Endogenous central kappa opioid systems augment renal sympathetic nerve activity to maximally retain urinary sodium during hypotonic saline volume expansion

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Running Head: Hypotonic saline volume expansion and central kappa opioids

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ABSTRACT

Intracerebroventricular (i.c.v.) injection of kappa opioid agonists produces diuresis, antinatriuresis and a concurrent increase in renal sympathetic nerve activity (RSNA). The present study examined whether endogenous central kappa opioid systems contribute to the renal excretory responses produced by the stress of an acute hypotonic saline volume expansion (HSVE). Cardiovascular, renal excretory and RSNA responses were measured during control, acute HSVE (5% BW, 0.45 M saline over 30-min) and recovery (70-min) in conscious rats pre-treated i.c.v. with vehicle or the kappa opioid receptor antagonist, nor-binaltorphimine (nor-BNI). In vehicle pretreated rats, HSVE produced a marked increase in urine flow rate, but only a low magnitude and delayed natriuresis. RSNA was not significantly suppressed during the HSVE or recovery periods. In nor-BNI-treated rats, HSVE produced a pattern of diuresis similar to that observed in vehicle-treated rats. However, during the HSVE and recovery periods RSNA was significantly decreased and urinary sodium excretion increased in nor-BNI treated animals. In other studies performed in chronic bilateral renal denervated rats, HSVE produced similar diuretic and blunted natriuretic responses in animals pretreated i.c.v. with vehicle or nor-BNI. Thus, removal of the renal nerves prevented nor-BNI from enhancing urinary sodium excretion during HSVE. These findings indicate that in conscious rats, endogenous central kappa opioid systems are activated during hypotonic saline volume expansion to maximize urinary sodium retention by a renal sympathoexcitatory pathway that requires intact renal nerves.

Keywords: urine flow rate, urinary sodium excretion, antinatriuresis, renal excretory function, central nervous system, endorphin, nor-binaltorphimine, renal nerves, Sprague-Dawley rats.
INTRODUCTION

The intracerebroventricular (i.c.v.) administration of kappa opioid agonists (e.g., U-50,488H, dynorphin A(1-17), etc.) produces a profound increase in urine flow rate and renal sympathetic nerve activity (RSNA) and a concurrent decrease in urinary sodium excretion in conscious rats (7,10,14). The renal excretory and RSNA responses produced by central kappa opioid agonists are blocked by the i.c.v. pre-treatment of animals with the selective kappa opioid receptor antagonists, nor-binaltorphimine (nor-BNI) (10). Thus, activation of central kappa opioid receptor pathways evoked by the exogenous administration of kappa agonists produces a unique solute-free water diuresis (6,10,15,16,21,23,24). The mechanisms by which kappa opioids evoke diuretic and antinatriuretic responses has been shown to involve inhibition of the release/secretion of vasopressin (15,16,22,24,25) and augmentation of central sympathetic outflow to the kidneys (10,11), respectively.

In contrast to the above findings, the central injection of the kappa opioid receptor antagonist, nor-BNI, fails to elicit any change in baseline cardiovascular or renal function in conscious rats (10). These data indicate that under the basal conditions studied, central kappa opioid systems do not exert a tonic influence on cardiovascular or renal excretory function. However, it is possible that in conscious rats, opioid systems remain quiescent during basal conditions and do not affect cardiovascular or renal function (or other biological processes) until activated by an appropriate manipulation, stress, or pathology. For example, based on their water diuretic profile it would be anticipated that endogenous central kappa opioid systems would be activated during acute stressful or pathological states that cause disturbances in fluid and electrolyte balance which lead to volume overload and/or hyponatremia. Under these conditions, it would be anticipated that endogenous central kappa opioid pathways would contribute in
mediating an increase in urine output and/or a decrease in urinary sodium excretion as a means to help restore intravascular volume and/or electrolyte/osmolar concentration to normal.

Based on these considerations, the present investigations were performed in conscious Sprague-Dawley rats to determine whether endogenous central kappa opioid systems are activated and contribute to the renal excretory responses produced by the stress of an acute hypotonic saline volume expansion (HSVE). In these studies, the highly selective kappa opioid receptor antagonist, nor-binaltorphimine (nor-BNI), was used as a pharmacological tool to elucidate a role for endogenous central kappa opioid systems on cardiovascular and/or renal excretory function during HSVE. Participation of the renal sympathetic nerves in mediating HSVE-induced changes in renal excretory function was also examined by directly measuring RSNA and by repeating the experimental protocol in rats that had the influence of the renal nerves on kidney function removed by chronic bilateral renal denervation. HSVE was selected as a stressor for these studies since it was predicted that this fluid and electrolyte challenge would activate native central nervous system (CNS) pathways (e.g., kappa opioid) to excrete water and maximally retain sodium in an attempt to prevent water overload and ultimately hyponatremia.

