Renal Excretory Responses Produced by Central Administration of Opioid Agonists in Ketamine and Xylazine-Anesthetized Rats

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ABSTRACT

This study examined the renal excretory responses produced by the intravenous (i.v.) infusion of the alpha-2 agonist, xylazine, in ketamine-anesthetized rats. In addition, the renal responses produced by the intracerebroventricular (i.c.v.) injection of opioid agonists were also examined with use of this anesthetic paradigm. In male Sprague-Dawley rats, the i.v. infusion of isotonic saline (55 \( \mu \)l/min) containing ketamine alone (1.0 mg/kg/min) produced low levels of urine flow rate (6.3 \( \pm \) 1.3 \( \mu \)l/min/gkidw) and urinary sodium excretion (0.28 \( \pm \) 0.08 \( \mu \)eq/min/gkidw). However, after adding xylazine (50 \( \mu \)g/kg/min) to the ketamine infusate, these renal excretory responses were significantly augmented. Steady-state levels of urine flow rate and urinary sodium excretion were attained approximately 120 min after starting the xylazine infusion and were similar in magnitude to the levels of water and sodium excretion previously observed in untreated, conscious rats. In ketamine/xylazine-anesthetized rats, the i.c.v. injection of the mu opioid agonist, dermorphin (0.1 nmol/kg), or the kappa opioid agonist, U-50488H (1 \( \mu \)g total), produced profound and concurrent diuretic and antinatriuretic responses. The pattern (direction and magnitude) of these opioid-induced renal excretory responses was similar to those previously reported in conscious rats. Together, these results indicate that the i.v. infusion of xylazine enhances the renal excretion of water and sodium in ketamine-anesthetized rats. Moreover, the renal responses produced by i.c.v. administration of opioids are similar in ketamine/xylazine-anesthetized and conscious rats. Thus, it appears that the ketamine/xylazine infusion protocol may provide a valid and useful approach to investigate various aspects of the central opioid control of renal function in rats during experimental procedures that require anesthesia.

Peripheral administration of opioid agonists produces marked changes in the renal excretion of water and sodium (for review see Kapusta, 1995). These opioid-induced renal excretory responses result, at least in part, via an action of the opioid within the CNS (Tseng et al., 1978; Huidobro-Toro and Huidobro, 1981; Cowan and Khunawat, 1986; Danesh and Walker, 1988; Salas et al., 1989; Brooks et al., 1993; Kapusta et al., 1993; Kapusta and Obih, 1993). This premise is supported by the demonstration that opioid agonists alter the renal excretion of water and sodium after their injection into the CNS. For example, i.c.v. administration of kappa opioid agonists (e.g., U-50488H, bremazocine) produce a profound diuretic response (Cowan and Khunawat, 1986; Salas et al., 1989; Brooks et al., 1993; Kapusta and Obih, 1993). On the other hand, i.c.v. administration of mu opioid agonists produces either a diuretic or antidiuretic response depending on the experimental conditions of the study (Tseng et al., 1978; Huidobro-Toro and Huidobro, 1981; Danesh and Walker, 1988; Kapusta et al., 1993). Despite the variable effects on urine flow rate, i.c.v administration of mu or kappa opioid agonists consistently produce a decrease in urinary sodium excretion (Huidobro-Toro and Huidobro, 1981; Danesh and Walker, 1988; Kapusta et al., 1993).

Although it is apparent that opioids can affect renal function via an action in the CNS, the specific brain nuclei and physiological regulatory mechanisms involved in producing these changes are largely unknown. The lack of knowledge in this area is primarily caused by the technical difficulties involved in implanting chronic CNS cannula and microinjecting drugs into single or multiple brain regions in conscious animals. In contrast, although stereotaxic techniques for microinjecting drugs in anesthetized animals (i.e., glass...
multibarrel pipette microinjection methods) are well established, this experimental approach has not been used to study the central control of renal function because the basal renal excretory levels for water and sodium are substantially reduced by the anesthesia and surgery (DeBodo and Prescott, 1945; Bachman, 1955; Papper et al., 1960; Mazze et al., 1963; Papper and Papper, 1964; Deutsch et al., 1969; Maddox et al., 1977). The reduced basal levels of urine flow rate and urinary sodium excretion present particular problems when studying the renal responses (e.g., antidiuresis or diuresis, antinatriuresis) produced by the central administration of opioids.

