Cardiovascular and renal responses produced by central orphanin FQ/nociceptin occur independent of renal nerves

Kapusta, Daniel R., and Velga A. Kenigs. Cardiovascular and renal responses produced by central orphanin FQ/nociceptin occur independent of renal nerves. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R987–R995, 1999.—The present study investigated the role of the renal nerves in mediating the cardiovascular and renal responses produced by the central administration of the opioid-like peptide orphanin FQ/nociceptin (OFQ/N) in conscious Sprague-Dawley rats. In conscious rats, OFQ/N (10 µg icv) produced a transient bradycardia and hypotension (nadir 20 min). Although renal sympathetic nerve activity (RSNA) initially remained unchanged, a delayed renal sympathoinhibitory response occurred after recovery (30 min) of blood pressure. By 30 and 70 min postinjection, RSNA decreased to 75 and 66% of control, respectively. Coinciding with the decrease in RSNA, central OFQ/N elicited a diuresis and antinatriuresis that occurred independent of changes in renal hemodynamics. In other studies, intracerebroventricular OFQ/N produced similar cardiovascular and renal excretory responses in bilaterally renal-denervated rats. Finally, in conscious sinoaortic deafferentiated rats, intracerebroventricular OFQ/N produced a rapid decrease in RSNA (55% of control, 10 min; 38% of control, 20 min) that paralleled the onset of the hypotension and bradycardia. These studies demonstrate that in conscious rats, intracerebroventricular OFQ/N produces a centrally mediated inhibition of RSNA which, due to activation of baroreflex mechanisms, is temporally dissociated from the hypotensive and bradycardia responses. As revealed in renal-denervated rats, the cardiovascular and renal excretory responses produced by central OFQ/N occur by a pathway that is independent of intact renal nerves or changes in renal hemodynamics.

renal sympathetic nerve activity; conscious Sprague-Dawley rats; urine flow rate; urinary sodium excretion; glomerular filtration rate; renal plasma flow; intracerebroventricular

ORPHANIN FQ/nociceptin (OFQ/N) is an endogenous heptadecapeptide that shares sequence homology most similar to that of the endogenous opioid peptides, particularly to the putative ligand of the kappa opioid receptor dynorphin A (32, 37). Whereas OFQ/N shares substantial sequence homology to classical opioids (the endorphins), this endogenous peptide does not interact with the mu, delta, or kappa opioid receptor (32, 37). Instead, OFQ/N binds in a saturable manner and with high affinity to a novel opioid-like receptor referred to as opioid receptor-like one (ORL1) (37). Both OFQ/N and ORL1 are located throughout the central nervous system (CNS), including in brain regions known to be involved in the regulation of cardiovascular and renal function (4, 7, 16, 28, 32, 33, 37, 44, 45).

In previous studies, we have demonstrated that the central administration of OFQ/N produces profound changes in cardiovascular and renal excretory function in conscious rats (19, 25). Intracerebroventricular injection of OFQ/N evokes significant reductions in heart rate, mean arterial pressure, and urinary sodium excretion and a marked increase in urine flow rate (i.e., a free-water diuresis) (19, 25). The centrally mediated changes in renal excretory function produced by intracerebroventricular OFQ/N are similar to those produced by central kappa opioids (22, 25). Thus, at equivalent intracerebroventricular doses, both OFQ/N and the kappa opioid agonist dynorphin A evoke a similar magnitude increase in urine flow rate and a sustained antinatriuresis (25). Despite these similar renal responses, central OFQ/N and kappa opioid systems appear to affect the renal handling of water and sodium via different receptor pathways. This premise is supported by the observation that the intracerebroventricular pretreatment of animals with the selective kappa-opioid receptor antagonist nor-binaltorphimine completely prevents the renal responses produced by central dynorphin A but does not alter the diuretic or antinatriuretic response elicited by central OFQ/N (25).

The physiological mechanisms by which central OFQ/N affects the renal handling of water and sodium are unknown. Of particular interest to this topic is whether central OFQ/N affects renal excretory function via a pathway that involves intact renal nerves and alterations in renal sympathetic outflow. This is of interest since in conscious rats, central kappa opioids mediate an increase in renal tubular sodium reabsorption via a central neural mechanism that is dependent on intact renal nerves and augmentation of renal sympathetic nerve activity (22, 23). Unlike activation of central kappa (or mu) opioid systems, central OFQ/N also elicits a concurrent reduction in heart rate and mean arterial pressure at doses that affect renal excretory function (19, 25). Whether these changes in cardiovascular function modulate the renal excretory responses produced by central OFQ/N and are mediated by alterations in central sympathetic outflow to the periphery remain to be established.

