Nociceptin inhibits rat sympathetic preganglionic neurons in situ and in vitro

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Lai, Chih-Chia, Su Ying Wu, Chiung-Tong Chen, and Nae J. Dun. Nociceptin inhibits rat sympathetic preganglionic neurons in situ and in vitro. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R592–R597, 2000.—In vitro and in situ experiments were conducted to evaluate the hypothesis that the nonclassical opioid peptide nociceptin acting on sympathetic preganglionic neurons (SPNs) inhibits spinal sympathetic outflow. First, whole cell patch recordings were made from antidromically identified SPNs from immature (12–16 day old) rat spinal cord slices. Nociceptin (0.1, 0.3, and 1 µM) concentration dependently suppressed the excitatory postsynaptic potentials (EPSPs) evoked by focal stimulation and hyperpolarized a population of SPNs; these effects were naloxone insensitive. L-Glutamate-induced depolarizations were not significantly changed by nociceptin. Results from this series of experiments indicate that nociceptin inhibits the activity of SPNs by either a presynaptic or postsynaptic site of action, whereby the peptide reduces, respectively, the amplitude of EPSPs or the excitability of SPNs. Second, intrathecal injection of nociceptin (3, 10, and 30 nmol) to urethane-anesthetized rats dose dependently reduced the mean arterial pressure and heart rate; these effects were not prevented by prior intravenous administration of naloxone (1 mg/kg). Physiological saline given intrathecally was without appreciable effects. These results, together with earlier observations of the detection of nociceptin-immunoreactive nerve fibers and nociceptin receptor immunoreactivity in the rat intermediolateral cell column, raise the possibility that the opioid peptide, which may be released endogenously, reduces spinal sympathetic outflow by depressing the activity of SPNs.

NOCICEPTIN OR ORPHANIN FQ, a 17-amino acid, noncyclized peptide, is thought to be the endogenous ligand for the opiate-like receptor (ORL-1; 2, 5, 15, 16, 19, 24). Despite sequence homology with the known µ-, δ-, and κ-opioid receptors, initial studies in rodents revealed that activation of ORL-1 by nociceptin produces pharmacological actions distinct from other known opiate peptides. For example, nociceptin injected intracerebroventricularly in mice produced hyperalgesia in behavioral tests rather than analgesia, as one might expect from an opiate peptide, and the hyperalgesic effect was not reversed by the classic opioid receptor antagonist naloxone (15, 19). The biological role of nociceptin in various brain regions and its behavioral correlate are largely unknown at the present time. Recent immunohistochemical studies revealed an extensive distribution of nociceptin-like and ORL-1-like immunoreactivity throughout the rodent brain and spinal cord, suggesting that activation of ORL-1 receptors by endogenously released peptide may participate in a wide range of physiological functions, including neuroendocrine, learning and memory, motor control, nociception, and other sensory functions (1, 17).

Opiate peptides, in addition to playing a major role in pain processing, have been shown to produce a number of physiological and/or pharmacological responses, including cardiovascular and respiratory effects (10, 18). The initial report that nociceptin relaxes phenylephrine-precontracted cat arterial ring preparations raises the possibility that the peptide may alter cardiovascular functions (9). A significant decrease in blood pressure, heart rate (HR), and cardiac output after intravenous injection of nociceptin to anesthetized rat has been reported (3, 4, 8). The bradycardic and hypotensive effect was nearly eliminated by a combination of vagotomy and guanethidine, suggesting that nociceptin may stimulate vagal afferents and inhibit sympathetic nerves (8). Several earlier studies have also demonstrated a vagally mediated bradycardia induced by morphinelike compounds after peripheral and central administration (13, 20, 25).

Because nociceptin-immunoreactive fibers (7) and immunoreactivity to ORL-1 receptors (1) have been detected in the rat intermediolateral cell column (ML), the present study was undertaken to explore the potential involvement of spinal sympathetic preganglionic neurons (SPNs) with respect to the bradycardia and hypotensive action of nociceptin.