Because of the advantages of the use of CNS microinjection techniques to identify the brain regions involved in the central opioid control of renal function, we contemplated potential means by which urine flow rate and urinary sodium excretion may be augmented in anesthetized rats. Of relevance to this issue, central and peripheral administration of alpha-2 agonists (e.g., clonidine, guanabenz, BH-T 933, rilmenidine, etc.) produce diuretic and natriuretic responses in conscious and anesthetized rats (and other species) (Roman et al., 1979; Miller, 1980; Strandhoy et al., 1982; Strandhoy, 1985; Gellai and Ruffolo, 1987; Dawson and Wallace, 1989; Blandford and Smyth, 1990; Brooks et al., 1991; Kline et al., 1994). Despite these findings, alpha-2 agonists have not been used in conjunction with anesthesia to augment basal levels of urine flow rate and urinary sodium excretion for the purpose of performing studies of renal function in anesthetized animals. Instead, most studies have been directed toward determining the renal responses produced by administration of alpha-2 agonists, or have attempted to investigate the neurohumoral mechanism(s) by which alpha-2 agonists affect renal function.

With these considerations, the present investigations were designed to establish an experimental approach by which the renal responses produced by the central administration of opioid agonists could be studied in anesthetized rats. As mentioned previously, the development of such an experimental approach could ultimately be combined with established CNS microinjection techniques to investigate the brain sites and mechanisms involved in central opioid control of renal function. For this purpose, studies were performed in male Sprague-Dawley rats to evaluate the time course and renal responses elicited by the continuous intravenous (i.v.) infusion of ketamine in combination with the alpha-2 agonist, xylazine. Whereas other agents (e.g., ADH antagonists, other alpha-2 agonists, atrial natriuretic peptide, furosemide, etc.) may enhance the renal excretion of water and/or sodium in states of anesthesia and surgery, the administration of ketamine and xylazine offers the advantage of providing adequate levels of anesthesia (via ketamine) and analgesia (via xylazine) while maintaining a continuous alpha-2 agonist influence on renal function. Moreover, ketamine and xylazine are commonly used in combination and are approved for veterinary use as an anesthetic and analgesic, respectively. The validity of this method of anesthesia as a means to investigate central opioid-induced changes in renal excretory function was also tested by examining the renal responses produced by the i.c.v. injection of the selective mu opioid agonist, dermorphin (Broccardo et al., 1981), or the kappa opioid agonist, U-50488H (Slizgi et al., 1984). The renal responses obtained in these groups of ketamine/xylazine-anesthetized rats were then compared with responses observed previously when these opioids were administered i.c.v. to conscious rats.

Methods

Subjects

Male Sprague-Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, IN) weighing 280 to 310 g were used. All rats were fed normal sodium diets (Na+ content 163 meq/kg) and allowed tap water ad libitum. All experimental procedures were in accordance with the Louisiana State University Medical Center and National Institutes of Health guidelines for the care and use of experimental animals.

General Surgical Procedures

For studies that involved the administration of drugs into the CNS, the rats were anesthetized (ketamine, 30 mg/kg i.m. in combination with xylazine, 3 mg/kg i.m.) and implanted with a stainless steel cannula (23 gauge) into the right lateral cerebral ventricle 6 to 7 seven days before experimentation. The cannula was implanted 0.3 mm posterior to the bregma, 1.3 mm lateral to the midline and 4.5 mm below the skull surface (Paxinos and Watson, 1986). The cannula was fixed into position with jeweler’s screws and cranioplast cement. Verification of cannula position was made by observing the leakage of cerebrospinal fluid from the cannula after removal of the obturator (Kapusta et al., 1993; Kapusta and Obih, 1993).

On the day of the experiment, rats were anesthetized with sodium methohexital (Brevital, 35 mg/kg i.p.). Catheters (PE-50 fused to PE-10) were then implanted in the femoral artery and vein for the recording of arterial pressure and i.v. administration of drugs, respectively. The catheters were tunneled subcutaneously to the back of the neck, flushed and plugged. A suprapubic incision was then made and a bladder catheter (flanged PE-240) inserted and sutured into the urinary bladder. The bladder catheter was then exteriorized and secured by suturing to adjacent muscle, tissue and skin. After surgical preparation, the rat was placed in a rat holder which permitted the collection of urine samples. The arterial and venous catheters were connected to a pressure transducer (model P23Db, Statham, Oxnard, CA) and an infusion pump (model 944, Harvard Apparatus, South Natick, MA), respectively. Mean and pulsatile AP were recorded on a Grass 7D polygraph (Grass Instruments, Quincy, MA). HR was determined from the arterial pressure signal by a Grass model 7P4 tachograph. During surgery and the experimental protocol, the body temperature of rats was monitored by a rectal probe and maintained at 37°C using a heat lamp.