METHODS

Subjects

Experiments were performed using male Sprague-Dawley rats (270-350 g, Harlan inc., Indianapolis, IN). The rats were group housed in a temperature and humidity-controlled room with a 12 hours light/dark cycle. Standard rat chow (sodium content 163 meq/kg) and tap water were available ad libitum. All procedures were conducted in accordance with the National Institutes of Health guidelines for the Care and Use of Animals and were approved by the
General Surgical Procedures

Rats were implanted with a chronic i.c.v. cannula at least 5-7 days before experimentation. Chronic i.c.v. cannula (23-gauge stainless steel) implantation was performed in rats anesthetized with ketamine (100 mg/kg i.p.) and xylazine (10 mg/kg i.p.) using standard stereotaxic procedures (10,11,12). Custom cut and fabricated guide, dummy (obturator) and internal cannula were purchased from Plastics One, Inc. Roanoke, Virginia, USA. The coordinates for i.c.v. cannula implantation were 0.3 mm posterior to the bregma, 1.3 mm lateral to the midline and 4.5 mm below the skull surface (19). The cannula was fixed into position with jeweler’s screws and cranioplast cement. Verification of cannula position in the lateral cerebroventricle was made by observing spontaneous flow of cerebrospinal fluid (CSF) from the tip of the cannula after removal of the obturator or by observing injected dye in the lateral ventricle following completion of the study and subsequent postmortem brain section (8,10-14).

On the day of the experiment, rats were anesthetized with sodium methohexital (Brevital; 35 mg/kg, i.p., supplemented with 10 mg/kg, i.v. as needed; King Pharmaceuticals, Bristol, TN) and implanted with chronic indwelling catheters (PE-10 connected to PE-50, Becton Dickinson and Company, Sparks, MD) into the femoral artery and vein for measurement of arterial blood pressure and infusion of isotonic saline vehicle/drugs, respectively. Through a suprapubic incision, a catheter (flanged PE-240, Becton Dickinson and Company, Sparks, MD) was also implanted into the urinary bladder for collection of urine samples. All catheters were exteriorized and securely sutured to adjacent muscle and skin. 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. The mean and pulsatile arterial pressures were recorded on a grass model 7 polygraph (Grass instruments, Quincy, MA). Heart rate was determined from the arterial pressure signal by a Grass model 7P4 Tachograph.

After implantation of these catheters, certain rats (still anesthetized with sodium methohexital) were also implanted with a recording electrode on a renal nerve bundle for direct measurement of multifiber RSNA using techniques described previously (6,10-12). The left kidney was exposed through a left incision via a retroperitoneal approach. With the use of a dissecting microscope (25 X), a renal nerve branch from the aorticorenal ganglion was isolated and carefully dissected. The renal nerve branch was then placed on a bipolar platinum wire (Cooner Wire Company, Chatsworth, CA) electrode. Renal sympathetic nerve activity was amplified (10,000-50,000 X) and filtered (low, 30; high, 3000 Hz) with a Grass P511 Bandpass Amplifier (Quincy, MA). When an optimal RSNA signal was observed, the recording electrode was fixed to the renal nerve branch with a dentistry impression material (Coltene President). The electrode cable was then secured in position by suturing it to the abdominal trunk muscles. Finally, the electrode cable was exteriorized, and the flank incision was closed in layers. The amplified and filtered signal was channeled to a Tektronix 5113 Oscilloscope (Tektronix, Beaverton, OR) and Grass model 7DA polygraph for visual evaluation, to an audio amplifier-loudspeaker (Grass model AM 8 Audio Monitor) for auditory evaluation, and to a rectifying voltage integrator for quantification (Grass model 7P10). The integrated voltage signals were displayed on the Grass polygraph and data acquisition for RSNA measurements were performed with a commercially available software package (Acknowledge for Windows, Biopac Inc., Santa Barbara, CA). Integrated RSNA was expressed as microvolt-seconds per 1-sec intervals. For
each 10-min experimental period, the values for integrated RSNA were sampled over the entire collection period and the numbers were averaged. The data for RSNA are expressed as the percent of the baseline value obtained during the control period alone, with this being expressed as 100% for each animal. The quality of the RSNA signal was assessed before and after the experimental protocol by i.v. bolus injections of norepinephrine (3 µg). The level of postmortem background noise observed after completion of the study was subtracted from all control and experimental values of RSNA.

Other studies were performed in conscious rats in which the influence of the renal nerves on kidney function was removed. For these experiments, rats were anesthetized with ketamine (100 mg/kg i.p.) and xylazine (10 mg/kg i.p.) and underwent chronic bilateral renal denervation 5 to 7 days before experimentation. Through flank incisions, the left and right kidneys were exposed and the renal arteries and veins were stripped of any nerve bundles visible using a 25X Olympus microscope. To increase the efficiency of the renal denervation, each renal artery and renal vein were brushed with 10% phenol. After the surgery, the rats were treated with Ambi-Pen (Butler, .3 cc, i.m.), and placed in individual cages. Because our laboratory has previously and repeatedly verified that this renal denervation procedure completely removes the influence of the renal nerves in kidney function (8,10,11,17), verification of renal denervation was not repeated in these studies.