The purpose of the present studies was to investigate the role of the renal nerves in mediating the cardiovascular and renal responses produced by the endogenous opioid-like peptide OFQ/N. For this purpose, we examined whether the intracerebroventricular administration of OFQ/N produces changes in renal sympathetic nerve activity as recorded from conscious Sprague-
Dawley rats. In addition, we compared the cardiovascular and renal responses produced by central OFQ/N administration in rats with an intact renal innervation with those from rats that had undergone chronic bilateral renal denervation. Finally, in other studies, we examined the direct central effects of OFQ/N on sympathethic outflow to the kidneys by measuring changes in renal sympathetic nerve activity in sinoaortic deafferentiated (SAD) rats. We have previously reported preliminary findings regarding the sympathoinhibitory effects of central OFQ/N in conscious rats (26).

MATERIALS AND METHODS

Subjects. Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing between 275 and 300 g were used in these studies. All rats were fed with normal sodium diets (Na content, 163 meq/kg) and were allowed tap water ad libitum. All experimental procedures were conducted in accordance with the Louisiana State University Medical Center and the National Institutes of Health Guidelines for the Care and Use of Animals.

Surgery. Five to seven days before experimentation, certain rats were implanted with a stainless steel cannula into the right lateral cerebral ventricle under anesthesia (ketamine, 30 mg/kg im, in combination with xylazine, 3 mg/kg im). The coordinates used for cannula implantation were derived from the atlas of the rat brain by Paxinos and Watson (35): 0.3 mm posterior to the bregma, 1.3 mm lateral to the midline, and 4.5 mm below the skull surface. Custom cut and fabricated guide, dummy (obturator), and internal cannula were purchased from Plastics One (Roanoke, VA). The guide cannula was fixed into position by jeweler's screws and cranioplastic cement. Verification of cannula position in the lateral cerebroventricle was made by observing spontaneous flow of cerebrospinal fluid from the tip of the cannula after removal of the obturator (after cannula implantation and before experimentation) and after the completion of the experiment by intracerebroventricular dye injection and subsequent postmortem brain section verification of dye placement (19, 22–25, 27).

On the experimental day, rats were anesthetized with methohexital sodium (Brevital, 20 mg/kg ip, supplemented with 10 mg/kg iv as needed; Lilly, Indianapolis, IN). Polyethylene catheters (PE-10 tubing attached to PE-50; Becton Dickinson, Sparks, MD) were then implanted into the left femoral artery and vein for recording of arterial pressure and infusion of isotonic saline, respectively. Through a suprapubic incision, a flanged polyethylene cannula (PE-240, Becton Dickinson) was inserted into the urinary bladder. The bladder catheter was then exteriorized and secured by suturing to adjacent muscle, tissue, and skin.

After implantation of these catheters, some rats were also implanted with a renal sympathetic nerve-recording electrode. This was performed by exposing the left kidney through a retroperitoneal flank incision. With the use of a dissecting microscope (×25), a renal nerve branch from the aortorenal ganglion was isolated and carefully dissected free. The renal nerve branch was then placed on a bipolar platinum wire (Cooner Wire, Chatsworth, CA) electrode. Renal sympathetic nerve activity was amplified (×10,000–50,000) and filtered (low, 30 Hz; high, 3,000 Hz) with a Grass PS11 Bandpass Amplifier (Grass Instrument, Quincy, MA). The amplified and filtered signal was channeled to a Tektronix S113 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 (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 examining the magnitude of decrease in recorded renal sympathetic nerve activity during sinoaortic baroreceptor loading with an intravenous injection of norepinephrine (3 µg). The renal nerve activity remaining after maximum inhibition following norepinephrine administration was similar to the background noise observed 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 suturing it to the abdominal trunk muscles. Finally, the electrode cable was exteriorized, and the flank incision was closed in layers.

