Effects of Opioid Peptides on Neural Control of Renal Function in Spontaneously Hypertensive Rats

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The aims of the present study were to examine the effects of opioid receptor agonists and antagonists on the renal vascular (renal blood flow) and tubular (urinary sodium excretion) responses to renal nerve stimulation and norepinephrine in anesthetized spontaneously hypertensive rats (SHR). Graded frequency renal nerve stimulation (0.5–4.0 Hz) and doses of norepinephrine (10–80 ng/kg) produced frequency and dose-dependent decreases in renal blood flow. The renal vasoconstrictor responses were not altered by intravenous infusion of the opioid receptor agonists methionine enkephalin (μ and δ, 75 μg/kg/min) or U-50488H (κ, 20 μg/kg/min) or administration of the opioid receptor antagonist naloxone (1 mg/kg i.v.). The antinatriuretic response to low frequency (<1.0 Hz) electrical renal nerve stimulation was prevented by naloxone but not affected by methionine enkephalin administration without changes in glomerular filtration rate or effective renal plasma flow. These studies suggest that endogenous opioid receptor mechanisms are involved in the increased renal tubular sodium reabsorption response to low frequency renal nerve stimulation but not in the renal vasoconstrictor response to either renal nerve stimulation or norepinephrine. This might occur by facilitation of the renal nerve terminal release, the direct renal tubular action, or both, of norepinephrine to influence renal tubular sodium reabsorption.

In conscious spontaneously hypertensive rats (SHR), the acute environmental stress stimulus of air jet produces sustained increases in heart rate, mean arterial pressure, and regional hemodynamic alterations characteristic of the defense reaction.1 Additionally, air jet stress increases efferent renal sympathetic nerve activity that in turn decreases urinary sodium excretion.2-4 The stress-induced antinatriuretic response is prevented by bilateral renal denervation.2 Moreover, intravenous administration of the opioid antagonist naloxone also prevents the antinatriuresis but does not affect the renal sympathoexcitatory response.5 In bilaterally renal-denervated SHR exposed to air jet stress, the urinary sodium excretion response is not altered by naloxone.5 Thus, these findings suggest that expression of the antinatriuretic response evoked by the air jet stress involves activation of endogenous opioidergic systems. Furthermore, it appears that these two systems, a functional opioidergic system and an intact renal innervation, interact to mediate the antinatriuretic response to this acute environmental stress stimulus.

The manner by which endogenous opioidergic systems interact with the renal sympathetic nerves to influence the renal tubular reabsorption of sodium during conditions of air jet stress–induced increases in efferent renal sympathetic nerve activity is unknown. One hypothesis is that opioid peptides might modulate neurotransmitter release from the renal sympathetic nerve terminals and thus influence renal functional responses to increased efferent renal sympathetic nerve activity. Presynaptic opioid receptors on postganglionic sympathetic nerve fibers are reportedly present in a number of nonrenal tissues where they function to modulate neurotransmitter release.6-15 Thus, the present studies were performed to examine the renal blood flow and urinary sodium excretory responses to electrical stimulation of the renal sympathetic nerves and norepinephrine administration before and after intravenous administration of opioid agonists and antagonists.
Methods

Subjects

Male SHR (Taconic Farms, Inc., Germantown, New York) weighing 275–335 g were used in these studies. All rats were fed a normal sodium diet (sodium content, 163 meq/kg) and allowed tap water ad libitum. All experimental procedures were in accordance with the University of Iowa and National Institutes of Health guidelines for the care and use of experimental animals.

