Microinjection of Nociceptin (Orphanin FQ) into Nucleus Tractus Solitarii Elevates Blood Pressure and Heart Rate in Both Anesthetized and Conscious Rats

LIMIN MAO and JOHN Q. WANG
Division of Pharmacology, School of Pharmacy, University of Missouri-Kansas City, Kansas City, Missouri

ABSTRACT

The role of nociceptinergic transmission in the nucleus tractus solitarii (NTS) in the central modulation of cardiovascular activity was investigated in pentobarbital-anesthetized and conscious rats. Pharmacological activation of nociceptin receptors with a unilateral injection of synthetic nociceptin into the NTS, wherein injection of L-glutamate (1 nmol) caused typical depressor responses, elevated blood pressure and heart rate (HR) in most of the anesthetized rats. The elevation of blood pressure and HR by nociceptin was dose-dependent (0.04, 0.2, and 1 nmol) with a threshold dose of 0.2 nmol. At 1 nmol, changes in blood pressure and HR were evident at 5 min, and remained for 45 min after injection. Pretreatment with the selective nociceptin receptor antagonist nocistatin (1 nmol) into the NTS abolished the nociceptin-induced hypertension and tachycardia. In contrast, the nonselective opioid receptor antagonist naloxone (5 nmol) did not modify the cardiovascular responses to nociceptin. Intra-NTS injection of nocistatin (0.04 and 1 nmol) and naloxone alone had no significant effect on baseline blood pressure and HR. In chronically cannulated and conscious rats, similar pressor and tachycardic responses were induced by intra-NTS injection of 1 nmol of nociceptin. However, changes in blood pressure and HR were rapid, and quickly returned to normal levels within 10 min. These data suggest that the newly discovered nociceptinergic transmission in the NTS has a powerful influence on peripheral hemodynamic activity. This influence is inhibitory and may not be tonically active under normal physiological conditions. Moreover, the cardiovascular responses to exogenous nociceptin were mediated through activation of specific nociceptin receptors rather than typical naloxone-sensitive opioid receptors.

Molecular studies on opioid receptors have made remarkable progress in recent years. Soon after successful cloning of cDNAs encoding the classic opioid receptors μ (Chen et al., 1993; Thompson et al., 1993), δ (Evans et al., 1992; Kieffer et al., 1992), and κ (Minami et al., 1993; Yasuda et al., 1993), a novel opioid-related receptor was cloned by several groups through low-stringency screening technology (for review, see Henderson and McKnight, 1997). This new receptor is named opioid receptor-like 1 (ORL1) receptor or orphan opioid receptor and is almost as homologous to each of the μ-, δ-, or κ-receptors as they are among themselves. However, despite the high sequence homology, traditional opioid ligands (β-endorphin, dynorphins, and enkephalins) display low affinity for the expressed ORL1 receptors in binding studies (Molleureau et al., 1994; Lachowicz et al., 1995). In searching for a natural ligand that interacts with the ORL1 receptor, Meunier et al. (1995) and Reinscheid et al. (1995) recently isolated and identified a biologically active heptadecapeptide from rat brain tissue that was named nociceptin (used in this study) by Meunier et al. (1995) and Orphanin FQ by Reinscheid et al. (1995). Nociceptin is structurally comparable to the existing opioid peptides, especially dynorphin A. However, it shows virtually no affinity for the three traditional opioid receptors and high preference for the ORL1 receptor (Meunier et al., 1995; Reinscheid et al., 1995).