Certain experiments were performed in which the influence of the renal nerves on kidney function was removed. For these studies rats were subjected to chronic bilateral renal denervation 6–7 days before experimentation. Bilateral renal denervation was performed via bilateral flank incisions and stripping the renal arteries and veins of adventitia, cutting the renal nerve bundles, and coating the vessels with a solution of 10% phenol in absolute ethanol as previously described (12). This renal denervation procedure prevents the renal vasconstrictor response to suprarenal lumbar sympathetic nerve stimulation, prevents the antiinhibitory response to environmental stress, reduces renal catecholamine histofluorescence to nondetectable levels, and reduces renal tissue norepinephrine concentration to <5% of control for up to 15 days postdenervation (12). Because our laboratory has previously and repeatedly verified that this renal denervation procedure completely removes the influence of the renal nerves on kidney function (22–24), verification of renal denervation was not repeated in these studies. After completion of the bilateral renal denervation procedure, the same rat (still anesthetized) was implanted with an intracerebroventricular cannula as previously described.

Certain studies, the central effects of OFQ/N on renal sympathetic nerve activity were studied in rats having undergone prior SAD. SAD was performed as described by Vasquez and Krieger (41). Briefly, after the induction of anesthesia, the external and internal branches of the carotid arteries were exposed. The vagus nerve and surrounding connective tissue were gently dissected away from the vessels. The superior laryngeal, aortic depressor, carotid sinus nerves, and the sympathetic trunk were then cut, and the superior ganglia were removed. After SAD surgery, the same animal (still anesthetized) was implanted with an intracerebroventricular cannula as described. SAD rats were then treated with benzethion penicillin, 100,000 U im, to minimize infection and allowed to recover for 4–6 days. On the day of the experiment catheters were implanted into the femoral artery and vein and the effectiveness of the SAD procedure was tested. For this purpose, conscious rats were administered sodium nitroprusside (4 µg/kg iv). The SAD was considered adequate if the animal responded with no change in heart rate after a decrease in diastolic pressure of 30–50 mmHg. Only animals meeting this criterion for SAD were included in our study.

After surgical preparation and recovery from anesthesia, rats were placed in rat holders to minimize movement and damage to the renal nerve electrode preparation and permit steady-state urine collection. An infusion (50 µl/min iv) of
isotonic saline containing sufficient quantities of inulin and p-aminohippurate (PAH) for determination of inulin and PAH clearance, respectively, was then started and continued for the duration of the experiment. Four to six hours after recovery and the start of isotonic saline infusion, the arterial catheter was flushed and attached to a pressure transducer (model P23 Db, Statham, Oxnard, CA), 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 injection of norepinephrine (3 µg iv), 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. Heart rate was derived from the pulse pressure by a tachograph (Grass model 7 P4H), and arterial pressure and heart rate were recorded on a Grass model 7 polygraph.

Experimental protocols. After stabilization of cardiovascular, renal excretory, and renal sympathetic nerve activity parameters, urine was collected during a 20-min control period. After this, the opioid-like peptide OFQ/N was injected (10 µg icv total in 5 µl isotonic saline vehicle, n = 10). Immediately after central administration, urine was collected during seven consecutive 10-min experimental OFQ/N urine samples.

The role of intact renal nerves in mediating the cardiovascular, renal excretory, and renal sympathetic nerve activity parameters, urine was collected during a 20-min control period. After this, the opioid-like peptide OFQ/N was injected (10 µg icv total in 5 µl isotonic saline vehicle, n = 10). Immediately after central administration, urine was collected during seven consecutive 10-min experimental OFQ/N urine samples.

The dose of OFQ/N used in the present investigations (10 µg icv total, 5.527 nmol, mol wt 1809) was derived from previous dose-response studies performed in conscious Sprague-Dawley rats (25). In these studies, the administration of OFQ/N (1, 10, or 30 µg icv) produced profound reductions in heart rate, mean arterial pressure, and urinary sodium excretion (all responses rapid in onset) and a concurrent increase in urine flow rate (delayed) (25). The diuretic response elicited by OFQ/N was shown to be dose dependent, with 10 µg icv producing the largest magnitude increase in urine flow rate (25). In addition, the intravenous injection of 10 µg OFQ/N, the same dose as administered intracerebroventricularly, did not alter any cardiovascular or renal parameter, thereby excluding the possibility that centrally administered OFQ/N affects cardiovascular and renal function subsequent to its leakage into the periphery (25). Finally, the increase in urine flow rate produced by 10 µg icv OFQ/N was shown to be similar to that produced by the injection of 10 µg icv dynorphin A, an endogenous ligand of the kappa opioid receptor (25). Based on these previous findings, a dose of 10 µg OFQ/N icv was also selected for use in the present investigations.