Surgery

On the experimental day, rats were anesthetized with pentobarbital (50 mg/kg i.p.) (Nembutal, Abbott Labs., North Chicago, Illinois) and maintained with an intravenous infusion of pentobarbital (10 mg/kg/hr). Rats were instrumented with catheters (Tygon, Fisher Scientific International, Chicago, Illinois) in the left carotid artery for measurement of arterial pressure and in the jugular vein for infusion of isotonic saline and drugs. For certain experiments, inulin and para-aminohippurate (PAH) were added to the isotonic saline in sufficient quantities for determination of inulin and PAH clearances. The left kidney was exposed through a retroperitoneal flank incision. The left ureter was cannulated with polyethylene tubing (PE-10, Clay-Adams, Parsippany, New Jersey) for urine collection. A precalibrated electromagnetic flow probe was placed around the left renal artery. The arterial catheter was attached to a pressure transducer (model P23Db, Gould-Statham, Oxnard, California). Renal blood flow and mean arterial pressure were measured as the urinary clearance of inulin and PAH, filtration rate and effective renal plasma flow were determined by the anthrone16 and ethylenediamine17 methods, respectively. Glomerular filtration rate and effective renal plasma flow were measured as the urinary clearance of inulin and PAH, respectively. Changes in renal blood flow were calculated as the maximum change from the prestimulation renal blood flow value and are expressed as percentage of decrease in renal blood flow.

Miscellaneous

Urinary volume was determined gravimetrically. Urine sodium concentration was measured by flame photometry (model 143, Instrumentation Labs., Lexington, Massachusetts). Urine and plasma inulin and PAH concentrations were determined by the anthrone16 and ethylenediamine17 methods, respectively. Glomerular filtration rate and effective renal plasma flow were measured as the urinary clearance of inulin and PAH, respectively. Changes in renal blood flow were calculated as the maximum change from the prestimulation renal blood flow value and are expressed as percentage of decrease in renal blood flow.

Drugs

The following drugs were used: U-50488H (Upjohn Co., Kalamazoo, Michigan), methionine enkephalin acetate salt (Sigma Chemical Co., St. Louis, Missouri), naloxone hydrochloride (Sigma Chemical Co.), and norepinephrine bitartrate (Winthrop-Breon Labs., New York, New York). Stock solutions of U-50488H (10 ml total, 25 µg/100 µl), methionine enkephalin (500 µg/100 µl), and norepinephrine (100 ml, 25 µg/100 µl) were prepared, stored cold (methionine enkephalin stored frozen), and diluted to the desired concentration with isotonic saline immediately before infusion. Naloxone was prepared fresh and injected and then repeated 10 minutes after beginning the intravenous infusion of the opioid receptor agonists methionine enkephalin (75 µg/kg/min, n=6) or U-50488H (20 µg/kg/min, n=5), or 10 minutes after the intravenous bolus injection of the opioid receptor antagonist naloxone (1 mg/kg, n=5). Heart rate, mean arterial pressure, and renal blood flow were continuously monitored.

Second, graded doses of norepinephrine (10, 20, 40, and 80 ng/kg) were injected into the left renal artery during a control period of intravenous isotonic saline infusion, and repeated 10 minutes after the intravenous infusion of methionine enkephalin (75 µg/kg/min, n=5), or 10 minutes after the intravenous bolus injection of naloxone (1 mg/kg, n=6). Heart rate, mean arterial pressure, and renal blood flow were continuously monitored.

Third, after stabilization of urine flow rate and sodium excretion in rats instrumented for urine collection, urine was collected during consecutive 15-minute periods of control, low frequency renal nerve stimulation (<1.0 Hz), and recovery, before and after the intravenous bolus administration of isotonic saline vehicle (0.2 ml, n=4) or naloxone (100 µg/kg in 0.2 ml isotonic saline, n=6), and during the intravenous infusion of methionine enkephalin (75 µg/kg/min, n=6). The frequency of renal nerve stimulation used in each rat was that which was subthreshold for producing decreases in renal blood flow. Heart rate, mean arterial pressure, and renal blood flow were continuously monitored.

Experimental Procedure

The following three experimental protocols were used: First, the efferent renal nerves were stimulated (17 V, 0.2 msec) at graded frequencies (0.5–4.0 Hz) for 1 minute at each frequency during a control period of intravenous isotonic saline vehicle infusion,
intravenously in 0.2 ml isotonic saline. All doses refer to the forms of the drugs previously cited.

**Data Analysis**

The data were statistically analyzed with repeated-measures analysis of variance for main effects and interactions and with Scheffe's test for pairwise comparisons among means. Statistical significance was defined as p values of less than 0.05.

