = 7) were measured in rats that had undergone chronic bilateral renal denervation 7 to 10 days before experimentation. The experimental protocol for these studies was the same as previously described for studies in which BW was administered to rats with intact kidneys.

The participation of endogenous delta opioid systems in the tonic regulation of renal excretory function in conscious rats was investigated by examining the cardiovascular and renal responses produced by the i.v. administration of the selective delta opioid receptor antagonist, naltrindole (1 mg/kg; n = 7). After collection of isotonic saline control urine samples (2 consecutive 10 min periods), naltrindole was administered. Immediately after injection, consecutive 10 min experimental urine samples were then collected for a total of 120 min.

In the present studies the effects of three different i.v. infusion doses (10, 30, 50 μg/kg/min) of BW were examined on renal excretory function. BW was infused i.v. to achieve a sustained activation of delta opioid receptors in the periphery, kidneys and central nervous system. Since behavioral changes were observed in some animals with a dose of 50 μg/kg/min, higher doses of BW were not tested in these investigations. The doses of BW tested in the present study were selected for use as they are similar to those used in a study by Lee et al. (1993). In these investigations, subcutaneous miniosmotic pump infusion of BW (0.1 to 3 mg/kg/hr) attenuated the intensity of naloxone-induced abstinence syndrome in morphine treated rats. The effects of BW at these doses were shown to be antagonized by the selective delta opioid antagonist, naltrindole.

**Analytical techniques.** Urine volume was determined gravimetrically. Urine sodium concentration was measured by flame photometry (model 943, Instrumentation Laboratories, Lexington, MA). Renal excretery data were normalized per gram kidney weight. Urine osmolality was measured by the vapor pressure method (Wescor 5500, Wescor Inc., Logan, Utah). Osmolar clearance (C\text{osm}) was measured as: C\text{osm} = (V\text{Uosm} \times V\text{Posm}) / P\text{osm}, where V\text{Uosm} and P\text{osm} are urine and plasma osmolality, respectively, and V is urine flow rate. Free-water clearance (C\text{fW}) was measured as: C\text{fW} = V - C\text{osm} where V is urine flow rate and C\text{osm} is osmolar clearance.

**Data analysis.** The data were analyzed statistically by using repeated measures analysis of variance (ANOVA) for main effects and interactions. A Bonferroni test was used for pairwise comparisons between mean. Statistical significance was defined as P < .05.

**Drugs.** The following drugs were used in this study: BW373U86 hydrochloride (generous gift from Dr. Robert McNutt, Burroughs Wellcome Co.), SNC80 (Tocris), naltrindole hydrochloride (Sigma), dermorphin acetate salt (Sigma) and U50,488H (Upjohn). All drugs were dissolved in isotonic saline and prepared on the day of the experiments. SNC80 was dissolved in isotonic saline and prepared on the day of the experiment, consecutive 10 min experimental urine samples were collected for 120 min beginning 5 min after the start of BW infusion.