The physiological effects produced by the in vivo administration of OFQ/N have been studied in other investigations in mice and rats using a similar intracerebroventricular dose (10 µg = 5.527 nmol) as that used in the present investigations (37, and see Ref. 19 for additional studies).

Analytic techniques. Urine volume was determined gravimetrically. Urine sodium concentration was measured by flame photometry (model 943, Instrumentation Laboratories, Lexington, MA). Urine osmolality was measured by the vapor pressure method (Wescor 5500, Wescor, Logan, UT). Urine and plasma inulin and PAH concentrations were determined by the anthrone (15) and ethylenediamine (2) methods, respectively. Glomerular filtration rate was measured as inulin clearance: C_{IN} = (U_{IN}P_{IN})/V, where U_{IN} and P_{IN} are urine and plasma inulin concentrations, respectively, and V is urine flow rate. Effective renal plasma flow was determined by PAH clearance: C_{PAH} = (U_{PAH}P_{PAH})/P_{OSM}, where U_{PAH} and P_{PAH} are urine and plasma PAH concentrations, respectively. Osmolar clearance (C_{osm}) was measured as C_{osm} = (V_{osm}/P_{osm}), where U_{osm} and P_{osm} are urine and plasma osmolality, respectively. Free water clearance (C_{H2O}) was measured as C_{H2O} = V_{osm} - C_{osm}. Data acquisition for renal sympathetic nerve activity measurements were performed with a commercially available software package (Labtech Notebook, version 6.1.1; Laboratory Technologies, Wilmington, MA). Integrated renal sympathetic nerve activity is expressed as µV s/1-s interval. Because of the limitations of comparing values for multifiber renal sympathetic nerve activity between animals, the data are expressed as percentage control with the control values for each animal taken as 100%.

Drugs. OFQ/N (1—17) was obtained from Phoenix Pharmaceuticals (Mountain View, CA). Stock solutions of OFQ/N for intracerebroventricular drug administration were prepared fresh in isotonic saline vehicle and stored frozen. Injection of drugs in isotonic saline vehicle (5-µl volume) was made via a 10-µl Hamilton syringe.

Data analysis. The data were statistically analyzed by using repeated measures ANOVA for main effects and interactions and a Scheffé’s test for pairwise comparisons between means (43). Statistical significance was defined as P < 0.05.

RESULTS

In the present and previous (19, 25, 26) investigations, the injection of 10 µg OFQ/N icv did not produce any overt sedative or behavioral effects (e.g., excitation, enhanced exploratory activity, convulsions, etc.), and the rats remained calm and conscious throughout the protocol.

The cardiovascular and renal responses produced by the intracerebroventricular administration of the opioid-like peptide OFQ/N in conscious Sprague-Dawley rats are shown in Fig. 1. Mean values for each parameter are depicted during control (control, 20 min), and during consecutive 10-min experimental urine collections (time periods 10–70 min) beginning immediately after the administration of OFQ/N (10 µg icv total/5 µl isotonic saline vehicle). In previous investigations, we have demonstrated that the intracerebroventricular injection of isotonic saline vehicle does not produce a change in any cardiovascular or renal parameter, thus demonstrating the stability of the parameters measured under these experimental conditions (24). Compared with respective group control values for each parameter (Fig. 1), the intracerebroventricular injection of OFQ/N produced significant reductions in heart rate, mean arterial pressure, urinary sodium excretion, and renal sympathetic nerve activity. Both the decrease
in heart rate (control, 417 ± 11 beats/min; 20 min, 335 ± 11 beats/min) and mean arterial pressure (control, 117 ± 3 mmHg; 20 min, 101 ± 5 mmHg) were immediate in onset and peaked by 20 min after injection. The bradycardia and hypotensive responses produced by central OFQ/N were transient and returned to control levels by 40 and 30 min, respectively. Although intracerebroventricular OFQ/N also produced an antinatriuresis, this renal excretory response was delayed in onset and persisted after the cardiovascular responses had recovered. Urinary sodium excretion was significantly reduced over periods 20–50 min. Similarly, intracerebroventricular OFQ/N produced a delayed renal sympathoinhibitory response, with significant reductions in renal sympathetic nerve activity not occurring until 30–70 min postinjection (30 min, 75% of control; 70 min, 66% of control; longer time periods not studied). In addition to the reductions in heart rate, mean arterial pressure, urinary sodium excretion, and renal sympathetic nerve activity, intracerebroventricular OFQ/N produced a profound diuresis. The increase in urine output was delayed in onset (30 min) and attained peak magnitude at 40 min (control, 54 ± 6 µl/min; 40 min, 191 ± 15 µl/min) before returning to control levels (60 min). In previous studies, we have shown that the cardiovascular and renal responses produced by the intracerebroventricular injection of OFQ/N (10 µg) resulted from a central site of action, since the intravenous bolus administration of the same dose of the drug did not alter any cardiovascular or renal parameter in conscious rats (25).