METHODS

Animals. A breeding colony of Sprague-Dawley rats purchased from Harlan (Indianapolis, IN) was established at the Division of Laboratory Animal Resources, East Tennessee State University. Animals were housed two per cage in a room maintained at 22 ± 1°C with an alternating 12:12-h light-dark cycle. Food and water were available ad libitum. Male rats at desired body weight and age were selected from the colony for use in the present study. Animal protocols were approved by the University Animal Care and Use Committee.

Whole cell recording techniques. Immature rats aged 12–16 days were used in this series of experiments. Procedures used in obtaining 500-µm transverse thoracolumbar spinal cord...
slices were similar to those described earlier. The spinal cord slice held between two nylon meshes in an organ chamber was continuously perfused with a Krebs solution of the following composition (in mM): 117 NaCl, 2.0 KCl, 1.2 KH₂PO₄, 2.3 CaCl₂, 1.3 MgCl₂, 26 NaHCO₃, and 10 glucose. The solution was saturated with 95% O₂ and 5% CO₂. Whole cell recordings were made from antidromically identified SPNs under current-clamp mode with the use of an Axoclamp 2A. Patch electrodes filled with a solution containing (in mM) 130 K gluconate, 2 MgCl₂, 2 CaCl₂, 4 ATP, 10 EGTA, and 10 HEPES had a resistance of 3–5 MΩ. A bipolar stimulating electrode (Frederick Haer) was placed near the ventral root exit for antidromic identification of SPNs, and a second electrode was placed dorsolateral to the IML to activate SPNs synaptically. Recordings were made at the room temperature (20 ± 1°C).

Nociceptin, naloxone, and tetrodotoxin (TTX) were applied to the spinal cord slices by superfusion in known concentrations. L-Glutamate was applied by pressure ejection from a glass micropipette containing 1 mM L-glutamate solution placed close to the recording neuron and downstream from the inlet of perfusing Krebs solution with the use of a Picospritzer (General Valve), as described earlier. In some experiments, nociceptin (10 µM) was pressure ejected to SPNs in a manner similar to that described for L-glutamate.

In situ experiments. Procedures for intrathecal administration to anesthetized rats were similar to those described earlier. Adult male rats weighing 250–275 g were used in this series of experiments. Under urethane anesthesia (1.2 g/kg ip), the left femoral artery was cannulated with a polyethylene tubing and connected to a pressure transducer with its output to a Gould pen recorder. The blood pressure signal was used to trigger a Biotach amplifier (Gould ECG/Bi tac) for HR recording. The right femoral vein was cannulated for intravenous injection of naloxone and nociceptin in one series of experiments. In the case of intrathecal injection, a polyethylene tubing (Intramedic PE-10) was passed through a slit in the dura at the atlanto-occipital junction to the T2-T3 segments; the position of the tubing was visually verified at the end of the experiment. Nociceptin was dissolved in saline and frozen in aliquots. A few minutes before administration, nociceptin was thawed and dissolved in phosphate-buffered saline (pH 7.4). Intrathecal injections were made at a volume of 10 µl, which was followed by 10 µl saline to wash in the peptide. Not more than two doses of nociceptin were administered to any one animal.

Drugs and statistical data analysis. Nociceptin was purchased from Bachem (Torrance, CA); bicuculline methobromide, l-glutamate, and strychnine hydrochloride were from Sigma (St. Louis, MO); and naloxone hydrochloride and TTX were from Research Biochemicals International (Natick, MA). For in situ experiments, the mean arterial pressure (MAP) was calculated using the equation [(S − D)/3 − D], where S is systolic blood pressure and D is diastolic blood pressure. The systolic and diastolic pressure was measured directly from the precalibrated chart recorder. Data were analyzed statistically using the ANOVA, with P < 0.05 considered statistically significant. Results are expressed as means ± SE.