Experimental Protocols

Effects of xylazine on renal excretory function. After surgical preparation and placement into the rat holder, rats received ketamine (40 mg/kg i.v.) over a 5-min period. An i.v. infusion of ketamine (1.0 mg/kg/min) in isotonic saline (55 μl/min) was then started and continued for the duration of the study. The experimental protocol then began immediately with the collection of three consecutive ketamine control urine samples (10 min each). AP and HR were measured simultaneously. After the third ketamine control urine collection, xylazine (50 μg/kg/min, n = 8, or 25 μg/kg/min, n = 4) was added to the isotonic saline/ketamine solution and infused for the duration of the protocol. Fifteen minutes after starting the xylazine infusion, urine samples were collected for 11 consecutive experimental periods (10 min each), thus completing the protocol. In other rats (n = 4), extended time-control studies were performed in which the same experimental protocol was repeated, with the exception that consecutive 10-min urine samples were collected immediately after the start of the i.v. infusion of xylazine, 50 μg/kg/min, for 210 min (3.5 hr).

As demonstrated in time-controlled studies (Figs.1 and 2), urine
flow rate and urinary sodium excretion tended to stabilize 100–120 min after starting the xylazine infusion. Therefore, subsequent experimental protocols were initiated at least 120 min after starting the ketamine/xylazine infusion.

Effects of i.c.v. administration of opioids in ketamine/xylazine-anesthetized rats. After surgical preparation and placement into the rat holder, rats received ketamine (40 mg/kg i.v.) over 5 min. A supplemental i.v. infusion (55 μl/min) of isotonic saline containing ketamine (1 mg/kg/min) and xylazine (50 μg/kg/min) was then started and continued for the duration of the experiment. Anesthetized rats were then allowed at least 120 min for equilibration of urine flow rate and urinary sodium excretion. Urine was then collected during two consecutive 10-min control periods. The selective μ opioid agonist, dermorphin (0.1 nmol/kg; n = 5) or the κ opioid agonist, U-50488H (1.0 nmol/kg; n = 8) was then injected i.c.v. Dermorphin and U-50488H were allowed to distribute for 15 and 10 min, respectively. Continuous 10-min experimental urine samples were then collected for 1 hr. The i.c.v. doses of dermorphin (0.1 nmol/kg) and U-50488H (1 μg total) and the respective times allotted for their CNS distribution (15 and 10 min, respectively), were derived from previously published studies performed in conscious rats (Kapusta et al., 1993; Kapusta and Obih, 1993).

Effects of pentobarbital on renal excretory function. For comparative purposes, the cardiovascular and renal responses produced by continuous i.v. infusion of isotonic saline vehicle were examined in pentobarbital-anesthetized rats. Rats (n = 4) were anesthetized with pentobarbital sodium (50 mg/kg i.p.; Nembutal, Abbott Laboratories, North Chicago, IL). After surgical preparation (femoral artery and vein; bladder catheter) and placement in the rat holder, rats received a continuous i.v. infusion (55 μl/min) of isotonic saline vehicle containing pentobarbital (10 mg/kg/hr). The experimental protocol then began immediately with the collection of consecutive experimental urine samples (10 min each) for 210 min (3.5 hr). AP and HR were measured continuously.

Effects of i.c.v. administration of opioids in pentobarbital-anesthetized rats. After surgical preparation and placement into the rat holder, pentobarbital-anesthetized (50 mg/kg i.p.) rats received an i.v. infusion (55 μl/min) of isotonic saline containing supplemental pentobarbital (10 mg/kg/hr i.v.). After 100 min equilibration and continued pentobarbital infusion, three consecutive control urine samples (10 min each) were collected. The selective μ opioid agonist, dermorphin (0.1 nmol/kg; n = 4) was then injected i.c.v., and continuous 10 min experimental urine samples immediately collected for 1 hour.

Analytical Techniques

Urine volume was determined gravimetrically. Urine sodium concentration was measured by flame photometry (model 943, Instrumentation Laboratories, Lexington, MA). After all experiments, the kidneys were removed, decapsulated and weighed to normalize urinary excretory data per gram of kidney weight.