Functional studies on physiological roles of the nociceptinergic system have emerged rapidly during past several years (for review, see Henderson and McKnight, 1997). Similar to the typical opioids, nociceptin is intimately involved in pain modulation (Meunier et al., 1995; Reinscheid et al., 1995; Xu et al., 1996; King et al., 1997). In addition, nociceptin appears to exert a strong influence over cardiovascular activity. For example, an i.v. injection of nociceptin or its analog [Tyr⁴]-nociceptin, decreased arterial blood pressure (ABP) in anesthetized rats (Champion and Kadowitz, 1997a,b) and conscious mice (Madeddu et al., 1999). The decrease in ABP was probably mediated by the selective stimulation of nociceptin receptor ORL1 because nociceptin-induced responses were

ABBREVIATIONS: ORL1, opioid receptor-like 1; ABP, arterial blood pressure; RVL, rostral ventrolateral medulla; NTS, nucleus tractus solitarii; HR, heart rate; PE, polyethylene; MAP, mean arterial pressure; bpm, beats per min.
resistant to blockade of the traditional opioid receptors with the nonselective antagonist naloxone (Champion and Kadowitz, 1997). The effects of systemic nociceptin may be related to inhibition of cardiovascular neurons in the rostral ventrolateral medulla (RVL), a key site and integrated center in the central nervous system controlling peripheral hemodynamic activity (Dampney, 1994; Spyer, 1994). This is because 1) nociceptin inhibited spontaneous discharges of neurons recorded from the RVL in vitro (Chu et al., 1998, 1999a); 2) i.c.v. or local injection of nociceptin into the RVL consistently induced depressor and bradycardic responses (Chu et al., 1999; Kapusta et al., 1999); and 3) nociceptinergic system (peptide and receptors) is densely presented in the RVL according to several morphological studies (Anton et al., 1996; Houtani et al., 1996; Nothacker et al., 1996; Schulz et al., 1996). Centrally mediated cardiovascular effects of nociceptin may not be limited to the RVL because nociceptin receptors also are concentrated in the nucleus tractus solitarii (NTS; Bunzow et al., 1994; Anton et al., 1996), another medullary area important for control of cardiovascular activity and reflex (Lawrence and Jarrott, 1996). However, until now, no attempt has been made to investigate the role of nociceptinergic transmission in the NTS in the regulation of cardiovascular activity.

This study was therefore designed to examine whether the nociceptin receptor in the NTS is involved in the regulation of peripheral ABP and heart rate (HR). Pharmacological activation or blockade of nociceptin receptors was achieved by a unilateral injection of a synthetic peptide agonist nociceptin or antagonist nocistatin (Nicol et al., 1998; Okuda-Ashitaka et al., 1998) into the NTS in acutely prepared and pentobarbital-anesthetized rats, respectively. The effects of the two agents on ABP and HR were detected after injections. In addition, cardiovascular effects of nociceptin were tested in chronically cannulated and conscious freely moving rats.

Materials and Methods

Animals. Adult male Wistar rats (250–300 g; Charles River Breeding Laboratories, New York, NY) were housed three to four in a plastic cage in a colony room maintained on a 12-h light/dark cycle in a controlled environment at a constant temperature of 23°C and humidity of 50 ± 10% with food and water provided ad libitum. Animals were kept in animal facility for at least 5 days before being used in experiments. All animal use procedures were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

Surgery and Intra-NTS Injection. Rats were anesthetized with i.p. injection of sodium pentobarbital (40 mg/kg). Supplemental doses were given through a femoral i.v. catheter whenever needed. The trachea was cannulated with polyethylene (PE)-240 tubing to keep the respiratory passage patent. In all rats, rectal temperature was monitored continuously and maintained within normal physiological limits by means of a heating pad. Left or right femoral vein and artery were cannulated with heparinized PE-50 tubing for i.v. injection and measurement of ABP, respectively. The tubing for ABP measurement was connected to a CE 344 pressure transducer (Maxim Medical). Mean arterial pressure (MAP) and HR were derived electronically from the ABP pulses. The three parameters (ABP, MAP, and HR) were monitored and recorded through a “real-time” ADInstruments PowerLab/8s data recording and analysis system on a Power Mac computer (7200/120).

The rat head was fixed on a stereotaxic frame in a prone position. A midline incision was made on back of the skull to expose the cranium. A hole was drilled through the cranium by targeting the NTS region. A 31-gauge injection cannula (external diameter 0.25 mm, internal diameter 0.11 mm, length 22 mm) was mounted unilaterally lowered through the hole to the injection site in the NTS (13.2 mm caudal to bregma, 0.5 mm lateral to midline, 8.0 mm dorsal to surface of skull).