After surgical preparation and recovery from anesthesia, the rat was placed in a rat holder (a chamber of stainless steel rods connected by plexiglas ends; the metal rods formed an inverted U shape and a flat base in which the rat would sit) which permits forward and backward movement of the rat, allows for steady-state collection of urine, and protects the renal nerve recording preparation. An i.v. infusion (20 µl/min) of isotonic saline was then started and
continued for the duration of the experiment except for during the HSVE period (see below). One to 2 hours after recovery and start of isotonic saline infusion the experiment commenced.

*Experimental protocols*

Studies were performed to determine the cardiovascular and renal excretory responses produced by the stress of a HSVE in conscious rats that were pre-treated i.c.v. with nor-BNI or isotonic saline vehicle. After stabilization of cardiovascular and renal excretory parameters in conscious rats infused (20 µl/min) with isotonic saline (approximately two hours), two consecutive ten-minute control urine samples were collected. Rats then received i.c.v. injection of vehicle (5 µl isotonic saline; n=6) or the kappa opioid receptor antagonist, nor-BNI (1 µg; n=6). Nor-BNI was chosen for use in the current studies because of its high selectivity for the kappa opioid receptor (26,27). Further, we have demonstrated that at this dose (1 µg, i.c.v.) central nor-BNI completely prevents the renal excretory responses produced by i.c.v. administration of the selective kappa opioid receptor agonist, U50488H, but not the renal excretory responses to the mu-opioid agonist, dermorphin (10,13) or the opioid-like peptide, nociceptin/orphanin FQ (14). After i.c.v. injection, ten minutes was allowed for distribution of nor-BNI or vehicle during which time a control urine sample was collected. Next, the i.v. isotonic saline infusate was stopped and an acute i.v. hypotonic saline volume expansion (HSVE, 5% body weight; 0.45 M saline) was then started and carried out for 30-minutes, with experimental urine samples being collected every ten minutes. After 30 minutes, the HSVE challenge was stopped and the i.v. infusate was switched back to isotonic saline at a rate of 20 µl/minute for the remainder of the protocol. A 70-minute recovery period was then allowed with urine samples being collected every ten minutes. At the end of the experiment, the collected
urine samples were weighed, analyzed for sodium and potassium concentration (IL943 automatic flame photometer, Instrumentation Laboratory), and stored at 10° C.

Additional studies were performed to determine the role of the renal nerves in mediating the cardiovascular and renal excretory responses to the HSVE challenge in vehicle and nor-BNI treated animals. Two types of studies were conducted for this purpose. In the first, the acute HSVE study described above was repeated in rats pretreated i.c.v. with isotonic saline vehicle (5 µl; n=6) or nor-BNI (1 µg; n=6) with the exception that animals in each group were chronically instrumented with an electrode for measurement of changes in RSNA. In other studies, investigations were performed to establish whether an intact renal innervation was required to elicit the cardiovascular and/or renal responses to the acute HSVE challenge. For these studies, the acute HSVE protocol was conducted in chronic bilaterally renal denervated rats that were pre-treated i.c.v. with vehicle (5 µl; n=6) or nor-BNI (1 µg; n=5).

**Analytical techniques**

The arterial and venous catheters were connected to a pressure transducer (model P23Db, Stathan, Oxnard, CA) and an infusion pump (model 944, Harvard apparatus, South Natick, MA) respectively. The mean and pulsatile arterial pressure, heart rate, and renal nerve activity (raw neurogram and integrated signal) signals were recorded on a Grass model 7 polygraph (Grass instruments, Quincy, MA).

Measurements of heart rate, mean arterial pressure, and RSNA were measured directly from the polygraph tracings and Biopack software (Acknowledge version 3.7.2, Santa Barbara, CA). The urine volume was determined gravimetrically and the urine sodium concentration was measured by flame photometry (Instrumentation Laboratories, model IL943).
Data analysis

Data are expressed as means ± S.E (figures). The response was calculated for each rat and each analyzed parameter as a difference (delta) between the actual observed value at each time point and the value at baseline. For the analysis, the two-way repeated measures approach was used with treatment being one fixed effect and time another, with the interaction included. The time (minutes) was the repeated factor. For modeling the covariances, the autoregressive type I covariance matrix was assumed. The Kenward-Roger type F-test and the denominator degrees of freedom calculations were used in the analysis. For multiple comparisons the Tukey-Cramer adjustments were used. The baseline was taken as the value for each parameter and each rat during the 10-min control period immediately prior to the i.c.v. injection of vehicle or drug. The area under the curve (AUC) was calculated with the trapezoid method after starting HSVE and continuing over the first 30-min (HSVE period only) or the entire 100-min experimental protocol (HSVE and recovery periods). The means of AUC were compared allowing for different variability for the treatment groups. The significance level was set to 0.05.