Drugs

The selective μ and κ opioid agonists, dermorphin (Broccardo et al., 1981) and U-50488H (Slizgi et al., 1984), respectively, were used for these studies. Stock solutions of dermorphin and U-50488H were prepared in isotonic saline and kept refrigerated. In previous investigations (Kapusta et al., 1993; Kapusta and Obih, 1993), the i.c.v. injection of dermorphin (0.1 nmol/kg) or U-50488H (1 μg total) has been shown to evoke concurrent centrally mediated diuretic and antinatriuretic responses in conscious Sprague-Dawley rats that were infused with isotonic saline at the same rate (i.e., 55 μl/min).

Data Analysis

The data were statistically analyzed using repeated measures analysis of variance for the main effects and interactions and Scheffè’s test was used for pairwise comparisons between means (Wallenstein et al., 1980). Statistical significance was defined as P < .05.

Results

Figure 1 illustrates the time-course effects of i.v. xylazine infusion on systemic cardiovascular and renal excretory function in ketamine-anesthetized Sprague-Dawley rats. Mean values for each parameter are depicted during continuous i.v. infusion of isotonic saline vehicle (55 μl/min) containing ketamine alone (ketamine control, C, 30 min) (40 mg/kg i.v. bolus over 5 min, and subsequently 1.0 mg/kg/min), and during consecutive 10-min experimental urine samples beginning 15 min after adding xylazine to the infusate (50 μg/kg/min) (25–125 min). Intravenous infusion of ketamine/xylazine produced adequate levels of anesthesia and analgesia as assessed by the lack of corneal and tail-pinch reflex responses. Compared with ketamine infusion alone (C), the addition of xylazine produced a significant reduction in HR and MAP that was immediate in onset and sustained over the duration of study. Before xylazine administration, the levels of urine flow rate and urinary sodium excretion during ketamine infusion alone (C) were 6.3 ± 1.3 μl/min/gkw and 0.28 ± 0.08 μeq/min/gkw, respectively. The subsequent administration of xylazine (50 μg/kg/min) produced an immediate increase in urine flow rate and a gradual increase in urinary sodium excretion. Urine flow rate and urinary sodium excretion tended to equilibrate and reach steady-state.

Fig. 1. Effects of i.v. xylazine infusion on cardiovascular and renal excretory responses in Sprague-Dawley rats anesthetized with ketamine. Values are means ± S.E. illustrating the systemic cardiovascular and renal effects of xylazine (50 μg/kg/min, n = 8; 25 μg/kg/min, n = 4) in rats anesthetized (40 mg/kg i.v. bolus over 5 min) and infused i.v. (55 μl/min) with ketamine (1.0 mg/kg/min). Urine samples were collected during ketamine control (C, 30 min), and 15 min after the start of xylazine infusion (consecutive 10-min urine samples). V, urine flow rate; UNaV, urinary sodium excretion.
levels 95 to 125 min after starting the xylazine infusion. The i.v. infusion of a lower dose of xylazine (25 \( \mu g/kg/min \)) produced a slow onset diuretic response but failed to alter urinary sodium excretion at any time point (fig. 1). Infusion of xylazine at 25 and 50 \( \mu g/kg/min \) produced similar reductions in HR; however, the lower dose of xylazine evoked a greater hypotensive response. Based on these observations, all subsequent experimental protocols were performed with an i.v. infusion dose of 50 \( \mu g/kg/min \) xylazine, in combination with ketamine (40 mg/kg i.v. over 5 min, and subsequently 1.0 mg/kg/min i.v.).

Figure 2 demonstrates the cardiovascular and renal responses produced over an extended time course of i.v. infusion of 50 \( \mu g/kg/min \) xylazine in ketamine-anesthetized (40 mg/kg i.v. bolus over 5 min, and subsequently 1.0 mg/kg/min) rats. For purposes of comparison, the cardiovascular and renal responses produced by continuous i.v. infusion of isotonic saline in rats anesthetized with an alternative anesthetic, pentobarbital (50 mg/kg i.p. followed by 10 mg/kg/hr i.v.), are also shown. In ketamine-anesthetized rats, xylazine produced a pattern of changes in cardiovascular and renal excretory function similar to that illustrated in figure 1. Moreover, after stabilization (approximately 120 min), urine flow rate and urinary sodium excretion tended to remain constant for an additional 90 min (time points, 120–210 min). In contrast to these enhanced renal excretory responses observed in ketamine/xylazine-anesthetized rats, urine flow rate and urinary sodium excretion remained substantially low during the continuous i.v. infusion of isotonic saline in pentobarbital-anesthetized animals. The renal excretory levels for water and sodium observed in these pentobarbital-anesthetized rats were similar to the substantially reduced levels for urine flow rate and urinary sodium excretion observed in rats anesthetized with ketamine anesthesia alone (fig. 1C).