Figure 2 shows the cardiovascular and renal responses produced by the intracerebroventricular injection of OFQ/N in nine rats having undergone chronic bilateral renal denervation. As previously observed in rats with an intact renal innervation (Fig. 1), the intracerebroventricular injection of OFQ/N in renaldenervated rats produced a significant decrease in heart rate, mean arterial pressure, and urinary sodium excretion and an increase in urine flow rate. In general, the pattern of changes in each cardiovascular and renal excretory parameter produced by central OFQ/N in renal-denervated rats (Fig. 2) were similar to those produced in intact animals (Fig. 1). There are two exceptions. In renal-denervated rats (Fig. 2), the peak increase in urine flow rate occurred earlier, at 30 min (control, 56 ± 7 µl/min; 30 min, 172 ± 21 µl/min). In addition, the antinatriuresis was further postponed,
with significant reductions attained over periods 40–70 min.

Depicted in Fig. 3 are the changes in glomerular filtration rate and effective renal plasma flow produced by the intracerebroventricular injection of OFQ/N in the same intact and bilaterally renal-denervated Sprague-Dawley rats. Values are means ± SE and illustrate renal hemodynamic effects of OFQ/N (10 µg icv total) for the same rats illustrated in Fig. 1 with intact renal innervation (n = 10) or Fig. 2 with bilaterally denervated kidneys (n = 9). Urine samples were collected during control (C, 20 min) and immediately after intracerebroventricular OFQ/N injection, denoted time periods 10–70 min (consecutive 10-min urine samples). GFR, glomerular filtration rate; RPF, renal plasma flow; gKw, g kidney wt.

Table 1. Changes in free water clearance produced by the intracerebroventricular administration of OFQ/N in conscious rats with intact and denervated kidneys

<table>
<thead>
<tr>
<th>Group</th>
<th>C</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>−60 ± 7</td>
<td>−51 ± 9</td>
<td>−6 ± 9*</td>
<td>75 ± 11*</td>
<td>117 ± 9*</td>
<td>28 ± 19*</td>
<td>−42 ± 12</td>
<td>−72 ± 10</td>
</tr>
<tr>
<td>RDNX</td>
<td>−61 ± 5</td>
<td>−55 ± 9</td>
<td>−9 ± 15</td>
<td>73 ± 25*</td>
<td>27 ± 14*</td>
<td>−9 ± 12</td>
<td>−35 ± 5</td>
<td>−51 ± 7</td>
</tr>
</tbody>
</table>

Values are means ± SE. Changes in free water clearance (C_{H2O}) produced by administration of the opioid-like peptide orphanin FQ/nociceptin (OFQ/N, 10 µg icv total) in conscious rats with intact and denervated (RDNX) kidneys. Values presented are calculated from renal excretory data obtained from same groups of rats depicted in Figs. 1 and 2, respectively. Urine samples were collected during control (C, 20 min) and immediately after injection of OFQ/N (time points 10–70 min, consecutive 10-min urine samples). *P < 0.05, significantly different from corresponding control.
min, respectively. As depicted in Fig. 4, the sympathetic inhibitory response produced by central OFQ/N in SAD rats tended to parallel the onset of the hypotension and the bradycardia. This pattern of response is in contrast to the delayed reduction in renal nerve activity produced by intracerebroventricular OFQ/N in rats with intact baroreceptors (Fig. 1). In SAD rats, intracerebroventricular injection of OFQ/N also produced a significant decrease in urinary sodium excretion and a marked diuretic response (delayed).