**Results**

Table 1 lists the renal blood flow responses, expressed as percentages of decrease in renal blood flow, to graded frequency electrical renal nerve stimulation before and after intravenous administration of the opioid receptor agonists methionine enkephalin and U-50488H and the opioid receptor antagonist naloxone. Graded renal nerve stimulation produced frequency-dependent decreases in renal blood flow in all groups studied. Renal nerve stimulation did not alter heart rate or mean arterial pressure before or after opioid receptor agonist or antagonist administration. Methionine enkephalin, U-50488H, and naloxone did not alter the vasoconstrictor responses to renal nerve stimulation.

Table 2 lists the renal blood flow responses to graded doses of norepinephrine (10, 20, 40, and 80 ng/kg) injected into the renal artery before and after intravenous infusion of methionine enkephalin and bolus injection of naloxone. Graded doses of norepinephrine produced dose-dependent decreases in renal blood flow in both groups studied. Administration of these doses of norepinephrine into the renal artery did not alter heart rate or mean arterial pressure. The vasoconstrictor responses to norepinephrine were not altered by administration of methionine enkephalin or naloxone.

Figure 1 (n=6) shows the results of time-control studies in which urinary sodium excretion was examined during continuous intravenous infusion of isotonic saline during periods of control, low frequency renal nerve stimulation (<1.0 Hz) and recovery, and before and after intravenous bolus injection of isotonic saline vehicle. Heart rate, mean arterial pressure, and renal blood flow were not significantly altered by renal nerve stimulation before or after isotonic saline vehicle injection. Urinary sodium excretion was significantly and reversibly decreased by low frequency renal nerve stimulation. The percentages of decrease in urinary sodium excretion were 28±6% before and 25±6% after isotonic saline vehicle administration. Glomerular filtration rate (n=4) (Table 3) and effective renal plasma flow (n=4) (Table 4) were not significantly altered by low frequency renal nerve stimulation before or after isotonic saline vehicle administration.

Figure 2 (n=11) illustrates the results from similar studies in which the urinary sodium excretion response to low frequency renal nerve stimulation was examined before and after intravenous bolus injection of the opioid antagonist naloxone (100 μg/kg). Low frequency renal nerve stimulation reversibly reduced urinary sodium excretion by 27±3% before naloxone administration, whereas after naloxone administration, the neurogenic anti-
FIGURE 1. Graphs showing effect of bolus intravenous injection of isotonic saline vehicle on responses to low frequency renal nerve stimulation. Values are mean±SEM for responses of heart rate (HR), mean arterial pressure (MAP), renal blood flow (RBF), and urinary sodium excretion (UNaV) obtained during 15-minute periods of control (C), low frequency renal nerve stimulation (RNS), and recovery (R), before and after (indicated by arrow) intravenous bolus isotonic saline (0.2 ml) in six anesthetized spontaneously hypertensive rats. *p<0.05, significantly different from corresponding control.

The present study examined the effects of administration of the opioid receptor agonists methionine enkephalin (native μ and δ opioid receptor agonist) and U-50488H (synthetic κ opioid receptor agonist) and the opioid receptor antagonist naloxone on the renal vascular (renal blood flow) and tubular (urinary sodium excretion) responses to renal nerve stimulation in anesthetized SHR. Intravenous infusion of methionine enkephalin, U-50488H, and bolus injection of naloxone did not alter the renal vasoconstrictor responses to graded frequencies of renal nerve stimulation or graded doses of norepinephrine. In contrast, the antinatriuresis evoked by low frequency renal nerve stimulation was abolished by naloxone but not affected by methionine enkephalin administration. Thus, these studies suggest that endogenous opioid peptides are involved in mechanisms that modulate the renal tubular sodium reabsorption but not the vascular responses to electrical stimulation of the renal sympathetic nerves in SHR.

In conscious SHR, the decrease in urinary sodium excretion evoked by acute environmental stimulation-induced increases in efferent renal sympathetic nerve activity is prevented by bilateral renal denervation or by central administration of drugs that prevent the renal sympathoexcitatory response. The antinatriuresis is thought to result from an increase in renal tubular sodium reabsorption because glomerular filtration rate and effective renal plasma flow were not altered by air jet stress in conscious SHR.