The renal responses produced by i.c.v. opioid agonist administration were studied in ketamine/xylazine-anesthetized rats. Shown in figure 3 are the cardiovascular and renal responses produced by i.c.v. injection of the selective \( \mu \) opioid agonist, dermorphin, in five rats receiving a ketamine/xylazine infusion for 120 min. Consecutive 10-min experimental urine samples beginning 15 min after i.c.v. administration of dermorphin (0.1 nmol/kg) are denoted D1–D6. The

![Fig. 2. Time-course changes in cardiovascular and renal excretory function produced by i.v. infusion of isotonic saline containing xylazine in ketamine-anesthetized rats, or i.v. infusion of isotonic saline alone in pentobarbital-anesthetized Sprague-Dawley rats. Format and abbreviations are the same as in figure 1. Values are means ± S.E. illustrating the cardiovascular and renal responses produced by continuous i.v. infusion (55 \( \mu l/min \)) of isotonic saline containing xylazine (50 \( \mu g/kg/min \)) in ketamine-anesthetized (40 mg/kg i.v. bolus over 5 min, and 1 mg/kg/min i.v.) rats. Urine samples were collected during ketamine control (C, 30 min), and immediately after the start of the xylazine infusion (consecutive 10-min urine samples for 210 min).●-Values are means ± S.E. illustrating the cardiovascular and renal responses produced by continuous i.v. infusion (55 \( \mu l/min \)) of isotonic saline in pentobarbital-anesthetized (50 mg/kg i.p. and 10 mg/kg/hr i.v.) rats. Urine samples were collected immediately after the start of i.v. infusion of isotonic saline containing supplemental pentobarbital anesthesia (consecutive 10-min urine samples for 210 min).

![Fig. 3. Cardiovascular and renal responses produced by i.c.v. dermorphin in ketamine/xylazine-anesthetized Sprague-Dawley rats. Values are means ± S.E. illustrating systemic cardiovascular and renal responses produced by i.c.v. administration of the selective \( \mu \) opioid agonist, dermorphin (0.1 nmol/kg), in five ketamine/xylazine-anesthetized rats. During continuous i.v. infusion of isotonic saline (55 \( \mu l/min \)) containing ketamine (1 mg/kg/min) and xylazine (50 \( \mu g/kg/min \)), urine samples were collected during control (C1–C5: 10 min each), and 15 min after i.c.v. dermorphin denoted D1–D6 (consecutive 10-min urine samples). Format and abbreviations are same as in figure 1. *P < .05, significantly different from C1 and C2.]
i.c.v. administration of dermorphin did not significantly change HR or MAP at any time. In contrast, i.c.v. dermorphin significantly increased urine flow rate over the first five experimental periods (D₁–D₅), after which levels returned to control by period D₆ (75 min after dermorphin injection).

Concurrent with the diuretic response, i.c.v. dermorphin produced an immediate and sustained decrease in urinary sodium excretion. In contrast to the diuretic and antinatriuretic responses produced by i.c.v. dermorphin in ketamine/xylazine-anesthetized rats, i.c.v. injection of the same dose of dermorphin did not alter urine flow rate or urinary sodium excretion in pentobarbital-anesthetized rats (table 1).

Figure 4 shows the cardiovascular and renal excretory responses produced by i.c.v. administration of the selective kappa opioid agonist, U-50488H (1 µg total), in eight ketamine/xylazine-anesthetized rats. Two hours after starting the ketamine and xylazine infusion, consecutive 10-min experimental urine samples, beginning 10 min after i.c.v. U-50488H administration, were collected. The i.c.v. administration of U-50488H failed to change HR or MAP. In contrast, i.c.v. U-50488H significantly increased urine flow rate over the first three experimental periods. Urine flow rate returned to control levels by the fourth experimental period (50 min after injection). I.c.v. U-50488H significantly decreased urinary sodium excretion for up to 30 min after injection (U1, U2).