RESULTS

Nociceptin depressed excitatory postsynaptic potentials. Stable recordings were made from 35 SPNs that could be antidromically activated by stimulation of the ipsilateral ventral roots (see Fig. 1; Ref. 21). These neurons had a mean resting potential of −56 ± 4 mV and input resistance of 645 ± 39 MΩ, which are comparable to those reported earlier.

A single electrical stimulus (0.1 ms, 5–10 V) applied to the white matter dorsolateral to the IML elicited an excitatory postsynaptic potential (EPSP) and/or inhibitory postsynaptic potential in SPNs. The glycine receptor antagonist strychnine (1 µM) and the GABA<sub>A</sub> receptor antagonist bicuculline (10 µM) were routinely added to the perfusing Krebs solution to isolate the EPSPs pharmacologically. Under these conditions, electrical stimulation elicited a monophasic EPSP, which can be largely blocked by the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (22).

Superfusion of nociceptin (0.1–1 µM) to SPNs consistently suppressed EPSPs in a concentration-dependent manner. Nociceptin (0.1 µM) reduced the amplitude of EPSPs without causing a change of membrane potential; the mean reduction of EPSPs was 9.4 ± 4.0% (n = 6), which is not statistically significant (P > 0.05). At a higher concentration (0.3 µM), the peptide reversibly depressed excitatory postsynaptic potentials (EPSPs) and L-glutamate-induced depolarizations recorded in sympathetic preganglionic neurons (SPNs) of spinal cord slices. A: EPSPs (small upward deflections) were evoked by electrical stimulation applied to an area dorsolateral to the intermediolateral cell column. Nociceptin (300 nM), applied to spinal slice by superfusion (between 2 arrows), depressed EPSPs to −65% of control and effect was reversible upon wash. Top 2 traces are continuous chart recording, interrupted by a period of 10-min wash as shown. Bottom traces are responses of 4 averaged EPSPs taken at times marked on continuous chart recording; a and b are 2 superimposed responses of 4 averaged EPSPs taken at times before and after addition of nociceptin to bath, and c and d are 2 superimposed responses taken before and after recovery from depressant effect of nociceptin. B: membrane depolarizations induced by pressure application of L-glutamate (1 mM) remained relatively constant before, during, and after nociceptin (300 nM) superfusion. Recordings in A and B are taken from 2 different SPNs.
suppressed the EPSPs by an average of 28.1 ± 3.3% (n = 10, P < 0.01); a representative experiment is shown in Fig. 1A. Nociceptin hyperpolarized four SPNs by 2–6 mV and caused no detectable membrane potential change in the other six neurons (Fig. 1A). Nociceptin (1 µM) depressed the EPSPs and hyperpolarized all four neurons; the mean reduction of EPSPs was 46 ± 7% (P < 0.01), and the hyperpolarization ranged from 5–16 mV. Prior superfusion of the opiate receptor antagonist naloxone (1 µM) to the spinal cord slices did not antagonize the synaptic depressant action of nociceptin. For example, nociceptin (0.3 µM) reduced the EPSPs by 32 ± 7 and 33 ± 5% (n = 9, P > 0.05) in the presence of naloxone.

Nociceptin hyperpolarized SPNs. A second conspicuous effect of nociceptin was a slow hyperpolarization in a population of SPNs. At the lower concentration (0.1 µM), nociceptin caused no detectable membrane potential change in any of the six neurons tested. At higher concentrations (0.3 or 1 µM), nociceptin hyperpolarized 8 of 14 SPNs tested; the amplitude of hyperpolarizations varied considerably among these cells, ranging from 2 to 16 mV. As reported earlier, a population of SPNs discharged spontaneously in spinal cord slices harvested from immature rats (23). An example is shown in Fig. 2A, where nociceptin (0.3 µM) by superfusion caused a slow hyperpolarization of ~10 mV and nearly abolished spontaneous discharges in this neuron. Nociceptin by pressure ejection elicited a hyperpolarization of relatively fast onset and offset and abolished spontaneous discharges in another neuron (Fig. 2B).