Drugs

The drugs used in this study were nor-BNI (Research Biochemicals, Natick, MA), sodium methohexital (Monarch Pharmaceuticals Inc., Bristol, TN), and ketamine hydrochloride (Phoenix Scientific, Inc., St. Joseph, MO), and xylazine (Butler Corp., Columbus, OH).
RESULTS

Figure 1 depicts the changes in cardiovascular and renal excretory function produced by an acute i.v. hypotonic saline volume expansion (HSVE, 30-min) in Sprague-Dawley rats pre-treated i.c.v. with the selective kappa opioid receptor antagonist, nor-BNI, or isotonic saline vehicle. In accord with previous observations (10,11,13), the i.c.v. injection of either nor-BNI or isotonic saline vehicle (Fig. 1, V/N) itself did not significantly alter cardiovascular or renal excretory function. In addition, heart rate and mean arterial pressure were not affected throughout the protocol by the stress of the HSVE. However, as compared to respective control levels for each group, HSVE significantly increased (p<0.001) urine flow rate in animals treated with vehicle (C, 32±5 µl/min; HSVE 30-min peak, 255±20 µl/min) or nor-BNI (C, 58±16 µl/min; HSVE 30-min peak, 327±14 µl/min). Concurrent with the diuresis, the HSVE challenge also increased urinary sodium excretion but there were differences in the pattern of the response between groups (Fig. 1). In rats treated i.c.v. with vehicle, there was a slow but progressive increase in urinary sodium excretion which was significantly elevated (p<0.01) above the respective control level in the last 10-min of the HSVE period (C, 2.46 ± 1.08 µeq/min; HSVE 30-min; 8.4±2.5 µeq/min) and at each time point of the recovery after the HSVE was terminated (recovery period time points 40-100 min). In contrast, rats pre-treated i.c.v. with the kappa opioid receptor antagonist nor-BNI showed a rapid increase in urinary sodium excretion which was already significantly (p<0.001) elevated above respective control levels in the first 10-min of the HSVE period (C, 3.71 ± 1.36 µeq/min; HSVE 10-min, 10.22 ± 1.99 µeq/min) and which was sustained for the remainder of the HSVE and recovery periods. Further, the levels for urinary sodium excretion during the HSVE and recovery periods were significantly (p<0.001) increased in nor-BNI treated animals above those attained in the i.c.v. vehicle treated group.
Additional studies were performed in a separate group of rats that were instrumented with a renal nerve recording electrode to investigate the role of the renal nerves in mediating the renal excretory responses to HSVE. The results of these studies are shown in Figure 2 which demonstrates the cardiovascular, renal excretory and RSNA responses produced by acute i.v. HSVE in rats that were pre-treated i.c.v. with nor-BNI or isotonic saline vehicle. As previously observed (Fig. 1, Filled circles), HSVE also produced a pronounced increase in urine flow rate and a slow onset and low magnitude natriuresis in vehicle-treated rats instrumented with a renal nerve-recording electrode (Fig. 2, Filled circles). In these studies (Fig. 2), the levels for urinary sodium excretion attained during the HSVE (10-30-min) and recovery periods (40-100 min) in rats treated i.c.v. with nor-BNI were significantly (p<0.005) increased above those for vehicle-treated rats during the same time periods. Further, as compared to respective control levels, RSNA failed to significantly decrease during the HSVE or recovery periods in rats pre-treated i.c.v. with vehicle. In contrast, in rats treated i.c.v. with nor-BNI, RSNA was significantly (p<0.01) reduced below respective control levels during the 20 and 30-min HSVE periods with the renal sympathoinhibition continuing throughout the recovery phase. The level of renal sympathoinhibition attained in nor-BNI treated animals during the HSVE and recovery periods was significantly (p<0.01) greater than that attained in vehicle treated rats.

Table 1 illustrates the peak and area under the curve (AUC) changes in cardiovascular and renal function produced by acute i.v. HSVE for the same rats in which data is presented in Figure 2. Prior to the administration of vehicle or nor-BNI the baseline values for heart rate, mean arterial pressure urine flow rate, urinary sodium excretion and RSNA were 424±12 bpm, 116±2 mmHg, 42±6 µl/min, 2.5±0.5 µeq/min and 100 % control, respectively. Following i.c.v. vehicle administration, peak changes (i.e., delta) in RSNA (20-min time point), urine output and
urinary sodium excretion (30-min time point) attained during the HSVE challenge (total 30-min) were significantly (p<0.001) increased above respective baseline control values and were: -10±7 % control, 212±16 µl/min and 2.4±1.4 µeq/min, respectively. In comparison, in rats pretreated centrally with nor-BNI, HSVE evoked peak changes in these parameters at the same time points that were: -27±7 % control, 226±16 µl/min, and 7.5±1.4 µeq/min, respectively, with these peak natriuretic and renal sympathoinhibitory responses being significantly (p<0.05) greater in magnitude than those observed in i.c.v. vehicle treated animals. In addition, as compared to vehicle treatment, nor-BNI significantly increased the AUC for urinary sodium excretion during the 30-min HSVE challenge (UNaV 30-min HSVE AUC; i.c.v. vehicle, 58±11 µl/30-min vs. i.c.v. nor-BNI, 139±15 µeq/30-min) and the total 100-min experimental protocol (UNaV 100-min AUC; i.c.v. vehicle, 428±120 µeq/100-min; i.c.v. nor-BNI, 761±102 µeq/100-min). In addition, the AUC for RSNA during the 100-min protocol was significantly (p<0.05) different from that attained in vehicle treated animals (RSNA 100-min AUC; i.c.v. vehicle, 93±497 % control/100-min; i.c.v. nor-BNI, -1804±529 % control/100-min).