**Discussion**

This study examined the renal responses produced by the i.v. infusion of the alpha-2 agonist, xylazine, in ketamine-anesthetized Sprague-Dawley rats, and determined the renal excretory responses produced by the central administration of opioid agonists under these anesthetic conditions. These studies demonstrated that the i.v. infusion of xylazine significantly enhanced basal levels of urine flow rate and urinary sodium excretion in ketamine-anesthetized rats. The levels of urine flow rate and urinary sodium excretion attained in these anesthetized animals were similar to those achieved in conscious, untreated rats receiving infusion of isotonic saline at similar rates (Kapusta et al., 1993; Kapusta and Obih, 1993). These higher basal levels of urine flow rate and urinary sodium excretion are in contrast to the low renal excretory levels for water and sodium observed in anesthetized rats in which an alpha-2 agonist was not administered. For instance, in the present studies the i.v. infusion of isotonic saline containing ketamine alone (40 mg/kg i.v. over 5 min, and subsequently 1.0 mg/kg/min) resulted in low basal levels of urine flow rate and urinary sodium excretion (fig. 1).

Similarly, in other studies urine flow rate and urinary sodium excretion remained low in pentobarbital-anesthetized (50 mg/kg i.p.) and maintained (10 mg/kg/hr i.v.) rats, despite the continuous i.v. infusion of isotonic saline vehicle (55 µl/min) (fig. 2). These observations are similar to those in previous reports which showed that when accompanied by the stress of the surgery, administration of virtually all anesthetic agents significantly reduced renal blood flow, glomerular filtration rate, urine flow rate and urinary sodium excretion (DeBodo and Prescott, 1945; Bachman, 1955; Papper et al., 1960; Mazze et al., 1963; Papper and Papper, 1964; Deutsch et al., 1969; Maddox et al., 1977).

It is well documented that i.c.v. administration of mu and

**TABLE 1**

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<th>cardiovascular and renal responses produced by i.c.v. injection of dermorphin in pentobarbital-anesthetized rats</th>
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<td>Values are means ± S.E.; n = 4. Sprague-Dawley rats were anesthetized (50 mg/kg i.p.) and maintained (10 mg/kg/hr i.v.) with pentobarbital in isotonic saline vehicle (55 µl/min). Control, three consecutive 10-min control periods beginning 100 min after the start of pentobarbital infusion; V, urine flow rate; U₀V, urinary sodium excretion; gkw, gram kidney weight.</td>
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kappa opioids can significantly affect urine flow rate and urinary sodium excretion in conscious rats (Kapusta, 1995). For example, the i.c.v. administration of kappa opioids (e.g., U-50488H, bremezocine) produces a profound diuretic response that is associated with an antinatriuresis (Cowan and Khunawat, 1986; Danesh and Walker, 1988; Salas et al., 1989; Brooks et al., 1993; Kapusta and Obih, 1993). Although the i.c.v. injection of mu opioids (e.g., β-endorphin, morphine, dermorphin) also decreases urinary sodium excretion in conscious rats, mu opioids produce either a marked diuretic or antidiuretic response (Tseng et al., 1978; Huidobro-Toro and Huidobro, 1981; Danesh and Walker, 1988; Kapusta et al., 1993). In contrast to the many studies performed in conscious rats (and other species), only a few investigations have examined the effects of opioids on renal function in anesthetized animals. The lack of data from anesthetized studies is most likely caused by the difficulties associated with the reductions in the basal levels of urine flow rate and urinary sodium excretion produced by the anesthesia (and surgery). This point is illustrated by our observation that in pentobarbital-anesthetized rats, urine flow rate and urinary sodium excretion remained at low levels during the i.v. infusion of isotonic saline containing pentobarbital. In other cases where the affects of opioids (central or peripherally administered) on renal excretory function have been studied in anesthetized animals, opioids tend to evoke only blunted changes in urine flow rate. Such low renal excretory levels can also prevent observation of the renal responses (antiuresis/diuresis, antinatriuresis) produced by administration of opioids. To examine this possibility, we also examined the renal responses produced by i.v. injection of the mu opioid agonist, dermorphin, in pentobarbital-anesthetized rats. In these studies, i.v. injection of 0.1 nmol/kg dermorphin, a dose that produces a profound diuretic and antinatriuretic response in conscious rats (Kapusta et al., 1993), failed to alter either renal excretory parameter (table 1). In related studies, anesthesia and surgery has been shown to alter the basal levels of water and sodium excretion and to alter the pattern of renal responses produced by kappa opioid agonists. For instance, Salas et al. (1992) reported that in conscious rats (implanted with a bladder catheter and infused i.v. with isotonic saline), the kappa opioid agonist, E-2078 (50 μg i.v.), produced a diuresis (control, 40 ± 7.5; 60 min, 94 ± 30 μl/min) and antinatriuresis (control, 3.0 ± 0.6; 60 min, 1.5 ± 0.5 μeq/min). In contrast, when these studies were repeated in pentobarbital-anesthetized rats, basal renal excretory levels were dramatically reduced and the same dose of E-2078 (50 μg) produced a blunted diuretic response (control, 4.0 ± 0.5; 60 min, 6.3 ± 1.0 μl/min). Moreover, in these same studies the antinatriuresis was no longer detectable (control, 0.2 ± 0.05; 60 min, 0.3 ± 0.1 μeq/min) (Salas et al., 1992).