More recent investigations have suggested that additional mechanisms might also be involved in the expression of the antinatriuretic response to acute

| Table 3. Effects of Isotonic Saline Vehicle, Methionine Enkephalin, and Naloxone on Glomerular Filtration Rate During Low Frequency Renal Nerve Stimulation |
|---------------------------------|------------------|------------------|
|                                  | Glomerular filtration rate (ml/min/g kidney wt) |
|                                  | Period | Vehicle | Drug |
| Isotonic saline (0.2 ml i.v.) (n=4) |       |         |      |
| C                               | 0.95±0.06 | 1.31±0.37 |
| RNS                             | 1.14±0.10 | 0.93±0.21 |
| R                               | 1.10±0.17 | 0.95±0.17 |
| Methionine enkephalin (75 μg/kg/min i.v.) (n=6) |       |         |      |
| C                               | 1.04±0.09 | 1.22±0.17 |
| RNS                             | 1.18±0.13 | 1.17±0.18 |
| R                               | 1.17±0.29 | 0.94±0.11 |
| Naloxone (100 μg/kg i.v.) (n=6)  |       |         |      |
| C                               | 0.97±0.09 | 0.99±0.12 |
| RNS                             | 1.02±0.13 | 0.95±0.15 |
| R                               | 0.99±0.10 | 1.01±0.14 |

Values are mean±SEM. Isotonic saline, methionine enkephalin, and naloxone were administered at the end of the vehicle recovery period (10 minutes before the drug control period). C, control period; RNS, low frequency renal nerve stimulation period (<1.0 Hz); R, recovery period.
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**TABLE 4. Effects of Isotonic Saline Vehicle, Methionine Enkephalin, and Naloxone on Effective Renal Plasma Flow During Low Frequency Renal Nerve Stimulation**

<table>
<thead>
<tr>
<th>Group</th>
<th>Effective renal plasma flow (ml/min/g kidney wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Isotonic saline (0.2 ml i.v.) (n=4)</td>
<td>3.78±0.16</td>
</tr>
<tr>
<td>Methionine enkephalin (75 µg/kg/min i.v.) (n=6)</td>
<td>3.75±0.46</td>
</tr>
<tr>
<td>Naloxone (100 µg/kg i.v.) (n=6)</td>
<td>3.97±0.43</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Isotonic saline, methionine enkephalin, and naloxone were administered at the end of the vehicle recovery period (10 minutes before the drug control period). C, control period; RNS, low frequency renal nerve stimulation period (<1.0 Hz); R, recovery period.

Image: **Figure 2.** Graphs showing effect of naloxone on responses to low frequency renal nerve stimulation. Values are mean±SEM for responses of heart rate (HR), mean arterial pressure (MAP), renal blood flow (RBF), and urinary sodium excretion (UNaV) obtained during 15-minute periods of control (C), low-frequency renal nerve stimulation (RNS), and recovery (R), before and after (indicated by arrow) intravenous bolus naloxone (100 µg/kg) in 11 anesthetized spontaneously hypertensive rats. *p<0.05, significantly different from corresponding control.

Image: **Figure 3.** Graphs showing effect of methionine enkephalin responses to low frequency renal nerve stimulation. Values are mean±SEM for responses of heart rate (HR), mean arterial pressure (MAP), renal blood flow (RBF), and urinary sodium excretion (UNaV) obtained during 15-minute periods of control (C), low frequency renal nerve stimulation (RNS), and recovery (R), before and during (indicated by arrow) intravenous infusion of methionine enkephalin (75 µg/kg/min) in six anesthetized spontaneously hypertensive rats. *p<0.05, significantly different from corresponding control.