Figure 3 depicts the cardiovascular and renal excretory responses produced by acute i.v. HSVE in chronic bilaterally renal denervated rats that were pre-treated i.c.v. with isotonic saline vehicle. For comparison, cardiovascular and renal excretory data from HSVE studies performed in intact rats depicted in Fig. 2 are superimposed in Fig. 3. Acute i.v. HSVE did not produce any significant change in heart rate or mean arterial pressure in renal denervated rats. In response to HSVE, renal denervated rats elicited a profound diuresis above respective group control levels (p<0.001) that was comparable in magnitude and pattern to the diuresis produced in intact rats. During the 30-min HSVE period, both intact and renal denervated rats produced only a slow onset and low magnitude natriuresis. However, during the recovery period (time points 50-100
min), renal denervated rats produced a significantly (p<0.005) greater magnitude natriuresis than that observed in intact rats.

Figure 4 depicts the changes in cardiovascular and renal excretory function produced by acute i.v. HSVE in chronic bilateral renal denervated rats that were pre-treated i.c.v. with nor-BNI. For comparison, superimposed are also data from HSVE studies (Fig. 3) in which renal denervated rats were pretreated i.c.v. with isotonic saline vehicle. As shown (Fig. 4), renal denervated rats pretreated i.c.v. with nor-BNI or vehicle did not produce any changes in cardiovascular function throughout the experiment. However, both renal denervated rats treated with nor-BNI or vehicle produced essentially the same pattern of diuresis and recovery from the HSVE challenge. Similar to vehicle treated animals, renal denervated rats treated i.c.v. with nor-BNI tended to slowly increase urinary sodium excretion during the 30-min HSVE, but in each case the natriuresis was not significantly elevated (p<0.01) above respective group control levels until the recovery phase (time points 50-100) of the protocol.

DISCUSSION

Opioid agonists have been shown to interact with neuronal, humoral, and hemodynamic mechanisms which control the renal handling of water and sodium (1,7,10,24,26). Endogenous opioid systems have also been demonstrated to be activated by various stressors (3,9,12,18,20,29). Based on these findings and observations that central kappa opioid administration evokes a concurrent diuresis and antinatriuresis (i.e., a selective water diuresis) (6,10,11,23), the present investigations examined whether native central kappa opioid systems contribute to the renal excretory responses mediated by the physiological stress of acute HSVE. Together, the findings of these studies demonstrated that endogenous central kappa opioid
systems are activated during HSVE and play an important role in maximally retaining sodium by augmenting central sympathetic outflow to the kidneys and overriding the volume-evoked suppression of RSNA.

In the present studies, the stress of acute i.v. HSVE did not change heart rate or mean arterial pressure in conscious rats. In contrast, in rats treated centrally with vehicle or the kappa opioid receptor antagonist, nor-BNI, the HSVE challenge produced a marked diuresis that progressively increased, peaked at 30-min, and then subsided as the volume expansion was terminated and the recovery period was initiated. While the pattern of change in urine flow rate was similar between groups, it was observed that rats treated centrally with nor-BNI produced a slight but significant increase in the magnitude of diuresis during the 30-min HSVE period as compared to levels attained in i.c.v. vehicle treated rats. On the surface this finding suggests that endogenous central kappa opioid systems contribute, at least to a minor extent, to the renal excretion of water during HSVE. However, when these studies were repeated in rats instrumented with a renal nerve recording electrode there were no significant differences observed in the magnitude or pattern of diuresis to the HSVE between nor-BNI and vehicle treated animals. While the reason(s) for this discrepancy in results is unknown, it is possible that underlying differences in the basal neurohumoral status of naïve animals (i.e., no nerve recording electrode) as compared to animals which had undergone surgical implantation of a nerve recording probe, were enough to prevent the full expression of the enhanced diuresis to the kappa antagonist from being observed.