To overcome the impairment of renal excretory function produced by the anesthesia and surgery, previous investigations have used strategies such as water and/or alcohol loading via gavage, or use of exceedingly high i.v. infusion rates of isotonic saline. Although these approaches may augment basal renal excretory levels in anesthetized animals, they also tend to allow only expression of unidirectional renal responses. Related to this issue, it is recognized that plasma volume decreases as a consequence of anesthesia and surgery (Maddox et al., 1977) and that correction of the intravascular hypovolemia by isoncotic plasma infusion tends to restore water and sodium excretion in anesthetized and surgically operated rats (Maddox et al., 1977).

In the present studies, we showed that the i.c.v. administration of opioids in ketamine/xylazine-anesthetized rats produces renal excretory responses that are similar in magnitude and direction to those previously observed in conscious rats. In conscious rats, i.c.v. microinjection of 0.1 nmol/kg dermorphin, a selective mu opioid agonist, produces a concurrent increase in urine flow rate and a reduction in urinary sodium excretion (Kapusta et al., 1993). In our ketamine/xylazine-anesthetized rats, i.c.v. injection of the same dose of dermorphin also produced a significant diuretic and antinatriuretic response (fig. 3). The absolute magnitude of the changes in urine flow rate and urinary sodium excretion were similar in ketamine/xylazine-anesthetized and conscious rats. In the present studies i.c.v. dermorphin increased urine flow rate from 33 ± 6 μl/min/gkw to a maximum of 58 ± 5 μl/min/gkw in ketamine/xylazine-anesthetized rats (fig. 3, period D2). In conscious rats, the same i.c.v. dose of dermorphin increased urine flow rate from 28 ± 4 μl/min/gkw to a maximum of 63 ± 7 μl/min/gkw (Kapusta et al., 1993). I.c.v. dermorphin also produced similar reductions in urinary sodium excretion in ketamine/xylazine-anesthetized (fig. 2) and conscious rats (Kapusta et al., 1993). Thus, these results indicate that ketamine/xylazine-anesthetized rats respond to the central administration of the mu opioid agonist, dermorphin, in a manner similar to conscious rats. Recall, however, that the diuretic or antinatriuretic response produced by this i.c.v. dose of dermorphin was not detected in pentobarbital-anesthetized rats.

Additional studies were also performed to determine whether the i.c.v. injection of selective kappa opioid agonists are similar in ketamine/xylazine-anesthetized and conscious rats. In ketamine/xylazine-anesthetized rats, the i.c.v. injection of the selective kappa opioid agonist, U-50488H (1 μg total), evoked a concurrent diuretic and antinatriuretic response without altering HR or MAP (fig. 4). The pattern of these renal excretory responses were similar in direction and magnitude to those observed after the i.c.v. administration of 1 μg total, U-50488H, in conscious rats (Kapusta and Obih, 1993). These results demonstrate that the central administration of kappa opioid agonists elicits similar renal excretory responses in ketamine/xylazine-anesthetized and conscious rats.