environmental stress. Administration of the opioid receptor antagonists naloxone (intravenously) or naltrexone methybenzilate (intracerebroventricularly) to conscious SHR abolishes the decrease in urinary sodium excretion without affecting the increase in efferent renal sympathetic nerve activity.5 These opioid receptor antagonists do not affect urinary sodium excretion during air jet stress under conditions in which the influence of the renal sympathetic nerves had been removed by previous bilateral renal denervation.5 Thus, an interaction between the renal sympathetic nerves and endogenous opioidergic systems is suggested in mediating the antinatriuretic response to acute environmental stimulation. From these cited findings it can be hypothesized that opioids might modulate neurotransmitter release from renal sympathetic nerves and thus modify renal function during conditions of increased efferent renal sympathetic nerve activity. Opioids modulate neurotransmitter release and consequently the response to sympathetic nerve impulses in various (all nonrenal) isolated tissues as well as in vivo preparations.6-15 In
the present investigations, however, use of opioid receptor agonists and antagonists in anesthetized SHR failed to alter renal blood flow responses to both electrical renal nerve stimulation and norepinephrine. These results suggest that norepinephrine release from renal nerve terminals had not been significantly altered to a degree capable of affecting the renal blood flow response and that these opioid receptor agonists and antagonists do not affect the action of norepinephrine at postsynaptic α1 adrenergic receptors in the renal vascular bed of SHR. The inability of naloxone to alter the renal vasoconstrictor action of norepinephrine is consistent with the view that naloxone is a specific opioid receptor antagonist without agonist properties.19 In nonrenal vessels, ring segments of rabbit ear, and saphenous arteries, Gan and Duckles20 found that both δ and κ selective opioid receptor agonists inhibited adrenergic nerve stimulation-evoked contractions. These differences might result from differences in opioid peptide action in different vessels, differences between in vivo and in vitro preparations, or both.

Although opioidergic mechanisms do not appear to influence the renal vascular responses to renal nerve stimulation, endogenous opioids might participate in modulating the renal tubular responses to low frequency renal nerve stimulation. This is suggested because the opioid receptor antagonist naloxone abolished the decrease in urinary sodium excretion produced by low frequency renal nerve stimulation. Inhibition of a response by naloxone is generally considered evidence for interaction of opioid receptors with endogenous opioids in the response,21 particularly if low doses are used, as in the present study. Thus, the results of these studies, together with the previous finding that naloxone also abolishes the antinatriuresis to air jet stress without altering the renal sympathoexcitatory response,8 provide further evidence that endogenous opioidergic systems influence mechanisms that regulate the renal tubular sodium reabsorption response during conditions of increased effluent renal sympathetic nerve activity.

Although not elucidated in these studies, endogenous opioids might act through facilitation of the nerve terminal release, the direct tubular action, or both, of norepinephrine to influence renal tubular sodium reabsorption and urinary sodium excretion. The opioid antagonist naloxone would block these facilitatory influences mediated by opioid receptors. Moreover, opioid receptors and peptides are reportedly present in the kidney.22–27 The inability of methionine enkephalin to alter the antinatriuretic response to low frequency renal nerve stimulation in these studies suggests that the acute stress of surgery, anesthesia, or both26,29 might have already produced maximally active amounts of endogenous opioids and that further effects caused by additional exogenous opioid administration could not be detected.

We have previously reported that the magnitude of the antinatriuretic response to low frequency renal nerve stimulation is similar in normotensive Wistar-Kyoto (WKY) rats and SHR.30 The extent to which endogenous opioid systems contribute to the renal functional responses to renal nerve stimulation in WKY rats, however, is not yet known. Thus, the possible contribution of these findings to the alterations in the neural control of renal function and the hypertension in SHR remains to be determined.

Opioid receptor agonist (methionine enkephalin and U-50488H) and antagonist (naloxone) administration to anesthetized SHR do not appear to significantly alter the release of norepinephrine from renal sympathetic nerve terminals leading to alteration in the renal vasoconstrictor response to renal nerve stimulation. The inhibition of the antinatriuretic response to low frequency renal nerve stimulation by naloxone, however, suggests that endogenous opioids might participate in the renal tubular reabsorption of sodium mediated by increased effluent renal sympathetic nerve activity by facilitation of the nerve terminal release, the direct tubular action, or both, of norepinephrine.

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**Key Words**  • naloxone  • sympathetic nervous system  • sodium excretion  • blood flow  • renal function
Effects of opioid peptides on neural control of renal function in spontaneously hypertensive rats.
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Hypertension. 1990;15:767-773
doi: 10.1161/01.HYP.15.6.767

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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