In contrast to the profound increase in urine output caused by HSVE, rats treated centrally with vehicle alone produced a concurrent and marked retention of sodium during the HSVE and recovery periods. The avid sodium retention that occurred during HSVE in these
animals is in opposition to the pronounced natriuresis that typically occurs in conscious rats given an acute i.v. isotonic saline load (4,5,10,28). For instance, when subjected to the same volume stimulus (5% body weight over 30-min), control rats (i.c.v. vehicle treated) infused with hypotonic saline (0.45 M) showed a blunted peak natriuretic response (30-min, 8.4±2.5 µeq/min; Fig. 1) as compared to that observed in rats which instead received an isotonic saline load (0.9 M) (30-min, 30 µeq/min; Kapusta and Obih, 1995). Thus, these differences demonstrate the effectiveness of the HSVE model in activating sodium retaining mechanisms. However, in comparison to the avid sodium retention observed during HSVE in rats treated centrally with vehicle, animals treated i.c.v. with the kappa opioid receptor antagonist nor-BNI exhibited an enhanced ability to excrete sodium throughout the HSVE (30-min, 16.0±1.4 µeq/min, Fig. 1) and recovery periods. Together, these findings demonstrate that central endogenous kappa opioid receptor systems are activated and contribute in maximally retaining sodium during acute i.v. HSVE and the recovery from this stressor. This observation is of interest considering that in other studies the administration of non-selective opioid receptor antagonists also revealed that endogenous central and peripheral opioid systems are activated as mechanisms to maximize renal tubular sodium reabsorption during the stress of food restriction (29), dietary sodium restriction (3,12) or acute psychoemotional stress (air jet stress) (9).

The diuretic effect produced by exogenous administration of kappa opioid agonists primarily results from a central action of the drug to inhibit vasopressin secretion (1,15,16,22,24,25), with the magnocellular region of the paraventricular nucleus of the hypothalamus being a specific brain site of action (6). In addition, a link between central kappa opioids and intact renal nerves in the control of urinary sodium excretion has been established (10,11). In this regard, it has been demonstrated that in conscious rats the central administration
of the kappa opioid agonist U-50488H evoked an increase in RSNA and decrease in urinary sodium excretion (10). In these studies, removal of intact renal nerves by bilateral renal denervation abolished the antinatriuretic response to central U-50488H (10). In related studies, the physiological and potentially pathological importance of this system was revealed by the observation that activation of central kappa opioid systems (e.g., exogenous i.c.v. injection of U-50488H) markedly blunted the natriuretic response elicited by an isotonic saline volume expansion by a renal nerve-dependent pathway (11). The results of these previous studies indicate that the pharmacological activation of central kappa opioid systems can elicit a significant antinatriuresis via augmenting central sympathetic outflow to the kidneys. Taken further, the findings from these present studies demonstrate that endogenous central kappa opioid systems are activated during the stress of acute HSVE and play an important physiological role in maximizing sodium retention and ultimately serving to minimize/prevent hyponatremia. Thus, the findings from these pharmacological and physiological studies demonstrate the importance of the central kappa opioid system as a novel control pathway involved in sodium homeostasis during acute stress conditions in which fluid and electrolyte status are perturbed.

Studies were performed to determine the mechanisms by which central kappa opioids affect renal excretory function during HSVE and the role of the renal nerves in this response. For these experiments, animals were implanted with a renal nerve recording electrode and changes in RSNA were measured in response to i.c.v. vehicle or nor-BNI treatment and the subsequent HSVE challenge. In rats pre-treated centrally with vehicle, RSNA showed only a tendency to suppress during the HSVE period, a response which correlated in time with the low magnitude natriuresis occurring in these animals. In contrast, in rats pre-treated centrally with the kappa antagonist, nor-BNI, RSNA was significantly suppressed during both the HSVE and recovery
periods, responses that were closely associated with the enhanced natriuresis resulting over this time. These findings provide strong evidence that in intact rats, HSVE evoked the central release of an endogenous kappa opioid ligand(s) (presumably dynorphin A (1-17)), which activated a nor-BNI sensitive kappa opioid receptor pathway to increase RSNA and maximize urinary sodium retention. We are currently examining the brain sites that kappa opioids alter c-Fos expression as an approach to determine which brain nuclei kappa opioid systems may produce their centrally evoked renal sympathoexcitatory and antinatriuretic responses.

The above mentioned findings with nor-BNI revealed an interaction between endogenous central kappa opioid systems and the renal nerves in the retention of sodium during HSVE. To further examine this relationship, additional studies were performed to determine whether intact renal nerves are essential for central kappa opioid systems to mediate enhanced renal tubular sodium reabsorption during HSVE. For these studies, the HSVE protocol with i.c.v. nor-BNI or vehicle was repeated in rats having undergone chronic bilateral renal denervation. Chronic bilateral renal denervation was used as a standard approach to remove the influence of the renal nerves on kidney function (2,4,5,9,10,11). The findings of these investigations (Fig. 4) demonstrated that renal denervated rats pretreated centrally with vehicle or nor-BNI produced essentially the identical pattern and magnitude of maximal sodium retention (and diuresis) during the HSVE and recovery periods. This is in opposition to the enhanced natriuresis observed during the HSVE and recovery periods in central nor-BNI treated rats which had an intact renal innervation (Figs. 1 and 2). Based on these findings it is concluded that central kappa opioids mediate sodium reabsorption in the face of HSVE by augmenting central sympathetic outflow to the kidneys via a renal nerve-dependent pathway. This hypothesis is also supported by the observation that in conscious rats, increased kappa opioid activity produced by i.c.v. U-50488H
injection significantly blunted the natriuretic response to isotonic saline volume expansion by a renal nerve-dependent pathway (11).

