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Bradycardia elicited by microinjections of nociceptin/orphanin FQ into the intermediolateral cell column at T1–T2 in the rat

Vineet C. Chitravanshi, Hreday N. Sapru *

Department of Neurological Surgery, MSB H-586, University of Medicine and Dentistry of New Jersey - New Jersey Medical School, 185 South Orange Ave., Newark, NJ 07103, USA

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Abstract

Microinjections (30 nl) of nociceptin/orphanin FQ (N/OFQ) into the intermediolateral cell column (IML) at T1 and T2 levels of the spinal cord elicited bradycardia. The decreases in HR were 12.3 ± 2.9, 17.3 ± 2.7, 26.7 ± 3.1, and 18.6 ± 3.4 beats/min in response to 0.075, 0.15, 0.62, and 1.25 mM concentrations, respectively. Maximally effective concentration of N/OFQ was 0.62 mM. No changes in BP were elicited by microinjections of N/OFQ into the IML at T1–T2. The bradycardic responses were completely blocked by prior microinjections of a N/OFQ receptor (NOP receptor) antagonist ([*N*-phe^1]-nociceptin-(1-13)-NH_2, 9 mM) into the IML at T1–T2. Blockade of myocardial β-1 adrenergic receptors also abolished the bradycardic responses elicited by microinjections of N/OFQ into the IML. It was concluded that activation of NOP receptors in right IML at T1–T2 by N/OFQ elicited bradycardic responses which were mediated via the sympathetic nervous system.

Keywords: Atenolol; Bradycardia; Denopamine; IML; NOP receptor

Nociceptin/orphanin FQ (N/OFQ) [18,19,25] is an endogenous ligand for a G-protein coupled receptor, named N/OFQ peptide (NOP) receptor (previously known as ORL1 receptor). N/OFQ has a high and selective affinity for NOP receptor and a very poor affinity for classical opioid receptors (mu, delta, and kappa receptors) [9,18]. Although NOP receptor shares a high sequence similarity with classical opioid receptors [3,5,6,13,14,20–22,24], it does not bind opioid peptides. Naloxone which is an antagonist at classical opioid receptors does not block the effects of NOP receptor agonists [3,5].

The sympathetic preganglionic neurons that provide innervation to the heart are located in the intermediolateral cell column (IML) of the thoracic cord at T1–T3 levels [30]. The presence of NOP receptors [2,24] and N/OFQ immunoreactivity [12,23,26], has been reported in the IML. Based on these reports [23,24,26] it was hypothesized that N/OFQ may elicit cardiac responses by activating NOP receptors in the IML. This hypothesis was tested in our above-mentioned model in which sympathetic preganglionic neurons providing innervation to the heart can be selectively stimulated [29].

Experiments were done in adult male Wistar rats (Charles River Laboratories, Wilmington, MA, USA), weighing 300–350 g ($n = 48$). The experimental procedures were performed in accordance with the NIH guidelines for research involving animals. Additionally, protocols for animal use in this investigation were approved by the Institutional Animal Care and Use Committee of this university.

Details of general procedures used in this study have been described by us previously [8,15,16]. Briefly, the rats were anesthetized with urethane (1.2–1.4 gm/kg, i.v.; Sigma Chemicals, St. Louis, MO). Pulsatile arterial pressure (PAP), mean arterial pressure (MAP) and heart rate (HR) were monitored by standard techniques. The rats were artificially ventilated with room air and end-tidal CO2 was maintained at 3.5–4%. Rectal temperature was monitored continuously and maintained at 37 ± 0.5 °C. All of the tracings were recorded on a polygraph (Grass Instruments, model 7D).

The rats were placed in a prone position in a stereotaxic instrument (model 1430) with a rat spinal unit attachment (model 980) (David Kopf Instruments, Tujunga, CA, USA) and the dorsal surface of the spinal cord from C7 to T5 was exposed [29]. Microinjections were made as described previously using a picospritzer (General Valve Corp, Fairfield, NJ, USA) and four barreled glass micropipettes (tip size 20–40 μm)

* Corresponding author. Tel.: +1 973 972 5876; fax: +1 973 972 5986.
E-mail address: sapru@umdnj.edu (H.N. Sapru).