**Results.**

**Effects of i.v. infusion of BW373U86.** Figure 1 shows the cardiovascular and renal excretory responses produced by the i.v. infusion of BW, a nonpeptide selective delta opioid receptor agonist, in conscious Sprague-Dawley rats. As compared to control levels, the i.v. infusion of BW at a dose of 50 μg/kg/min, produced a diuretic and natriuretic response. At this dose, BW significantly (P < .05) increased urine flow rate from 23.3 ± 3.2 μl/min/gkw, (C, predrug control) to 54.2 ± 4.9 μl/min/gkw, by 25 min after the start of drug infusion (B2). The diuretic response persisted for the duration of the experiment. Cumulative urine output (total urine collected for 60 min beginning 5 min after the start of BW infusion) produced by 50 μg/kg/min BW was 2.98 ± 0.25 ml/gkw. Concurrent with the increase in urine flow rate, this dose of BW produced an increase in urinary sodium excretion that was significantly (P < .05) elevated 35 min after the start of drug infusion. As shown in figure 1, values for urinary sodium excretion for predrug control (C) and experimental period B3, were 4.1 ± 0.6 and 6.9 ± 0.6 μeq/min/gkw, respectively. In comparison with these results, the i.v. infusion of 30 μg/kg/min BW (fig. 1) produced a similar magnitude diuresis 15 min after start of drug infusion (C; 24.9 ± 1.6 vs. B1; 53.1 ± 5.8 μl/min/gkw). This response, however, was transient and
returned to control levels by the next experimental period (B3). Over the 60 min experimental period, the cumulative urine output produced by 30 μg/kg/min BW was 1.93 ± 0.14 ml/gkw. At this lower dose, BW did not significantly change urinary sodium excretion (C; 5.1 ± 0.4 vs. B1; 5.3 ± 0.5 μeq/min/gkw). In other studies, there were no significant changes in any renal excretory parameter when BW was infused i.v. at a dose of 10 μg/kg/min (fig. 1). All doses of BW tested produced a slight, but not significant decrease in urine osmolality. As shown in table 1, both the 30 and 50 μg/kg/min infusion dose of BW produced an increase in free water clearance, however statistical significance was only achieved in rats infused with the lower dose during the first experimental period (B1). After the start of i.v. infusion, BW (10, 30, or 50 μg/kg/min, i.v.) did not alter heart rate or mean arterial pressure (fig. 1).

**Effects of naltrindole pretreatment on BW-induced renal responses.** Figure 2 shows the cardiovascular and renal excretory responses produced by the administration of BW in conscious rats pretreated with the selective delta opioid receptor antagonist, naltrindole. The renal responses typically produced by the i.v. infusion of 50 μg/kg/min BW (e.g., diuresis and natriuresis, fig. 1) were abolished by naltrindole (1 mg/kg, i.v.) pretreatment (fig. 2).

**Effects of i.v. administration of SNC80 in naïve or naltrindole pretreated rats.** Figure 3 shows the cardiovascular and renal excretory responses produced by the i.v. administration of SNC80 in conscious naïve rats, and in rats pretreated with the selective delta opioid receptor antagonist, naltrindole (1 mg/kg). After i.v. injection, SNC80 produced a significant increase in urine output over experimental periods S1 and S2, but thereafter returned to baseline values. Concurrent with the diuresis, SNC80 produced a transient, but significant increase in urinary sodium excretion. As shown in table 1, SNC80 tended to increase free water clearance, but these changes did not attain statistical significance. The diuretic and natriuretic responses produced by the i.v. administration of SNC80 were abolished in rats pretreated with naltrindole (1 mg/kg, i.v.)

**Effects of naltrindole pretreatment on U50,488H and dermorphin-induced renal responses.** Additional studies were performed to verify that the dose of naltrindole (1 mg/kg, i.v.) used in the antagonism studies mentioned above was selective for delta opioid receptors. As shown in table 2, the i.v. infusion of the selective kappa opioid agonist, U50,488H (20 μg/kg/min), produced a sustained diuresis in conscious naïve rats. U50,488H also produced a significant decrease in urinary sodium excretion. Pretreatment of rats with 1 mg/kg, i.v. naltrindole did not alter the diuretic or antinatriuretic response produced by U50,488H. In other studies (table 3), the administration of the selective mu opioid agonist, dermorphin (0.3 mg/kg, i.v.), produced a significant increase in urine output and decrease in urinary sodium excretion. The diuresis and antinatriuresis produced by dermorphin were not altered by naltrindole (1 mg/kg, i.v.) pretreatment (table 3).

**Effects of i.v. infusion of BW in chronic bilaterally renal denervated rats.** Figure 4 shows the cardiovascular and renal excretory responses produced by the i.v. infusion of BW in chronic bilaterally renal denervated rats. As previously shown in figure 1 (●), the i.v. infusion of 50 μg/kg/min BW produced an increase in urine flow rate and urinary sodium excretion in rats with intact renal innervation. In contrast, as compared to control levels for renal denervated animals (fig. 4, ○), this dose of BW did not produce a significant change in either urine flow rate (C, 21.2 ± 0.8 vs. B2, 24.1 ± 2.4 μl/min/gkw) or urinary sodium excretion (C, 3.7 ± 0.2 vs. B3, 3.4 ± 0.4 μeq/min/gkw). Likewise, in renal denervated rats (fig. 4, ○), the i.v. infusion of 30 μg/kg/min BW did