The nociceptin-induced hyperpolarization was associated with a small to moderate (10–25%) decrease of membrane input resistance, as shown in Fig. 2B. Prior superfusion of the spinal slices with naloxone (1 µM) did not prevent the nociceptin-induced hyperpolarizations in all three cells tested; an example is shown in Fig. 2B. TTX (0.3 µM) was added to Krebs solution to validate that the peptide hyperpolarizes SPNs directly. Nociceptin (1 µM) caused a membrane hyperpolarization of similar amplitudes before and after the addition of TTX to Krebs solution in all three neurons tested (not shown).

L-Glutamate-induced depolarizations. This series of experiments was conducted to analyze the possible site of synaptic depressant action of nociceptin. Membrane depolarizations were induced by L-glutamate (1 mM) released from a micropipette positioned close to the recording SPN as described (11). Nociceptin (0.1 or 0.3 µM) caused no significant decrease of L-glutamate-induced depolarizations (Fig. 1B); the mean amplitude of L-glutamate depolarizations before and after nociceptin (0.1 µM; n = 3 or 0.3 µM, n = 4) was 12.3 ± 2.5 and 12.0 ± 2.4 mV, respectively.

Nociceptin on MAP and HR. The MAP and HR in urethan-anesthetized rats was 80.7 ± 2.7 mmHg (n = 21) and 355 ± 15 beats/min (n = 16). Nociceptin (3, 10, and 30 nmol) in 10 µl by intrathecal injection consistently decreased the MAP and HR in a dose-dependent manner; the responses lasted for minutes to more than 1 h (Fig. 3, B and C). The bradycardic and vasodepressant effects of nociceptin were not antagonized by prior intravenous naloxone (1 mg/kg) in any of the five rats tested. The mean reduction in MAP and HR by intrathecal nociceptin (10 nmol) was 19 ± 4 and 15 ± 3 and 20 ± 3 and 14 ± 4% (P > 0.05), respectively, before and after intravenous naloxone (not shown).

Control experiments. Two sets of control experiments were performed. First, physiological saline of equal volume upon intrathecal injection produced little or no change of blood pressure and HR in any of the six animals. Second, intravenous nociceptin has been shown to lower blood pressure and HR in several studies (3, 4, 8). As a positive control, intravenous nociceptin (0.3–10 nmol/kg) was found to dose dependently lower MAP and HR. A representative experiment is shown in Fig. 4. Contrary to the slow time course after intrathecal
Injection, nociceptin by intravenous injection produced a relatively fast onset of decrease and recovery of MAP and HR (Fig. 4).

**DISCUSSION**

In vitro and in situ experiments conducted in this study support the thesis that the opiate peptide nociceptin is inhibitory to rat SPNs, as assessed by direct recording from these neurons in spinal cord slices and by monitoring the output of these neurons in the form of blood pressure and HR in situ.

First, results from whole cell recordings from SPNs of spinal cord slices demonstrate that nociceptin depressed the activity of these neurons by two possible sites and mechanisms of action. At lower concentrations (<0.1 µM), nociceptin attenuated EPSPs without causing a significant change of membrane potentials. At higher concentrations, nociceptin depressed EPSPs and hyperpolarized a portion of SPNs. It should be mentioned that SPNs recorded in the slice preparations may or may not be associated with cardiovascular activities. As the response to nociceptin is uniformly inhibitory, a cardiovascular SPN would have been inhibited by the peptide.

The observations that nociceptin at lower concentrations suppressed the amplitude of EPSPs without causing a detectable change of membrane potentials and L-glutamate-induced depolarizations are consistent with the idea that the peptide attenuates EPSPs by a presynaptic site of action whereby the release of excitatory transmitters, probably L-glutamate, is diminished. This is similar to the presynaptic inhibitory action of nociceptin reported in substantia gelatinosa neurons (11, 14). The mechanism by means of which nociceptin inhibits transmitter release is not known. The inhibitory action of nociceptin is not prevented by the opiate receptor antagonist naloxone, thus consistent with the earlier reports that the peptide interacts with a naloxone-insensitive site (15, 16).