Chronic implantation of a guide cannula that allowed microinjection to be made into the NTS of conscious, freely moving rats was performed as described previously (Mao and Abdel-Rahman, 1994, 1995). Briefly, after a rat was anesthetized with sodium pentobarbital (40 mg/kg i.p.), the head was fixed on a stereotaxic frame. A 24-gauge stainless steel guide cannula (20 mm in length) was implanted unilaterally from the dorsal surface of the cranium according to the following coordinates: 13.8 mm caudal to bregma, 0.5 mm lateral to midline, and 6 mm dorsal to surface of skull. The guide cannula was permanently secured to the skull by two jeweler’s screws and dental cement. An inner stainless steel wire of the same length (20 mm) was then lowered through the guide cannula, effectively sealing the channel until the day of the experiment. Two to three days after initial surgery, the animals were reanesthetized with sodium pentobarbital (40 mg/kg i.p.). PE-50 catheters, filled with heparinized saline (100 U/ml), were placed in the abdominal aorta and vena cave via left or right femoral artery and vein for measurement of blood pressure and i.v. injection, respectively. The catheters were tunneled s.c., exteriorized at the back of the neck, flushed with a solution of heparin (100 U/ml in saline), and sealed with stainless steel pins. The wound was closed by surgical clips and swabbed with povidone-iodine solution. The experiment was performed at least 3 to 4 days after the second surgery, i.e., 6 to 7 days after brain surgery. On the day of the experiment, the arterial catheter was connected for ABP, MAP, and HR measurements. A 31-gauge stainless steel injector with a length of 22 mm replaced the inner steel wire and protruded 2 mm beyond the guide cannula. The injections of drugs were made at a volume of 50 nl over 15 s in both anesthetized and conscious rats. After completing each injection, the injector was allowed to remain in the brain for an additional 3 min to reduce any possible backflow of the solution along the injection track.

Functional and Histological Examination of Injection Sites. Before or, in a few cases, after each of the experiments, L-glutamate (1 or 2 nmol/50 nl) was injected into the NTS site. The injection sites were functionally considered to be localized within the cardiovascular zone of the NTS if the typical depressor and bradycardic responses were induced after L-glutamate injection (Talman et al., 1986; Leone and Gordon, 1989). Histologically, after the end of the experiment, Puntamine Sky Blue (4%, 50 nl) was applied to the injection site. Animals were euthanized with a lethal dose of the anesthetic given i.v. and were then decapitated. The brain was removed and fixed in a solution of 10% formalin for 4 to 7 days. Frozen serial frontal sections (40 μm) of the brainstem were cut, mounted, and stained with neutral red, from which the actual injection sites were identified by referring to the Paxinos and Watson (1986) atlas. The locations of the blue spots in the NTS were plotted onto copies of standard sections of the medulla taken from the atlas.

Drugs. Sodium pentobarbital and L-glutamate were purchased from Sigma Chemical Co. (St. Louis, MO). Nociceptin (Phe-Gly-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Gln), nocistatin (Thr-Glu-Pro-Gly-Leu-Glu-Glu-Val-Gly-Ile-Glu-Gln-Lys-Gln-Leu-Gln), and naloxone hydrochloride were purchased from Tocris Cookson Inc. (Ballwin, MO). Pentobarbital was dissolved in 0.9% sodium chloride. L-Glutamate, nociceptin, nocistatin, and naloxone were dissolved in ACSF (123 mM NaCl, 0.86 mM CaCl₂, 3.0 mM KCl, 0.89 mM MgCl₂, 25 mM Na₂HCO₃, 0.50 mM NaH₂PO₄, and 0.25 mM Na₂HPO₄ aerated with 95% O₂, 5% CO₂, pH 7.4). All drugs were freshly prepared immediately before use.

Statistical Analysis. The results are presented as mean ± S.E. Data were analyzed with a nested two-way ANOVA followed by a
group comparison with least square-adjusted means. The criterion for statistical significance was $P < .05$.