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[7,8,15,16,28,29]. All of the solutions for the microinjections were freshly prepared in aCSF. The volume of all microinjections was 30 nl. The duration of microinjection was 10 s. Controls for microinjections consisted of aCSF. The coordinates for the IML at T1–T2 were: 0.8–1 mm lateral to the midline and 0.8–1.1 mm deep from the dorsal surface of the spinal cord.

Typical sites in the IML, where L-Glu and N/OFQ were microinjected, were marked by a microinjection of diluted India ink contained in one of the barrels of the glass micropipette used for microinjections. The details of perfusion of the animals, tissue fixation, and cutting and preparation of sections (30 μm) have been described previously [8,15,16].

For statistical analyses, the means and standard error of the means (S.E.M.) were calculated for maximum changes in heart rate (HR) in response to microinjections of N/OFQ. Comparisons of changes in HR elicited by different concentrations of N/OFQ (Sigma Chemicals) were made by using a one-way analysis of variance followed by Tukey–Kramer multiple comparison test. Comparisons of the maximum decreases in HR elicited by N/OFQ before and after the microinjections of NOP receptor antagonist, [*N*-Phe^1]-nociceptin-(1-13)-NH_2 (Phoenix Pharmaceuticals, Belmont, CA) [4], were made by using paired *t*-test. In all cases, the differences were considered significant at $p < 0.05$.

Baseline HR and MAP were 426 ± 9.8 beats/min and 110 ± 2.7 mmHg, respectively in the urethane-anesthetized rats ($n = 48$).

In each rat, right IML at T1 and T2 was selected at random for microinjections. As mentioned earlier, the IML site was identified by microinjections of L-Glu (5 mM); tachycardic responses (36.9 ± 4.1 bpm), with no accompanying changes in BP, were observed. Microinjections of aCSF at these sites did not elicit any response. The interval between the microinjections of L-Glu and other agents was 5 min. N/OFQ in different concentrations (0.075, 0.15, 0.62, and 1.25 mM) was microinjected into the right IML ($n = 12$). No more than three concentrations of N/OFQ were microinjected in random order in each rat and the interval between these microinjections was at least 30 min. The decreases in HR elicited by the afore-mentioned concentrations of N/OFQ were 12.3 ± 2.9, 17.3 ± 2.7, 26.7 ± 3.1, and 18.6 ± 3.4 beats/min, respectively (Fig. 1A). No changes in BP were elicited by microinjections of N/OFQ. Since the responses to 0.62 mM were greater than other concentrations, this concentration was selected for other experiments. The onset and duration of HR responses to 0.62 mM concentration were 12.8 ± 2.7 s and 5.31 ± 0.4 min, respectively. At T1–T2, microinjections of either L-Glu (5 mM) or N/OFQ (0.62 mM) outside IML (e.g., 0.5–0.6 mm lateral to the midline and 2.0–2.2 mm deep from the dorsal spinal surface) did not elicit any cardiovascular response. Intravenous injections of the same dose of N/OFQ (i.e., 0.62 mM, 30 nl) in another group of rats ($n = 12$) elicited no changes in HR.

In another group of rats ($n = 12$), N/OFQ (0.62 mM) was microinjected into IML three times, at 30 min intervals. The decreases in HR in response to these three consecutive microinjections of N/OFQ were 26.7 ± 3.1, 25 ± 3.4 and 27.5 ± 5.5 bpm, respectively; the differences between the bradycardic responses to repeated microinjections of N/OFQ were not statistically significant ($p > 0.05$).