At higher concentrations, nociceptin hyperpolarized a population of SPNs. This effect was also naloxone resistant. As the hyperpolarization persisted in a TTX-containing solution, nociceptin probably acted directly on SPNs. The ionic mechanism underlying nociceptin-
induced hyperpolarizations remains to be determined. The hyperpolarization was accompanied by a fall in membrane resistance, suggesting that nociceptin may increase membrane conductance, probably to K ions. The hyperpolarizing action of nociceptin renders SPNs less excitable and limits neuronal discharges, as illustrated in Fig. 2.

Collectively, the opioid peptide nociceptin may inhibit the activity of SPNs by interacting with ORL-1 receptors located at nerve fibers presynaptic to or on SPNs. Activation of presynaptic and postsynaptic receptors reduces, respectively, excitatory transmitter release and membrane excitability.

Results from in situ experiments corroborated the inhibitory action of nociceptin on SPNs observed in vitro. Nociceptin by intrathecal administration dose dependently lowered blood pressure and HR for minutes, whereas saline injection had no appreciable effects. The response was not antagonized by intravenous naloxone. Because SPNs in the spinal cord provide the known output to the sympathetic ganglia, which in turn, innervate the heart and blood vessels, the change in blood pressure and HR after intrathecal nociceptin should reflect a depressed activity of SPNs. The possibility that intrathecal nociceptin may enter the peripheral circulation and produce a vasodepressant action cannot be excluded.

As a positive control, we evaluated the effects of nociceptin on blood pressure and HR after intravenous injection. Similar to the results reported by others (3, 4, 8), nociceptin dose dependently lowered blood pressure and HR. Collectively, our results indicate that the fall in systemic blood pressure and HR after intrathecal nociceptin is probably caused by the peptide interacting with receptors within the spinal cord, possibly the IML, where the majority of SPNs is situated. The detection of ORL-1 receptor immunoreactivity in the rat IML provides the anatomical substrate for interaction with nociceptin (1).

The question of whether endogenously released nociceptin in the spinal cord may play a role in regulating sympathetic outflow remains to be addressed. Our immunohistochemical study shows that nociceptin-like immunoreactive fibers are present in the rat IML (7). This, in conjunction with the observation of the presence of ORL-1 receptors in the IML area (1), supports the hypothesis that the peptide may serve as a putative neurotransmitter/modulator to SPNs. Recently, nociceptin has been found to inhibit spontaneous discharges of rostral ventrolateral medulla (RVLM) neurons in brain stem slices and to lower blood pressure and HR upon bilateral microinjection into the RVLM of anesthetized rats (6). Viewed in this context, nociceptin or like substances may influence cardiovascular activity at several different levels of the neuraxis. The physiological condition under which the nociceptin system at different levels of the neuraxis may be differentially activated is currently unknown.

In summary, the opioid peptide nociceptin is found to exert a naloxone-insensitive inhibitory action on rat SPNs, thus negatively influencing the spinal sympathethic outflow.

Perspectives

Opioid peptides are thought to be mainly involved in pain perception. More recent studies have shown that opioid receptors are widely distributed in the central and peripheral nervous systems and that activation of these receptors exerts a depressor or pressor response, dependent on the subtype and location of opioid receptors (10, 18). The present study shows that the recently isolated, nonclassical opioid peptide nociceptin produces a depressor response by interacting with ORL-1 receptors situated in the IML area. Our result reinforces the idea that opioid peptides may play an important role in cardiovascular regulation by interacting with receptors at different levels of the neuraxis. Further studies will be needed to determine the pathophysiological condition under which various opioid systems at different levels of the neuraxis are activated.

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