As noted above, central pretreatment with the kappa opioid receptor antagonist nor-BNI failed to produce an exaggerated natriuresis during HSVE in chronic bilateral renal denervated rats (Fig. 4). These observations indicate that in under conditions of chronic renal denervation (10-14 days), other pathways independent of central kappa opioid systems are recruited to compensate for the loss of neural pathways and are activated to retain sodium during HSVE. While these non-neural pathways have yet to be explored, one can speculate that the renin-angiotensin-aldosterone systems and other humoral-hemodynamic systems may be involved. Upon initiation of the HSVE stimulus these non-neural (renal nerves) mechanisms, which under the conditions of chronic renal denervation have an accentuated influence on the renal handling of sodium and water, retain sodium relatively quickly and to a level equivalent to that observed in intact animals (see Figs. 3 and 4). Despite this ability, these non-neural systems appear to lack the fine-tuning that the renal nerves can have on the renal handling of sodium. This is suggested since during the recovery period, following termination of the HSVE stimuli, the renal denervated animals (Figs. 3 and 4) produced a marked natriuretic response which was in contrast to the continued and sustained urinary sodium retention observed in intact animals (Figs 1 and 2). Thus, in rats with intact kidneys the renal sympathetic nerves appear to be essential for rapid and maximal retention of sodium during both the HSVE and recovery periods. Similarly, an intact renal innervation has been shown to be required for rapid and maximal changes in urinary sodium excretion to other manipulations or stressful stimuli (2,4,5). Instead, the findings of the present study indicate that an intact and functional renal innervation is only required for optimal retention of sodium during the recovery period of the HSVE stressor.
PERSPECTIVES

In conclusion, it is recognized that during an acute i.v. isotonic saline volume expansion there is a pronounced suppression of RSNA which contributes to diuresis and natriuresis and restoration of normal total body water and sodium content (2,4,5,28). A novel finding of the present investigation is that during HSVE there is a failure to suppress RSNA and that a component of this blunted renal nerve response, which contributes to maximal sodium retention during this stressor, is mediated by activation of endogenous central kappa opioid systems which stimulate central sympathetic outflow to the kidneys. Based on these observations, endogenous central kappa opioid and receptor pathways may have an important physiological role in the acute-chronic homeostasis of body fluid and sodium balance when it is necessary to excrete water, yet retain sodium; i.e., during states that may provoke fluid overload and/or hyponatremia. Further, it may be speculated that in certain situations central kappa opioid systems may become deranged such that enhanced activity of this system may contribute to the avid sodium retention which occurs in different pathological states in which there is augmentation of sympathetic traffic to the kidneys and RSNA fails to fully suppress to a volume load (e.g., cirrhosis with ascites, congestive heart failure).

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REFERENCES


FIGURE LEGENDS

**Figure 1.** Cardiovascular and renal responses produced by acute i.v. hypotonic saline volume expansion (HSVE) in conscious rats pre-treated i.c.v. with nor-BNI or isotonic saline vehicle. Values are means ± S.E. Experiments were performed in conscious chronically instrumented rats that were continuously infused with isotonic saline (20 µl/min). Two hours after the start of the infusion, two consecutive 10-min urine samples were collected (C; control); Isotonic saline vehicle (5 µl) or the selective kappa opioid receptor antagonist, nor-BNI (1 µg total) was then injected i.c.v. and a 10 min urine sample collected (V/N). Time periods 10 - 30 = consecutive experimental periods (10 min each) collected during HSVE (5% BW, 0.45 M saline over 30 min). Time periods 40-100 = consecutive recovery periods (10 min each) collected after stopping the HSVE. HR, heart rate; MAP, mean arterial pressure; V, urine flow rate; U_{Na}V, urinary sodium excretion. *·\(^t\) P < 0.01, significantly different from respective control period. # P < 0.05, ## P < 0.001, significant difference between groups over the 100-min protocol.

**Figure 2.** Cardiovascular, renal excretory and RSNA responses produced by acute HSVE in conscious rats pre-treated i.c.v. with isotonic saline vehicle (●; 5 µl, n = 6) or nor-BNI (○; 1 µg, n = 6). Values are means ± S.E. Experiments were performed in conscious chronically instrumented rats that were implanted with a RSNA recording electrode. Experimental protocol, format and abbreviations same as in figure 1. *·\(^t\) P < 0.01, significantly different from respective control period. # P < 0.01, ## P < 0.005, significant difference between groups over the 100-min protocol.
Figure 3. Cardiovascular and renal excretory responses produced by acute i.v. HSVE in conscious chronic bilaterally renal denervated (DNX) rats pre-treated i.c.v. with isotonic saline vehicle (○; 5 µl, n = 7). For comparison, data from HSVE studies performed in intact rats depicted in Fig. 1 are superimposed in Fig. 3 (●; 5 µl, n = 6). Values are means ± S.E. Format and abbreviations same as in figure 1. * † \( P < 0.01 \), significantly different from respective control period. \# \( P < 0.005 \), significant difference between groups over the 100-min protocol.