---

**TABLE 1**

Changes in free water clearance produced by the administration of BW373U86 or SNC80 in conscious rats

<table>
<thead>
<tr>
<th>Period</th>
<th>C_H2O (50 μg/kg/min)</th>
<th>BW (50 μg/kg/min)</th>
<th>BW (30 μg/kg/min)</th>
<th>SNC80 (5 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1/S1</td>
<td>−31.6 ± 8.3</td>
<td>−29.7 ± 4.9</td>
<td>−19.1 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>B2/S2</td>
<td>−14.4 ± 1.1</td>
<td>−7.3 ± 4.5</td>
<td>−11.9 ± 3.7</td>
<td></td>
</tr>
<tr>
<td>B3/S3</td>
<td>−9.0 ± 3.3</td>
<td>−12.5 ± 3.3</td>
<td>−3.1 ± 7.1</td>
<td></td>
</tr>
<tr>
<td>B4/S4</td>
<td>−15.3 ± 4.8</td>
<td>−17.9 ± 1.7</td>
<td>−3.7 ± 4.0</td>
<td></td>
</tr>
<tr>
<td>B5/S5</td>
<td>−14.9 ± 3.1</td>
<td>−22.6 ± 8.9</td>
<td>−12.2 ± 3.7</td>
<td></td>
</tr>
<tr>
<td>B6/S6</td>
<td>−27.6 ± 9.5</td>
<td>−16.7 ± 3.4</td>
<td>−13.2 ± 3.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>−28.1 ± 4.3</td>
<td>−13.8 ± 7.1</td>
<td>−18.1 ± 2.4</td>
<td></td>
</tr>
</tbody>
</table>

Changes in free water clearance (C_H2O) produced by the administration of the delta opioid agonist BW373U86 (50 μg/kg/min, n = 5; 30 μg/kg/min i.v, n = 7), or SNC80 (5 mg/kg, i.e., bolus, n = 5). The values presented are calculated from renal excretory data obtained from the same groups of rats depicted in figures 1 and 3, respectively. Urine samples were collected during control (C, 20 min), and after the administration of either BW (B1 to B6, six consecutive 10 min urine samples beginning 5 min after the start of infusion) or SNC80 (S1 to S6, six consecutive 10 min urine samples beginning after i.v. injection). The values are mean ± S.E.

* P < .05, significantly different from corresponding control.

Abbreviations are the same as in figure 1.
not alter urine flow rate compared to respective control (C, 21.7 ± 1.7 vs. B1, 29.4 ± 3.4 µl/min/gkW).

Effects of i.v. administration of naltrindole. Figure 5 depicts the cardiovascular and renal responses produced by the administration of the selective delta opioid agonist dermorphin in naive or naltrindole pretreated rats. The values are mean ± S.E. and illustrate the cardiovascular and renal excretory parameter over 120 min.

TABLE 3
Renal responses produced by the administration of the mu opioid agonist dermorphin in naive or naltrindole pretreated rats

<table>
<thead>
<tr>
<th>Period</th>
<th>Dermorphin</th>
<th>Naltrindole + dermorphin</th>
</tr>
</thead>
<tbody>
<tr>
<td>V (µl/min/gkW)</td>
<td>UNaV (µeq/min/gkW)</td>
<td>V (µl/min/gkW)</td>
</tr>
<tr>
<td>C/N</td>
<td>20.9 ± 1.6</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>D2</td>
<td>30.3 ± 1.0</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>D4</td>
<td>31.5 ± 2.4</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>D6</td>
<td>32.5 ± 3.5</td>
<td>4.1 ± 0.5</td>
</tr>
</tbody>
</table>

Renal excretory responses produced by the administration of the selective mu opioid receptor agonist dermorphin (50 µg/kg i.v.) in conscious naive rats (n = 11; and in rats (n = 4) pretreated with naltrindole (1 mg/kg i.v.). In naive rats, urine samples were collected during control (C, 20 min) and 10 min after dermorphin administration (D1 to D6, six consecutive 10 min urine samples). In pretreated rats, two consecutive 10 min naltrindole control urine samples (N) were collected 20 min after administration of dermorphin. After control collections, dermorphin was administered and consecutive 10 min experimental urine samples were collected for 60 min (D1–D6). The values are mean ± S.E.