Results

Effects of Intra-NTS Injection of Nociceptin on ABP and HR. In time control experiments, without drug injection, continuous measurements of ABP and HR over 1 h, to which all of our experiments were confined, indicated only minimal fluctuations of the two parameters (data not shown). It was first observed that unilateral injections of nociceptin into the NTS at doses in the low picomolar range (0.1, 1, and 10 pmol, representing 0.18, 1.81, and 18.08 ng, respectively) did not cause any reliable change in cardiovascular activity when tested in three groups of rats ($n = 3$ to $4$ per group; data not shown). Nociceptin was then tested at doses in the higher range (0.04, 0.2, and 1 nmol, representing 0.07, 0.36, and 1.81 µg, respectively) in three other groups of animals ($n = 6$ per group). As can be seen from Fig. 1, dose-dependent and prolonged alterations in blood pressure and HR occurred after intra-NTS injection of nociceptin in this dose range. The lowest dose of nociceptin (0.04 nmol) did not produce any detectable change. At the middle dose (0.2 nmol), nociceptin started to increase MAP and HR. A statistically significant level was reached 15 (MAP) or 10 (HR) min after the injection compared with the corresponding values from the group of rats given ACSF. The significant increases remained until 30 (MAP) or 40 (HR) min after the injection. Greater responses were seen after nociceptin was injected at the highest dose (1 nmol). Significant elevation of MAP and HR was evident at 5 min and peaked at 25 min. At the peak point, MAP and HR were elevated from baseline 102.4 ± 6.5 mm Hg and 398.3 ± 8.1 beats per min (bpm) to 122.2 ± 6.3 mm Hg (+19.8 mm Hg, representing 19% of the baseline; $P < .05$) and 442.3 ± 7.1 bpm (+44.0 bpm, representing 11% of the baseline; $P < .05$), respectively. The effects of nociceptin were reversible. The elevated MAP and HR returned to the normal levels 50 min after the injection. Besides pressor and tachycardic responses, nociceptin also induced depressor and bradycardic responses in the four injection sites given with either 0.2 or 1 nmol of nociceptin (two per dose).

The cardiovascular effects of nociceptin were repeatable at all of six sites surveyed. Subsequent administration of 1 nmol of nociceptin into the same site 2 h after the initial injection caused marked elevation of blood pressure and HR (data not shown) comparable to those observed after the initial injection. Accordingly, tachyphylaxis to the effects of nociceptin did not appear to develop readily.

To examine whether the pressor and tachycardic responses to intra-NTS injection of nociceptin were due to leakage of the drug from the central injection site to peripheral circulation, we administered nociceptin (1 nmol/100 nl i.v.) to four rats. No changes in baseline MAP and HR were seen after systemic administration of nociceptin (data not shown).

Figure 2 illustrates actual traces of one example experiment showing the character of pressor and tachycardic responses to nociceptin injection. In contrast to nociceptin-induced elevation of MAP and HR, 1 nmol of l-glutamate at the same site induced typical depressor and bradycardic responses (Talman et al., 1980; Leone and Gordon, 1989). From histological examination, most of the injection sites from the above-mentioned studies were distributed in the ventral and medial parts of the NTS (Fig. 3). The four sites in which nociceptin induced hypotension overlapped with the sites in which nociceptin induced hypertension (Fig. 3).

