Role of Endogenous Peripheral Opioid Mechanisms in Renal Function\(^1, 2\)

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ABSTRACT
The role of endogenous peripheral opioid mechanisms in renal function was evaluated in normotensive and hypertensive rats. Intravenous naloxone, a quaternary opioid antagonist with limited ability to cross the blood-brain barrier, was used to inhibit endogenous peripheral opioid mechanisms. In normotensive rats, the opioid antagonist impaired the normal renal adaptive response to dietary sodium restriction. In spontaneously hypertensive rats, the opioid antagonist did not affect the renal functional responses to acute environmental stress. These data indicate that, depending on the nature of the intervention, a role for endogenous peripheral opioid mechanisms in the renal function responses may be identified.

Key Words: Renal sympathetic nerve activity, opioid agonists, opioid antagonists, acute environmental stress

Opioids are known to influence renal mechanisms regulating total body sodium and volume regulation (1). The iv administration of various natural and synthetic opioid receptor agonists affects urinary flow rate and sodium excretion. A portion of the renal responses to iv administered opioid receptor agonists depends on a central nervous system site of action. This results in the activation of peripheral neurohormonal mechanisms that act on the kidney to influence the renal excretory responses. For example, the antinatriuretic response to the iv administration of morphine, an opioid receptor agonist active at \(\mu\) opioid receptors, is abolished by bilateral renal denervation (2), and the central administration of dermorphin, a specific \(\mu\) opioid receptor agonist, is known to increase efferent renal sympathetic nerve activity (ERSNA) (3).

However, it is difficult for these studies involving the administration of exogenous opioid receptor agonists to provide insight into the role of endogenous opioid systems in the renal handling of sodium and water. Because the opioid systems are known to be stimulated under conditions of acute environmental stress and to be involved in central modulation of ERSNA, our laboratory has conducted studies in spontaneously hypertensive rats (SHR) using air jet stress, a form of acute environmental stress (4, 5). Evidence from these studies suggested that, in addition to a central nervous system site of action, endogenous peripheral opioid systems may influence renal function via a direct action within the kidney. It is known that the kidney contains opioid peptides (6) and opioid receptors (7). Because naloxone, a \(\mu\) opioid receptor antagonist that readily crosses the blood-brain barrier, prevented the antinatriuretic response to both low-frequency renal nerve stimulation (5) and air jet stress without affecting the concurrent increase in ERSNA (4), it was suggested that \(\mu\) opioids might exert their influence on renal function via an intrarenal action (8). In support of this, it was demonstrated that the intrarenal administration of the specific \(\mu\) opioid receptor agonist dermorphin elicited antidiuretic and antinatriuretic responses, which were inhibited by the prior intrarenal administration of naloxone or renal denervation (8). These results suggested that the intrarenal action of opioids might occur by the facilitation of the nerve terminal release (presynaptic) and/or the direct tubular action (postsynaptic) of norepinephrine to increase renal tubular sodium and water reabsorption (8).

To extend these direct and invasive studies implicating an intrarenal site of action of opioids, this study was undertaken to more fully examine the role of endogenous opioid systems located outside the central nervous system in renal responses to integrated physiologic stimuli. It was reasoned that fasting/dietary sodium restriction was known to activate peripheral endogenous opioid mechanisms in normotensive control rats (9) and that there was strong evidence for a role for central nervous system opioid mechanisms in the air jet stress responses in SHR (4, 5). However, there were also data (8) suggesting the possibility that peripheral (intrarenal) endoge-
nous opioid mechanisms might contribute to the renal responses to activation (by direct electrical stimulation) of the renal sympathetic nerves (which is known to occur during air jet stress in SHR). Two aspects remained unclear. First, did activation of the peripheral endogenous opioid mechanisms constitute a physiologic contribution to the renal response to dietary sodium restriction in normotensive control rats? Second, did peripheral endogenous opioid mechanisms contribute to the renal responses to the renal sympathetic nerve activation that occurs during air jet stress in SHR (i.e., is reflex activation the same as direct electrical stimulation with respect to opioid involvement)? Intravenous naltrexone methyldibromide (NMB), a quaternary opioid antagonist with limited ability to cross the blood-brain barrier (4,10), was used to inhibit endogenous peripheral opioid mechanisms.