The CNS sites and mechanisms mediating the renal responses produced by i.c.v. administration of opioid agonists are largely unknown. This lack of knowledge may reflect the necessity to perform these studies in conscious animals in which basal renal excretory function is maintained. However, studies of CNS function, especially those simultaneously examining multiple brain nuclei, are technically very difficult to perform in conscious animals. The ability of ketamine/xylazine anesthesia to augment basal renal excretory function (and produce anesthesia/analgesia) and allow full expression of the renal excretory responses produced by i.c.v. administration of opioids is unique and of interest experimentally. This experimental protocol may provide a viable model to examine the CNS sites and mechanisms involved in the central opioid control of renal excretory function with established CNS microinjection techniques (e.g., multibarrel glass pipette).
The observation that stimulation of \( \alpha-2 \) adrenoceptors by the i.v. infusion of xylazine produced diuresis and natriuresis in rats confirms previous observations with other \( \alpha-2 \) adrenergic agonists (Roman et al., 1979; Stanton et al., 1985; Kline and Mercer, 1990; Kline et al., 1994). Although not the focus of the present investigations, there are several mechanisms by which i.v. xylazine may act to increase urine flow rate and urinary sodium excretion in ketamine-anesthetized rats. These possibilities include an action of xylazine to inhibit: 1) central sympathetic outflow to the kidneys, 2) the intrarenal action of ADH on water permeability and electrolyte transport, 3) the central release of ADH and/or 4) the action of xylazine to promote the release of hormones with natriuretic/diuretic properties (e.g., atrial natriuretic factor, dopamine, etc.) (Roman et al., 1979; Miller, 1980; Strandhoy et al., 1982; Strandhoy, 1985; Stanton et al., 1985; Brooks et al., 1991; Kline et al., 1994). In the present studies, i.v. infusion of xylazine at a dose of 50 \( \mu \text{g/kg/min} \) in ketamine-anesthetized rats produced a prompt diuresis and natriuresis. As mentioned previously, these renal excretory responses tended to stabilize after approximately 120 min after the start of the xylazine infusion (figs. 1 and 2). In contrast, an i.v. infusion of a lower dose of xylazine (25 \( \mu \text{g/kg/min} \)) produced a gradual increase in urine flow rate, but failed to increase urinary sodium excretion (fig. 1). These results suggest that in ketamine-anesthetized rats, the diuretic and natriuretic effects of xylazine are dose-related, and that the threshold dose of xylazine required to produce a significant increase in urine flow rate and urinary sodium excretion is between 25 and 50 \( \mu \text{g/kg/min} \). In agreement with these findings, Blandford and Smyth (1988) demonstrated a dose-related selectivity of alpha-2 adrenoceptor stimulation for water and sodium excretion. As suggested by Strandhoy et al. (1982) and Strandhoy (1985), the dissociation of the renal sodium and water excretory responses may reflect differences in the alpha adrenergic mechanisms controlling renal function. It should also be noted that the 25 \( \mu \text{g/kg/min} \) infusion of xylazine reduced MAP to a level that approached the renal autoregulatory pressure-flow break point (fig. 1). Although not investigated, the delayed diuresis and failure of lower doses of xylazine to evoke a natriuresis, may be partly related to this hypotensive response. The central and peripheral mechanisms by which xylazine augments renal excretory function in ketamine-anesthetized rats are currently being investigated in our laboratories. In addition, we are attempting to determine whether the mechanisms mediating the renal responses elicited by i.c.v. administration of opioids in ketamine/xylazine-anesthetized and conscious rats are the same.

The present investigations have demonstrated that in Sprague-Dawley rats, ketamine anesthesia substantially reduced urine flow rate and urinary sodium excretion, and that the i.v. infusion of the alpha-2 agonist, xylazine, significantly augmented these renal excretory parameters. Further, in ketamine/xylazine-anesthetized rats, the i.c.v. injection of selective \( \mu \) or kappa opioid agonists produced profound diuretic and antinatriuretic responses that were similar in pattern to those observed in conscious rats. These findings suggest that the ketamine/xylazine protocol may provide a novel approach to study the central opioid control of renal function during conditions that require anesthesia (CNS microinjection). For this purpose, the ketamine/xylazine protocol provides the following advantages: 1) an augmentation of base-line levels of urine flow rate and urinary sodium excretion, 2) the ability to detect bidirectional changes in renal excretory function, 3) the capability to characterize central opioid-induced changes in renal excretory function that are similar to those responses observed in conscious rats and 4) the ability to study multiple central sites in each animal and to examine interactions and functional pathways between these CNS sites. Thus, this anesthetized preparation appears to be a practical experimental approach to study central opioid control of renal function because it possesses a level of renal function similar to that of a nonanesthetized rat.

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References


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