Effects of Intra-NTS Injection of Nocistatin on ABP and HR. Effects of pharmacological blockade of nociceptin receptors with the selective antagonist nocistatin on basal levels of blood pressure and HR were tested in three groups of rats ($n = 4$ per group). A unilateral injection of 0.04 nmol nocistatin was administered into the NTS. Nocistatin was then tested at doses in the higher range (0.04, 0.2, and 1 nmol, representing 0.07, 0.36, and 1.81 µg, respectively) in three other groups of animals ($n = 6$ per group). As can be seen from Fig. 1, dose-dependent and prolonged alterations in blood pressure and HR occurred after intra-NTS injection of nocistatin in this dose range. The lowest dose of nocistatin (0.04 nmol) did not produce any detectable change. At the middle dose (0.2 nmol), nocistatin started to increase MAP and HR. A statistically significant level was reached 15 (MAP) or 10 (HR) min after the injection compared with the corresponding values from the group of rats given ACSF. The significant increases remained until 30 (MAP) or 40 (HR) min after the injection. Greater responses were seen after nocistatin was injected at the highest dose (1 nmol). Significant elevation of MAP and HR was evident at 5 min and peaked at 25 min. At the peak point, MAP and HR were elevated from baseline 102.4 ± 6.5 mm Hg and 398.3 ± 8.1 beats per min (bpm) to 122.2 ± 6.3 mm Hg (+19.8 mm Hg, representing 19% of the baseline; $P < .05$) and 442.3 ± 7.1 bpm (+44.0 bpm, representing 11% of the baseline; $P < .05$), respectively. The effects of nocistatin were reversible. The elevated MAP and HR returned to the normal levels 50 min after the injection. Besides pressor and tachycardic responses, nocistatin also induced depressor and bradycardic responses in the four injection sites given with either 0.2 or 1 nmol of nocistatin (two per dose).

The cardiovascular effects of nocistatin were repeatable at all of six sites surveyed. Subsequent administration of 1 nmol of nocistatin into the same site 2 h after the initial injection caused marked elevation of blood pressure and HR (data not shown) comparable to those observed after the initial injection. Accordingly, tachyphylaxis to the effects of nocistatin did not appear to develop readily.

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(0.08 µg) of nocistatin induced no significant alteration in ABP and HR during a 30-min observation compared with the group of rats treated with ACSF (Fig. 4). At a higher dose (1 nmol, corresponding to 1.93 µg), nocistatin still did not display any detectable effects on either blood pressure or HR (Fig. 4).

**Effects of Administration of Nocistatin or Naloxone before Nociceptin on Nociceptin-Induced Cardiovascular Activity.** To probe the specificity of nociceptin receptors in mediating nociceptin-induced cardiovascular activity, effects of nocistatin and naloxone on nociceptin action were tested and compared in six groups of rats. In the presence of 5 nmol of naloxone, nociceptin preserved its ability to produce pressor and tachycardic responses to the extent parallel with those observed from the ACSF + nociceptin group (Fig. 5). Unlike naloxone, pretreatment with 1 nmol of nocistatin completely blocked nociceptin-induced responses (Fig. 5). In the rats treated with nocistatin + ACSF or naloxone + ACSF, no significant alteration in either MAP or HR was observed (Fig. 5). Figure 6 displays the representative traces illustrating the blockade of nociceptin-induced cardiovascular changes by nocistatin, but not by naloxone (right column versus middle column in Fig. 6).

**Effects of Intra-NTS Injection of Nociceptin on ABP and HR in Conscious Rats.** Similar to observations in anesthetized rats, a unilateral injection of 1 nmol but not 0.04 nmol of nociceptin in six conscious rats caused small-to-moderate increases in ABP and HR (Figs. 7 and 8). Injection of ACSF into the same site caused no alteration in MAP and HR (Figs. 7 and 8). However, the time course of cardiovascular responses to nociceptin in conscious rats differed from those in anesthetized rats. In conscious rats, the induced changes were seen immediately, peaked at 2 min, and quickly returned to preinjection levels 10 (MAP) and 5 (HR) min after the injection (Fig. 7). In addition to elevation of blood pressure and HR, nociceptin also induced depressor and bradycardic responses in two sites. Injection of 1 nmol of L-glutamate into the sites in which application of nociceptin increased ABP and HR caused decreases in the two parameters (Fig. 8). However, L-glutamate injection into the sites in which nociceptin injection decreased ABP and HR produced hypertension accompanied by bradycardia. This hypertensive response to L-glutamate is a similar pattern reported in the early studies in conscious, freely moving rats (Machado and Bonagamba, 1992; Colombari et al., 1994).