For comparative purposes, Table 2 summarizes the renal excretory and renal nerve responses produced by the intracerebroventricular administration of agonists selective for the kappa (U-50488H), mu (dermorphin), and ORL1 (OFQ/N) receptor in conscious rats (intact). In addition, the effects of chronic bilateral renal denervation on the changes in urine flow rate and urinary sodium excretion produced by the central administration of these selective agonists are also shown (renal denervated). The direction of changes depicted for central kappa and mu opioid agonists are from previous investigations in which U-50488H (1 µg total) (22) and dermorphin (0.1 nmol/kg) (24), respectively, were administered intracerebroventricularly to conscious rats using an experimental protocol similar to that employed in the present investigations. Although not depicted in Table 2, central U-50488H (1 µg total) and dermorphin (0.1 nmol/kg) did not alter heart rate, mean arterial pressure, glomerular filtration rate, or effective renal plasma flow in these studies (22, 24).

**DISCUSSION**

The results of the present study demonstrate that in addition to changes in cardiovascular and renal excretory function, the intracerebroventricular administration of OFQ/N produces a significant decrease in renal sympathetic nerve activity in conscious Sprague-Dawley rats. Note, however, that the reduction in renal sympathetic nerve activity did not coincide with the hypotensive response and, instead, was delayed in onset (30 min), tending to parallel the central OFQ/N-induced diuresis and antinatriuresis. Despite the apparent temporal correlation between the renal sympathoinhibitory and excretory responses, several findings indicate that the renal nerves are not involved in mediating the changes in urine flow rate or urinary sodium excretion produced by central OFQ/N. First, it is recognized that alterations in efferent renal sympathetic nerve activity produce reciprocal changes in the renal excretion of sodium and water (11). Based on this knowledge, it would be predicted that central OFQ/N would augment central sympathetic outflow to the kidneys if this peptide had affected urinary sodium excretion by a renal nerve-dependent pathway. Instead, intracerebroventricular OFQ/N evoked a renal sympathoinhibitory effect over the course of the antinatriuresis. In this manner and in contrast to the present findings with OFQ/N, the activation of central kappa opioid systems produces a sustained antinatriuresis by augmenting renal sympathetic nerve activity (22, 23, see Table 2). Further support for the premise that central OFQ/N does not affect the renal handling of sodium (or water) via a pathway that involves the renal nerves stems from the observation that chronic bilateral renal denervation did not alter the OFQ/N-induced antinatriuresis (or diuresis). Together these findings indicate that central OFQ/N evokes an increase in the renal tubular reabsorption of sodium via a pathway that is independent of intact renal nerves and thus changes in central sympathetic outflow to the kidneys.

Similar to the responses produced by OFQ/N, the intracerebroventricular injection of mu opioid agonists (e.g., dermorphin) also produces a decrease in urinary sodium excretion in chronic bilaterally renal-denervated rats (24, Table 2). Therefore, central mu opioid systems also affect the renal handling of sodium via a pathway that does not require intact renal nerves. Despite this similar feature, certain differences exist between the central OFQ/N and mu opioid control of renal function. For instance, unlike the renal sympathoinhibitory response evoked by intracerebroventricular OFQ/N, central mu opioids produce a marked increase in efferent renal nerve activity that occurs over the course of the antinatriuresis (24). Whereas an intact renal innervation is not required to decrease urinary sodium excretion, the increase in renal sympathetic outflow elicited by central mu opioids may, at least in part, contribute to the pattern (i.e., time course) of the antinatriuresis produced by these compounds (24, Table 2). Of further interest, the adrenal glands have also been shown to play a role in mediating the central mu opioid-induced antinatriuresis since this renal response is prevented by chronic bilateral renal denervation and adrenalectomy (20). Whether the adrenal glands also play a role in mediating the antinatriuresis elicited by central OFQ/N remains to be established.