METHODS

Animals

Male Sprague-Dawley rats and SHR weighing 275 to 300 g were used in these studies. All rats were fed normal sodium diets (Na content, 163 mEq/kg) and allowed tap water ad libitum until the beginning of the experimental protocol. All experimental procedures were in accordance with the University of Iowa and National Institutes of Health guidelines for the care and use of laboratory animals.

Sodium Balance

Male Sprague-Dawley rats were transferred to individual metabolism cages where they were allowed free access to a low-NaCl diet (Na < 2 mEq/kg) and 50 mM NaCl drinking solution for 1 wk. After this equilibration period, metabolic balance measurements consisting of daily measurements of body weight, urine volume, food consumed, and fluid drunk were begun (Day 1) and continued through Day 16. Because the rats never exhibited diarrhea, fecal collections were not made. On Days 1 to 4 and 9 to 12 rats received a low-NaCl diet and 50 mM NaCl drinking solution (normal dietary sodium intake period), and on Days 5 to 8 and 13 to 16, rats received a low-NaCl diet and distilled water drinking solution (zero dietary sodium intake period). In addition, all rats received a daily ip injection of 0.5 mL of distilled water on Days 1 to 8 and 1.0 mg of NMB in 0.5 mL of distilled water on Days 9 to 16.

It should be noted that randomization of NMB administration between the first and second periods of dietary sodium restriction was initially attempted in pilot studies. However, the effect of 8 days of NMB administration was slow to wear off. There was a residual antagonistic effect to the action of a μ opioid receptor agonist for up to 14 days after the cessation of NMB administration. As to the alternate approach of comparing NMB and vehicle in matched parallel groups during a single test period, one is left with not knowing whether the group that received NMB had a normal response to vehicle before receiving NMB. The advantage of using each rat as its own control is that one has the opportunity to demonstrate that each rat that is to receive NMB is known to be able to respond normally when untreated.

Daily dietary sodium intake (milliequivalents per day) = dietary sodium content (milliequivalents per gram) × daily consumption of food (grams per day) + drinking fluid sodium content (milliequivalents per milliliter) × daily fluid consumption (milliliters per day). Daily urinary sodium excretion (milliequivalents per day) = daily urine volume (milliliters per day) × urinary sodium concentration (milliequivalents per liter). Daily sodium balance (milliequivalents per day) = daily dietary sodium intake – daily urinary sodium excretion.

The procedures and measurements for metabolic balance measurements have been previously used in our laboratory (11–13).