**Discussion**

A series of experiments was conducted in this study to define the role of a newly discovered nociceptinergic system in the NTS in the regulation of peripheral cardiovascular activity. We found that pharmacological activation of nociceptin receptors in the NTS with a direct injection of synthetic nociceptin into this region significantly increased blood pressure and HR in both anesthetized and unanesthetized rats. The nociceptin-sensitive sites are histologically in good accordance with the classic cardiovascular area within the intermediate NTS as clarified by typical hypotensive and...
bradycardic responses to injection of an excitatory amino acid L-glutamate (Talman et al., 1980; Leone and Gordon, 1989). Thus, the NTS contains a biologically active nociceptinergic transmission to participate the modulation of hemodynamic activity. The nociceptin-induced cardiovascular activity was blocked by the selective nociceptin receptor antagonist nocistatin, but not by the opioid receptor antagonist naloxone, indicating the specificity of nociceptin receptors in mediating hypertension and tachycardia induced by exogenous administration of nociceptin. And last, pharmacological blockade of nociceptin receptors with nocistatin did not affect basal levels of blood pressure and HR. This suggests that the NTS nociceptinergic system may not be tonically active under normal physiological conditions.

Nociceptin is considered to be a novel member of the opioid peptide family based on its homology in amino acid sequence and similarity of its functional roles in the regulation of a variety of physiological activities, especially pain and cardiovascular modulation, with the typical opioid peptides. For pain modulation, the role of nociceptin peptide shows its complexity. Although some studies define a strong analgesic effect of nociceptin, the other studies find that the same peptide reverses opioid analgesia or produces hyperalgesia (Henderson and McKnight, 1997). The disparity of the biological actions of nociceptin in pain modulation may reflect, and result from, the heterogeneity of this peptide in distribution in the central nervous system and physiological profiles. For cardiovascular regulation, the majority of responses to nociceptin receptor stimulation in the NTS are increases in

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**Fig. 5.** Effects of pharmacological blockade of nociceptin receptors with nocistatin and opioid receptors with naloxone on nociceptin-induced pressor and tachycardic responses. A unilateral intra-NTS injection of vehicle, nocistatin (1 nmol), or naloxone (5 nmol) was made 10 min before 1-nmol nociceptin injection into the same NTS sites in anesthetized rats (n = 6 per group). Values are represented as the mean changes from baseline MAP and HR and vertical bars indicate S.E.M. *P < .05 compared between ACSF + nociceptin or naloxone + nociceptin versus ACSF + ACSF.

**Fig. 6.** Representative recordings illustrating the blockade of nociceptin-induced elevations in blood pressure and HR by nocistatin but not by naloxone. A unilateral intra-NTS injection of vehicle, nocistatin (1 nmol), or naloxone (5 nmol) was made 10 min before 1-nmol nociceptin injection into the same NTS sites in an anesthetized rat. The tracings from top to bottom are pulsatile ABP, MAP, and HR, respectively. Arrows indicate the start of the nociceptin injection.

**Fig. 7.** Time course of increases in MAP (A) and HR (B) after a unilateral injection of ACSF or nocistatin (0.04 or 1 nmol/50 nl) into the NTS in conscious rats (n = 6 per group). Values are expressed as mean changes from baseline MAP and HR and vertical bars indicate S.E.M. *P < .05 compared with the corresponding values taken from ACSF group.
Conscious rat