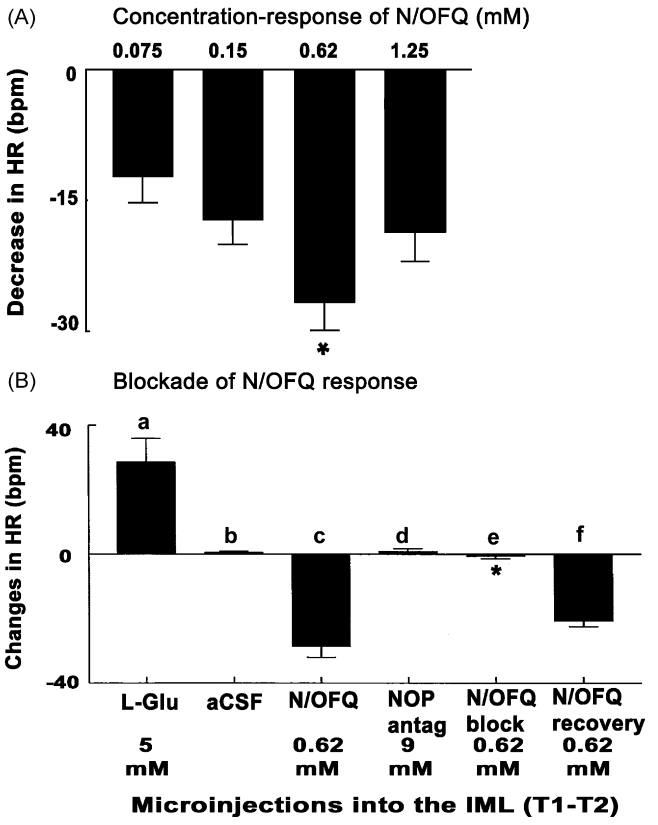

Fig. 1. (A) Concentration-response for HR. Microinjections (30 nl) of different concentrations of N/OFQ (0.075, 0.15, 0.62 and 1.25 mM) into the IML at T1–T2 elicited decreases in HR ($n = 12$). The responses to 0.62 mM were significantly ($^*p < 0.05$) greater compared to other concentrations. (B) Blockade of nociceptin responses. Microinjections of L-Glu (5 mM) into the right IML at T1–T2 elicited tachycardic responses ($n = 7$) (Fig. B, (a)). At the same site, microinjections of aCSF (30 nl) did not elicit any response (Fig. B, (b)). Microinjections of N/OFQ (0.62 mM) at the same site elicited a decrease in HR (Fig. B, (c)). After an interval of 30 min, microinjection of an NOP receptor antagonist, [*N*-phe^1]-nociceptin-(1-13)-NH_2 (9 mM) elicited no significant HR responses (Fig. B, (d)). An interval of about 5 min was allowed when N/OFQ (0.62 mM) was microinjected again at the same site; at this time, N/OFQ failed to elicit bradycardic responses (Fig. B, (e)). The difference in N/OFQ-induced decreases in HR before (Fig. B, (c)) and after (Fig. B, (e)) the microinjection of the NOP receptor antagonist was highly significant ($^*p < 0.001$). After an interval of 60 min, the bradycardic responses to microinjections of N/OFQ showed some recovery but it was incomplete (Fig. B, (f)).

The role of NOP receptors in mediating the responses to N/OFQ was tested in another group of rats ($n = 7$). In this group, microinjections of L-Glu (5 mM) into the right IML elicited tachycardic responses (28.6 ± 7.3 bpm) ($p < 0.01$) (Fig. 1B, (a). When HR returned to baseline levels, a 5 min interval was allowed. Microinjections of aCSF (30 nl) at the same site did not elicit any cardiovascular response (Fig. 1B, (b). Five minutes later, microinjections of N/OFQ (0.62 mM) at the same site elicited a decrease in HR (28.6 ± 3.4 bpm) ($p < 0.01$) (Fig. 1B, (c). When the HR returned to baseline levels, an interval of 30 min was allowed when an NOP receptor antagonist, [*N*-phe^1]-nociceptin-(1-13)-NH2 (9 mM), was microinjected at the same site. The antagonist by itself did not elicit significant