Acute Environmental Stress

On the morning of the experimental day, male SHR were anesthetized with methohexital sodium (20 mg/kg ip, supplemented with 10 mg/kg iv as needed) and instrumented with catheters in the left femoral artery and vein. The catheters were tunneled to the back of the neck, flushed and filled with heparinized normal saline (100 U/mL), and plugged with stainless steel pins. Through a suprapubic incision, a polyethylene urinary bladder catheter, modified from that of Gellai and Valtin (14), was flanged and sutured into the urinary bladder, exteriorized, and secured by suturing to adjacent muscle, tissue, and skin. After the implantation of these catheters, rats were also implanted with a renal sympathetic nerve activity recording electrode. This was performed by exposure of the left kidney through a left flank incision via a retroperitoneal approach. With the use of a dissecting microscope (magnification, ×25), a renal nerve branch from the aorticorenal ganglion was isolated and carefully dissected free. The renal nerve branch was then placed on a bipolar platinum wire electrode. Renal sympathetic nerve activity was amplified (×10,000 to 50,000) and filtered (low, 30; high, 3,000 Hz) with a Grass P511 Bandpass Amplifier (Grass Instruments Co., Quincy, MA). The amplified and filtered signal was channeled to a Tektronix 5113 Oscilloscope (Tektronix Inc., Beaverton, OR) and a 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 (Grass Model 7P10). The integrated voltage and renal neurogram signals were
displayed on the Grass polygraph. The quality of the renal sympathetic nerve signal was assessed by its pulse synchronous rhythmicity and by examination of the magnitude of decrease in recorded renal sympathetic nerve activity during sinoaortic baroreceptor loading with an iv injection of norepinephrine (3 μg). The renal nerve activity remaining after maximum inhibition after norepinephrine administration was similar to the background noise observed approximately 30 min postmortem; this value was subtracted from all experimental values of renal sympathetic nerve activity. When an optimal renal sympathetic nerve activity signal was observed, the recording electrode was fixed to the renal nerve branch with silicone cement (Wacker Sil-Gel 604; Wacker Chemie, Munich, Germany). The electrode cable was then secured in position by being sutured to the abdominal trunk muscles. Finally, the electrode cable was exteriorized, and the flank incision was closed in layers.

After surgical preparation and recovery from anesthesia, rats were placed in cylindrical rat holders which permitted forward and backward movement and steady-state urine collection. An iv infusion (50 μL/min) of isotonic saline was then started and continued for the duration of the experiment. Four to 6 h after recovery and the start of isotonic saline infusion, the arterial catheter was flushed and attached to a pressure transducer and the urinary bladder catheter was lead to a collection beaker. The quality of the renal sympathetic nerve activity recording was again tested with an iv injection of norepinephrine (3 μg), as previously described, to ensure the absence of noise due to mechanical movement, respiration, or heart rate. If the quality of the renal sympathetic nerve activity recording was the same as that observed when the electrode was implanted, then the experiment commenced.

The control period consisted of two consecutive 10-min urine collections. The acute environmental stress was applied for 20 min by the use of air jet stress. Five minutes was allowed after the start of air jet stress before the collection of two consecutive 10-min urine specimens (air jet stress period). Five minutes was allowed after the cessation of air jet stress before the collection of two consecutive 10-min urine specimens (air jet stress period). Venous blood samples (200 μL) were taken during the midpoints of the control, air jet stress, and recovery periods. Acute environmental stress stimulation consisted of an air jet delivered to the top of the rat's head through a tube located 4 to 5 cm in front of the rat. Repeated applications of air jet stress in the same rat result in similar increases in heart rate, mean arterial pressure, and ERSNA and in decreases in urinary flow rate and sodium excretion (3–5). At the end of the recovery period, each rat was given 0.5 mg of NMB in 0.2 mL of isotonic saline iv. After a 15-min equilibration period, the above sequence of control, air jet stress, and recovery periods and measurements was repeated. The quality of the renal sympathetic nerve signals was again assessed with iv injections of norepinephrine (3 μg) after the completion of the protocol. The rat was then euthanized, and postmortem renal sympathetic nerve activity was continuously recorded for 30 min as a measure of background noise. The kidneys were removed, decapsulated, drained, and weighed.

Analytical Techniques

Urine volume was determined gravimetrically. The sodium content of the diet was determined on 24-h ashed samples extracted for 24 h in concentrated hydrochloric acid and diluted appropriately with distilled water. The sodium concentration was measured by flame photometry. Data acquisition for renal sympathetic nerve activity measurements was performed with a commercially available software package (Labtech Notebook, version 6.1; Laboratory Technologies Corp., Wilmington, MA). Integrated renal sympathetic nerve activity is expressed as microvolts per second per 1-s interval. Because of the limitations of comparing values for multifiber renal sympathetic nerve activity between animals, the data are expressed as percent control, with the control values for each animal taken as 100%.

Data Analysis

The data were statistically analyzed by the use of repeated-measures analysis of variance for main effects and interactions and Scheffe's test for pairwise comparisons among means (15). Statistical significance was defined as P < 0.05. The data in the text and figures are expressed as means ± SE.