Figure 4. Cardiovascular and renal excretory responses produced by acute i.v. HSVE in chronic bilaterally renal denervated (DNX) rats pre-treated i.c.v. with isotonic saline vehicle (○; 5 µl, n = 6) or nor-BNI (●; 1 µg total, n = 6). Format and abbreviations same as in figure 1. * † \( P < 0.01 \), significantly different from respective control period.
Table 1. Effects of ICV nor-BNI pre-treatment on peak and area under the curve (AUC) changes in cardiovascular and renal function produced by acute I.V. hypotonic saline volume expansion in conscious Sprague-Dawley rats for which time course data is presented in Figure 2.

<table>
<thead>
<tr>
<th>ICV Pre-Treatment (n=6/group)</th>
<th>HR (beats)</th>
<th>MAP (mmHg)</th>
<th>V (µl)</th>
<th>UNaV (µeq)</th>
<th>RSNA (% Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peak Δ/min, during 30-min HSVE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>13±11</td>
<td>-2±1</td>
<td>212±16 *</td>
<td>2.4±1.4</td>
<td>-10±7</td>
</tr>
<tr>
<td>nor-BNI</td>
<td>-2±11</td>
<td>-1±1</td>
<td>226±16 *</td>
<td>7.5±1.4 *, †</td>
<td>-27±7 *, †</td>
</tr>
<tr>
<td><strong>AUC, Δ during 30-min HSVE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>331±388</td>
<td>-41±30</td>
<td>2316±193</td>
<td>58±11</td>
<td>-185±88</td>
</tr>
<tr>
<td>nor-BNI</td>
<td>-109±100</td>
<td>-22±39</td>
<td>2769±506</td>
<td>139±15 †</td>
<td>-570±162</td>
</tr>
<tr>
<td><strong>AUC, Δ during 100-min protocol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>1416±1522</td>
<td>-81±138</td>
<td>8661±559</td>
<td>428±120</td>
<td>93±497</td>
</tr>
<tr>
<td>nor-BNI</td>
<td>-596±417</td>
<td>-18±100</td>
<td>9136±1107</td>
<td>761±102 † †</td>
<td>-1804±529 † †</td>
</tr>
</tbody>
</table>

ICV, intracerebroventricular; I.V., intravenous; HSVE, hypotonic saline volume expansion (5% BW for 30-min); Baseline control values for each parameter: HR, heart rate = 424±12 bpm; MAP, mean arterial pressure = 116±2 mmHg; V, urine flow rate = 42±6 µl/min; UNaV, urinary sodium excretion = 2.5±0.5 µEq/min; RSNA, integrated RSNA = 100 % control; *, p<0.001 vs. respective baseline control; †, p<0.05, † †, p<0.005 vs. vehicle pre-treatment group.
Fig. 1

- **HR** (bpm)
  - C: 450 bpm
  - V/N: 450 bpm
  - 10-30 min: 450 bpm
  - HSVE: 450 bpm
  - RECOVERY: 450 bpm

- **MAP** (mmHg)
  - C: 150 mmHg
  - V/N: 150 mmHg
  - 10-30 min: 150 mmHg
  - HSVE: 150 mmHg
  - RECOVERY: 150 mmHg

- **V** (μl/min)
  - C: 120 μl/min
  - V/N: 120 μl/min
  - 10-30 min: 120 μl/min
  - HSVE: 120 μl/min
  - RECOVERY: 120 μl/min

- **UNaV** (μeq/min)
  - C: 8 μeq/min
  - V/N: 8 μeq/min
  - 10-30 min: 8 μeq/min
  - HSVE: 8 μeq/min
  - RECOVERY: 8 μeq/min

**i.c.v.:**
- Vehicle (5μl, n=6)
- Nor-BNI (1 μg, n=6)
Fig. 2

- **HR** (bpm)
- **MAP** (mmHg)
- **V** (μl/min)
- **UNaV** (μeq/min)
- **RSNA** (%)

**i.c.v.:**
- **Vehicle** (5μl, n=6)
- **Nor-BNI** (1 μg, n=6)

[Graph showing changes in HR, MAP, V, UNaV, and RSNA over time with HSVE and recovery phases indicated.]
Fig. 3

HR (bpm)

MAP (mmHg)

V (μL/min)

UNaV (μeq/min)

C V 10 20 30 40 50 60 70 80 90 100 min

i.c.v. HSVE RECOVERY

Vehicle: intact (n=6) DNX (n=6)
Fig. 4

Renal Denervated Rats

- HR (bpm)
- MAP (mmHg)
- V (μl/min)
- UNaV (μeq/min)

C V/N 10 20 30 40 50 60 70 80 90 100 min

↑ HSVE RECOVERY

i.c.v.:
- Vehicle (5 μl, n=6)
- Nor-BNI (1 μg, n=6)