* P < .05, significantly different from corresponding control.
Abbreviations are the same as in figure 1.


**Discussion**

This study examined the renal responses produced by the i.v. infusion of the selective *delta* opioid receptor agonist, BW373U86 (BW), in conscious Sprague-Dawley rats. BW was used to activate *delta* opioid receptor systems since this compound has been demonstrated to be a selective *delta* opioid receptor agonist in various *in vivo* and *in vitro* studies (Chang et al., 1993; Wild et al., 1993; Dykstra et al., 1993). In conscious rats, the i.v. infusion of BW produced a marked increase in urine flow rate at a dose of 30 and 50 μg/kg/min. Since 10 μg/kg/min BW did not alter urine flow rate in these studies, it appears that the threshold dose of BW to affect the renal handling of water lies between 10 and 30 μg/kg/min. Thus, in addition to *mu* and *kappa* opioid agonists, these findings indicate that selective *delta* opioid receptor agonists can also alter the renal handling of water.

*Mu* or *kappa* opioid agonists are recognized to produce a marked antinatriuretic response in conscious or anesthetized animals (Slizgi et al., 1984; Walker and Murphy, 1984; Kapusta et al., 1993; Kapusta et al., 1989b). Alternatively, under certain experimental conditions *kappa* opioid agonists evoke a change in urine output without altering urinary sodium excretion (Yamada et al., 1989; Salas et al., 1992). Regardless of whether a bona fide antinatriuresis is revealed, *mu* or *kappa* opioid agonists do not characteristically produce a natriuretic response (Kapusta, 1995). This point is of relevance since in the present studies, peripheral administration of the *delta* opioid agonists, BW (highest dose tested) or SNC80, produced a significant increase in urinary sodium excretion. Thus, it appears that activation of *delta* opioid receptor systems may, under appropriate conditions, have opposing effects on the renal handling of sodium as those produced by *mu* and *kappa* opioid systems.

In these studies the renal responses produced by the non-peptide *delta* opioid receptor agonist, SNC80, were also examined. As shown in these studies (fig. 3), the peripheral administration of SNC80 produced a profound diuretic and natriuretic response in conscious rats. Although SNC80 and BW produced similar magnitude increases in urine flow rate and urinary sodium excretion, the duration of these renal responses was shorter for SNC80. It is possible that these differences are related to variations in each drugs pharmacokinetic profile since SNC80 was administered as an i.v. bolus (due to limitation of drug) and BW as an i.v. infusion. In addition, differences in the potency and efficacy of each drug to affect water and sodium excretion may have contributed to the different renal excretory patterns observed. Of merit, however, is that the results obtained with SNC80 also support the notion that activation of *delta* opioid receptor systems can exert a significant influence on the renal handling of water and sodium.

BW is reported to be a selective agonist at *delta* opioid receptors (Chang et al., 1993; Comer et al., 1993). However, it has been suggested that certain behavioral effects (e.g. increased locomotor activity, hyperactivity) produced by BW may be mediated via activation of *mu* opioid receptors (Comer et al., 1993; Wild et al., 1993). In the present study, the renal responses (*i.e.* diuresis and natriuresis) produced by BW and SNC80 were abolished in rats pretreated with 1 mg/kg, i.v. naltrindole, a selective *delta* opioid receptor antagonist (Portoghese et al., 1988). The dose of naltrindole used in the present study (1 mg/kg, i.v.) is within range of that which antagonizes the analgesic effects produced by other *delta* selective agonists in other physiological systems (Ayres et al., 1990; Drower et al., 1991). Moreover, the dose of naltrindole (1 mg/kg, i.v.) used in our studies was shown to be selective for *delta* opioid receptors since the renal responses produced by the peripheral administration of selective *kappa* or *mu* opioid agonists were not altered (see tables 2 and 3, respectively). Together, these findings indicate that the renal responses produced by BW and SNC80 were, in fact, mediated by the activation of a *delta* opioid receptor pathway.