As noted in the preceding paragraphs, the stimulation of central kappa, mu, and ORL1 receptor systems
evoke similar renal excretory responses in conscious rats. Despite this resemblance, there are important differences in the neural and/or hormonal mechanisms by which these central opioid and opioid-like systems mediate their renal responses. To emphasize this point, Table 2 provides a summary of the renal excretory and renal nerve responses produced by the activation of central kappa, mu, and ORL1 receptor systems in conscious rats (intact). Data shown in Table 2 are from previous studies in which the selective kappa and mu opioid agonists, U-50488H (22) and dermorphin (24), respectively, were administered intracerebroventricularly to conscious rats using a similar protocol as that employed in the present studies. To illustrate the requirement for the renal nerves in mediating the renal responses elicited by each central system, the effects of renal denervation on each renal excretory parameter are also shown.

The central administration of OFQ/N evoked an increase in urine flow rate and decrease in urinary sodium excretion without altering effective renal plasma flow or glomerular filtration rate. Thus the renal excretory responses produced by central OFQ/N in conscious rats occur via a pathway(s) that is independent of changes in renal hemodynamics. Similar to these findings, the activation of central kappa or mu opioid systems produces a concurrent diuresis and antinatriuresis in conscious rats without altering renal hemodynamics (10, 22, 24). These findings imply that in each case the reduction in urinary sodium excretion produced by activation of central kappa, mu, or ORL1 systems results from the enhanced renal tubular reabsorption of sodium. The demonstration that central OFQ did not alter renal hemodynamics is of interest since intracerebroventricular OFQ/N produced an initial hypotensive response from 117 ± 3 (control) to 101 ± 5 mmHg (20 min). It is likely that autoregulatory mechanisms acting at the level of the afferent and efferent renal arterioles maintained renal blood flow and glomerular filtration rate relatively constant during the systemic hypotensive period. Although intracerebroventricular OFQ/N also evoked an antinatriuresis and delayed diuretic response in SAD rats, the affects on water excretion were of a different pattern than those observed in rats with intact baroreceptors. In SAD animals, central OFQ/N first produced a decrease in urine flow rate from 71 ± 3 µl/min during control to 30 ± 8 µl/min 20 min postinjection. Coinciding with the recovery of the hypotensive response, the antidiuretic response was converted to a profound diuresis that was greater in magnitude than that observed in rats with intact baroreceptors. Although not studied, it is likely that in SAD rats, changes in renal hemodynamics may have occurred secondary to the marked reduction in mean arterial pressure (to levels at the lower limit for autoregulation) and have contributed to the pattern of renal excretory responses observed.

The central administration of kappa (U-50488H) and mu (dermorphin) opioid agonists produces a profound diuresis in conscious rats by a pathway that is independent of the renal nerves (18, 22, 24, Table 2). As suggested in these and related studies, central kappa (3, 5, 14, 29, 30, 39, 40, 46) and mu opioids (1, 14, 21, 31, 40, 42) cause an increase in urine flow rate via inhibiting the secretion of antidiuretic hormone (vasopressin) into the peripheral circulation. Although not tested in the present studies, it is possible that the diuresis evoked by intracerebroventricular OFQ/N also resulted, at least in part, from a central action of the peptide to inhibit the secretion/release of antidiuretic hormone.