RESULTS

Sodium Balance

The results of the metabolic balance study are shown in Figure 1. During Days 1 to 8 when the rats received the NMB vehicle, daily sodium intake averaged approximately 1.5 mEq/day during the period of normal dietary sodium intake (Days 1 to 4). As rats continue to grow throughout their lifetime, the daily sodium balance was positive and averaged approximately 0.35 mEq/day. During the period of zero dietary sodium intake (Days 5 to 8), daily sodium balance became negative, more so on the first day of zero dietary sodium intake (−275 ± 39 μEq), with a rapid decline toward zero thereafter. During Days 9 to 16 when the rats received NMB, daily sodium intake averaged approximately 1.9 mEq/day during the
Figure 1. Sodium balance study in Sprague-Dawley rats. Daily sodium intake is plotted upward from the zero line. Daily urinary sodium excretion is plotted down from the level of daily sodium intake. Positive daily sodium balance is represented by the cross-hatched area above the zero line, whereas negative daily sodium balance is represented by the solid area below the zero line.

Figure 2 compares daily sodium balances of the same rats on zero dietary sodium intake when receiving vehicle and NMB. On both Days 1 (Day 13 versus Day 5 in Figure 1) and 2 (Day 14 versus Day 6 in Figure 1), mean daily sodium balance was significantly ($P < 0.05$) more negative in the NMB-treated rats than in the vehicle-treated rats. On Days 1 and 2, daily sodium balance was more negative during NMB than vehicle treatment in 7 of 10 and 10 of 10 rats, respectively. These changes in daily sodium balance during the early phase of the zero dietary sodium intake period were reflected in cumulative sodium balance. As seen in Figure 3, cumulative sodium balance was significantly ($P < 0.05$) more negative in NMB than in vehicle-treated rats for each day of the zero dietary sodium intake period.

As to the higher daily sodium balance on the first day of the catch-up phase (Day 9), this relates to the fact that rats grow continuously throughout their life. Thus, body weight increases each day, which means that daily sodium balance is positive, and therefore, cumulative sodium balance is on a continuous rising curve. Thus, even if one reverses the order of dietary sodium intake, cumulative sodium balance will always be higher during the second period because of the continued growth increase in body weight. It might be questioned whether the total body sodium content at the end of the second normal sodium period represents "excess sodium" or is an appropriate amount of sodium for the growth in body weight during the interval. Although total body sodium content was not determined, when the difference between cumulative sodium balance (Day 12 minus Day 4) is divided by the difference in body weight (Day 12 minus Day 4), a value of 52 μEq of Na/g body wt is obtained. That is, with each gram of increase in body weight, there was a retention of 52 μEq of Na. Of interest, values for total body exchangeable sodium

Figure 2. Daily sodium balance during the 4 days of zero dietary sodium intake in control (filled circles and solid line) and NMB (open circles and hatched line) periods.

Figure 3. Cumulative sodium balance during the 4 days of zero dietary sodium intake in control (filled circles and solid line) and NMB (open circles and hatched line) periods.
content range from 30 to 80 with a mean of 53 μEq of Na/g (16). Thus, although highly indirect, this would suggest that the increase in total body sodium content at the end of the second normal sodium period is not "excess sodium" but represents the amount of sodium that would be expected in view of the increase in body weight.

**Acute Environmental Stress**

The effects of an iv administration of a high dose of NMB on the responses to acute environmental stress in SHR are shown in Figure 4. Air jet stress elicited similar and significant (all \( P < 0.05 \)) increases in mean arterial pressure, heart rate (+31 ± 6 and +29 ± 7 beats/min, respectively) and ERSNA and decreases in urinary sodium excretion before and after NMB administration.