Due to limited studies, detailed pre- or postsynaptic mechanisms underlying the nociceptin effects in the complex in vivo system are unclear. Local injection of nociceptin might postsynaptically inhibit NTS neurons surrounding the injection site. This speculation is supported by the anatomical evidence that nociceptin receptors are abundantly expressed in NTS neurons (Bunzow et al., 1994; Anton et al., 1996). The inhibition could reduce responsiveness or excitability of NTS neurons to baroreceptor inputs, which could ultimately lead to an increase in blood pressure and tachycardia. A similar cardiovascular change occurs when baroreflex is suppressed at the NTS level. Alternatively, a presynaptic mechanism could, at least in part, contribute to the mediation of the nociceptin effects. In this scenario, intra-NTS nociceptin affects local transmitter release through presynaptically located receptors or interneuronal mechanisms. For example, nociceptin could directly inhibit release of excitatory transmitters, such as glutamate, a proposed transmitter of primary baroreceptor afferents at the level of the NTS (Talman et al., 1980; Reis et al., 1981; Leone and Gordon, 1989). Reduction of glutamate release can result in reduction of NTS neuronal activity and thus increases in blood pressure and HR. In support of this, blockade of glutamate receptors in the NTS produces nociceptin-like pressor and tachycardic responses (Ohta and Talman, 1994). In addition, nociceptin could indirectly result in an increase in release of inhibitory transmitter, such as $\gamma$-aminobutyric acid, an enriched transmitter in the NTS, which, like nociceptin, can induce hypertension and tachycardia after intra-NTS injection (Bousquet et al., 1982; De Wildt et al., 1994; Barron et al., 1997).

Nocistatin at the dose that blocked the nociceptin-induced hemodynamic activity did not affect baseline blood pressure and HR. This suggests that the endogenous ligand is not tonically active in interacting with nociceptin receptors in the NTS to control neuronal activity related to peripheral cardiovascular activity under normal physiological conditions. This inactive nature of nociceptinergic system seems in good accordance with the general concept that endogenous opioids are not significantly involved in maintaining basal activity of many functions. Due to low levels of basal receptor activity,
application of exogenous ligand (synthetic nociceptin) could readily enhance receptor activity to a degree adequate to suppress NTS neurons. The lack of tonic activity of nociceptinergic transmission raises a question as to whether the system is active in the regulation of excitatory responses of NTS neurons to increased baroreceptor inputs. Currently, no attempt has been made to clarify this issue. It is possible that the nociceptinergic system may serve as a compensatory mechanism to normalize or limit overexcitation of NTS neurons in response to strong or long-term stimulation of baroreflex.

Nociceptin was reported to possess the properties of peripheral vasodilators because 1) it decreased tension of isolated peripheral arterial rings from the cat (Gumusel et al., 1997); and 2) i.v. administration of the peptide decreased blood pressure (Champion and Kadowitz, 1997a,b; Madeddu et al., 1999). However, the hemodynamic changes induced by intra-NTS nociceptin were less likely due to a leakage of the drug into circulating system because direct i.v. injection of nociceptin at the effective dose in the NTS did not produce any detectable changes in blood pressure (this study). Moreover, intra-NTS nociceptin induced hypertension and tachycardia, whereas systemic nociceptin induced the opposite pattern of changes (hypotension and bradycardia).

Nociceptin injected into the RVL induced relatively delayed and prolonged cardiovascular responses in α-chloralose/urethane-anesthetized rats (Chu et al., 1999b). Similarly, injection of nociceptin into the NTS of pentobarbital-anesthetized rats caused long-lasting changes in ABP and HR. However, in unanesthetized rats, nociceptin caused a rapid and transient hemodynamic activity. Precise mechanism(s) underlying the distinct dynamic alterations in cardiovascular activities between anesthetized and conscious rats is not clear. Perhaps, the inhibitory influence of anesthetics on baroreflex sensitivity (Bedran-de-Castro et al., 1990; Kurihara et al., 1992) may delay recovery of changes induced by nociceptin. In addition, cellular responsiveness to nociceptin or local intercellular interactions might be shifted under the anesthetized conditions, which somehow contributes to the long-lasting effects of nociceptin.

In conclusion, a unilateral injection of a novel opioid-like neuropeptide nociceptin (orphanin FQ) into the NTS elevated blood pressure and HR in anesthetized and conscious rats. The elevation was sensitive to the nociceptinergic system may serve as a compensatory mechanism to normalize or limit overexcitation of NTS neurons in response to strong or long-term stimulation of baroreflex.

Send reprint requests to: John Q. Wang, Ph.D., Division of Pharmacology, School of Pharmacy, University of Missouri-Kansas City, 2411 Holmes St., Kansas City, MO 64108. E-mail: wangjq@umkc.edu