As reported in these and preliminary investigations (26), the central administration of OFQ/N produced a renal sympathoinhibitory response in conscious rats that was delayed in onset, with significant reductions in renal nerve activity not occurring until 30 min after peptide administration. The decrease in renal nerve activity did not show a temporal correlation with the bradycardia and hypotensive response produced by this peptide. In this regard, heart rate (control, 417 ± 11 beats/min; 10 min, 360 ± 12 beats/min) and mean arterial pressure (control, 117 ± 3 mmHg; 10 min, 105 ± 4 mmHg) were already markedly reduced 10 min after OFQ/N administration. In contrast, at this same time point (10 min), renal sympathetic nerve activity remained unaltered (control, 100%; 10 min, 98 ± 5%) and was not significantly reduced until periods 30–70 min, time points in which heart rate and mean arterial pressure had returned to predrug control levels. In part, the lack of a temporal correlation between the cardiovascular and renal nerve responses produced by central OFQ/N in conscious rats may be explained by opposing direct and indirect central effects of the drug on sympathetic outflow. It may be hypothesized that at the dose administered (10 µg), central OFQ/N has a direct CNS action to inhibit renal sympathetic nerve activity, yet this effect is initially masked by a baroreceptor-induced increase in central sympathetic outflow triggered by the systemic hypotension. The validity of this premise is provided by the observation that in SAD rats, intracerebroventricular OFQ/N produced an immediate decrease in renal sympathetic nerve activity (to 55 ± 4 and 38 ± 4% of control, by 10 and 20 min postinjection, respectively) that occurred simultaneously with the hypotension and bradycardia. In addition, the magnitudes of the bradycardia and hypotension produced by intracerebroventricular OFQ/N were greater in SAD rats than in animals with intact baroreceptors. It is interesting to speculate that the rostral ventrolateral medulla may participate in mediating the direct renal sympathoinhibitory effects produced by intracerebroventricular OFQ/N since this peptide has been shown to profoundly inhibit spontaneous discharges of neurons in the rostral ventrolateral medulla in rat brain slices (8). Aside from this possibility, the present findings demonstrate that the carotid baroreceptors are important as a physiological buffering mechanism to minimize the central effects of OFQ/N on changes in blood pressure and heart rate. These findings are also of interest from an alternative prospective. The temporal dissociation of the renal sympathoinhibitory response and the hypotension bradycardia...
suggest that central OFQ/N produces a differential pattern of changes in central sympathetic outflow to different peripheral organs (i.e., the kidneys, heart, and arteriolar resistance vessels). This is of merit since during the hypotension, OFQ/N elicited a decrease in heart rate in conscious rats, yet sympathetic outflow to the kidneys remained unchanged. After the central OFQ/N-induced hypotension and bradycardia recovered, sympathetic outflow to the kidneys was inhibited with this response being sustained. Further studies are clearly required to understand the complex neural and potentially humoral pathways by which central OFQ/N evokes changes in systemic cardiovascular and hemodynamic function in conscious animals.

Perspectives

The central or peripheral administration of OFQ/N produces a complex and unique pattern of changes in cardiovascular and renal function that are of importance from both a physiological and therapeutic standpoint. OFQ/N has been shown to produce a hypertensive response that occurs without a reflex tachycardia (19, 25), produce relaxation of vascular smooth muscle (6, 17), inhibit sympathetic outflow to the kidneys (26, and present investigations), and to be a free water diuretic (i.e., an aquaretic; 19, 25). The combination of these effects produced by the administration of a single drug suggests that OFQ/N, or OFQ/N analogs, may offer a unique approach toward the therapeutic management of fluid-retaining states such as congestive heart failure, liver cirrhosis with ascites, syndrome of inappropriate antidiuretic hormone secretion, and edematous states of the lung.

In summary, the results of the present studies demonstrate that in conscious Sprague-Dawley rats, intact renal nerves are not required to elicit the cardiovascular (bradycardia and hypotension) and renal (diuretic and antinatriuretic) responses to central OFQ/N. This premise is supported by the observation that the intracerebroventricular injection of OFQ/N to chronic bilaterally renal-denervated rats produced profound changes in cardiovascular and renal excretory function that were similar to those observed in intact animals. Moreover, central OFQ/N produced a decrease in renal sympathetic nerve activity, a response that is not consistent with a role for the renal nerves in augmenting the renal tubular reabsorption of sodium. Although reductions in sympathetic outflow to the heart and/or arterial resistance vessels may ultimately contribute to the hypotension and bradycardia produced by central OFQ/N in conscious rats, changes in directly recorded renal sympathetic nerve activity do not provide a useful indicator of this possibility. This is due to the fact that the renal nerve responses are temporally dissociated from the changes in heart rate and mean arterial blood pressure. As revealed in SAD rats, a temporal dissociation results because the direct central inhibitory effects of OFQ/N on renal sympathetic nerve activity are initially masked by a hypotension-induced activation of baroreflex pathways. Although these studies suggest that the central OFQ/N system may play an important role in the regulation of cardiovascular and renal function, further investigations are required to elucidate the pathways by which this novel central opioid system affects these physiological processes.

This work was supported by the National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-43337 and DK-02605 and the American Heart Association-Louisiana Affiliate (91–6–088) to D. R. Kapusta.

Address for reprint requests and other correspondence: D. R. Kapusta, Dept. of Pharmacology, Louisiana State Univ. Medical Center, 1901 Perdido St., New Orleans, LA 70112 (E-mail: dkapus@lsuhn.edu).

Received 24 March 1999; accepted in final form 28 May 1999.

REFERENCES