Opioids may evoke changes in the renal excretion of water and sodium by modulating neural and/or humoral pathways within the central nervous system, periphery and kidneys (Kapusta, 1995). Over the course of the experimental period, BW (50 μg/kg/min) did not produce a significant change in heart rate or mean arterial pressure. Despite the stability of blood pressure, it remains to be established whether changes in renal hemodynamics (glomerular filtration rate and/or renal plasma flow) contributed to the renal responses produced by BW.

A major finding of this study is the observation that the renal excretory responses produced by BW are dependent on intact renal nerves. The renal sympathetic nerves innervate the major structural components of the kidneys (*e.g.* vessels,
glomeruli, and tubules), and changes in effrent renal sympathetic nerve activity can evoke significant changes in renal hemodynamic and excretory function (DiBona and Kopp, 1997). In the present study, the diuresis and natriuresis produced by i.v. infusion of BW (50 μg/kg/min) were abolished in rats having undergone chronic bilateral renal denervation. This finding indicates that the renal responses produced by BW are mediated via a renal nerve-dependent pathway. Although not tested in these studies, BW may cause a diuretic and natriuretic response by altering (i.e., decreasing) sympathetic outflow to the kidneys, the release of neurotransmitter (e.g., norepinephrine and/or cotransmitters) from the renal nerve terminals, or the postsynaptic renal tubular (or vascular) action of norepinephrine.

Further studies are required to explore the role of these pathways in mediating the renal responses produced by BW and other delta opioid ligands.

Previous investigations have failed to reveal a role for endogenous mu or kappa opioid systems in the tonic regulation of renal function (Leander, 1983; Kapusta et al., 1993; Kapusta and Obih, 1993). Similar to these findings, the results of the present study suggest that in conscious rats endogenous delta opioid systems do not exert a tonic influence on the renal handling of water or sodium under our experimental conditions. Support for this premise is based on the observation that the administration of naltrindole (1 mg/kg, i.v.) did not alter urine flow rate or urinary sodium excretion (fig. 5). Should naltrindole have interrupted an endogenous influence of delta opioid systems on renal excretory function, it would have been anticipated that a change in either renal excretory parameter would have occurred. It should be noted that in the present studies 1 mg/kg, i.v. naltrindole was effective in preventing the renal responses produced by exogenous administration of the delta opioid agonists BW and SNC80, but not the mu and kappa opioid agonists, dermorphin and U50,488H, respectively. Based on these findings, it appears that delta opioid systems remain quiescent and may only affect renal excretory function when activated by a particular condition or stimuli. In fact, endogenous opioid systems have been shown to contribute to the changes in renal function that result from various types of stimuli or stress (e.g., air jet stress, dietary sodium restriction) (Kapusta et al., 1989c; Kapusta and Obih, 1995). Further studies are required to test this hypothesis and to determine the specific conditions or stimuli (e.g., stressors, pathophysiological states) in which endogenous delta opioid systems are activated to influence kidney function.

In conclusion, the present study demonstrated that the peripheral administration of the selective delta opioid receptor agonists, BW373U86 and SNC80 produce profound, naltrindole-sensitive, diuretic and natriuretic responses in conscious Sprague-Dawley rats. Furthermore, an intact renal innervation is required for the renal actions of BW. Despite the renal responses produced by exogenous administration of delta opioid agonists, endogenous delta opioid systems do not appear to exert a tonic influence on renal excretory function. Further studies are required to understand the role of delta opioid systems in the regulation of renal function under conditions in which these pathways may be activated, and to determine how delta mechanisms may interact with mu and/or kappa opioid systems in this regulatory process.

References


**Send reprint requests to:** Daniel R. Kapusta, Ph.D., Department of Pharmacology, LSU Medical Center, 1901 Perdido St., New Orleans, LA 70112.