**DISCUSSION**

**Sodium Balance**

These studies demonstrate that a completely normal renal adaptive response to dietary sodium restriction does not occur in rats treated with NMB, a predominantly \( \mu \) opioid receptor antagonist with limited ability to cross the blood-brain barrier and enter the central nervous system (4,10). It is known that fasting is a sufficient stress to activate peripheral endogenous opioid systems (9), and it is likely that severe dietary sodium restriction elicits a similar response. We have shown that, in anesthetized surgically operated stressed rats, the intrarenal administration of the \( \mu \) opioid receptor agonist dermorphin elicited an antinatriuretic response in the absence of changes in GFR, RBF, and mean arterial pressure (8). This response was blocked by pretreatment of the kidney with naloxone, a \( \mu \) opioid receptor antagonist, or by prior renal denervation (8). In addition, naloxone blocked the antinatriuretic response to low-frequency renal nerve stimulation (5). These results suggest that, under periods of stress, the activation of endogenous peripheral opioid systems occurs with the opioids acting on \( \mu \) opioid receptors located presynaptically on renal sympathetic nerve terminals to facilitate the release of the endogenous neurotransmitter norepinephrine, which acts throughout the nephron to augment sodium reabsorption (8). It is known that, when confronted with a maximal need for renal sodium conservation, the normal renal adaptive response is dependent on intact renal innervation (11). Thus, these studies suggest that, during the stress of severe dietary sodium restriction, there exists an opioid-dependent mechanism that interacts with increased ERSNA in an attempt to maximally increase renal tubular sodium reabsorption in order to achieve sodium balance. It is evident, however, that this effect is not of great magnitude and is more clearly evident early in the renal adaptive response to severe dietary sodium restriction.

**Acute Environmental Stress**

We had previously demonstrated that 5 μg of NMB, given into the lateral cerebral ventricle, abolished the renal sympathetic nerve and excretory responses to air jet stress (4). Although NMB is poorly permeable to the blood-brain barrier (10), to be certain that the centrally administered NMB was not having its effect by entering the systemic circulation, the effect of the same dose of NMB, 5 μg, given iv was examined. The iv administration of 5 μg of NMB had no effect. Thus, it was concluded that, even if the entire intracerebroventricular dose of 5 μg of NMB had entered the systemic circulation, this could not account for the blockade of the air jet stress responses (4). However, to more fully exclude the possibility that there existed endogenous peripheral opioid mechanisms that were involved in the responses to air jet stress, a 100 times greater dose of NMB, 500 μg, was administered iv. Because the responses to air jet stress were not affected, these results strengthen the conclusion that endogenous peripheral opioid mechanisms do not appear to be involved in the responses to air jet stress.
Further, because the iv dose of 500 µg of NMB had no effect, whereas the intracerebroventricular dose of 5 µg completely abolished the renal responses to air jet stress, these results confirm the view that NMB is poorly permeable across the blood-brain barrier (4,10).

Because both interventions, dietary sodium restriction (17) and air jet stress (4), are known to increase ERSNA, it may be queried as to why the effects of NMB were different. The normal renal adaptive response to dietary sodium restriction, including increases in ERSNA (17), develops over a period of hours to days and involves an integrated response with associated alterations in other hormonal systems (e.g., renin-angiotensin-aldosterone, atrial natriuretic peptide). However, the renal responses to air jet stress, including increases in ERSNA (4), are immediate in onset and are fully developed in so brief a period of time that substantial contributions from mechanisms other than ERSNA (e.g., hormonal systems) are minimized. The effects of renal denervation on the two interventions are also substantially different. Renal denervation has no effect on renal adaptation to dietary sodium intake when dietary sodium intake is normal but impairs it when dietary sodium intake is restricted (11). However, renal denervation abolishes the renal responses to air jet stress when dietary sodium intake is normal (18). These differences would suggest that the effect of NMB in the sodium balance study is rebated to an action over time that may alter presynaptic and/or postsynaptic mechanisms involved in the contribution of increased ERSNA to renal sodium conservation.

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REFERENCES