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Short Communication

Microinjections of nociceptin into the nucleus ambiguus elicit tachycardia in the rat

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Abstract

Cardiovascular effects of activation of opioid receptor like receptors (ORL1 receptors) in the nucleus ambiguus were studied in urethane-anesthetized, adult male Wistar rats. Microinjections of nociceptin (0.31, 0.62, 1.25 and 2.25 mmol/L) into the nucleus ambiguus elicited increases in heart rate (17.5 ± 4, 33.3 ± 2.9, 16.5 ± 1.5 and 13.9 ± 2.7 beats/min, respectively) which were blocked by an ORL1 receptor antagonist. These results indicate that activation of ORL1 receptors in the nucleus ambiguus elicits tachycardia.

Theme: Endocrine and autonomic regulation
Topic: Cardiovascular regulation

Keywords: Glutamate; Heart rate; ORL1 receptor

A G-protein coupled receptor, named opioid receptor like receptor (ORL1 receptor) or opioid receptor OP_4, has been identified in the central nervous system [1,3,7,17,18,20,22–24,26]. This receptor shares a high sequence similarity with other opioid (mu, delta and kappa) receptors [5,7,17,18,22–24,26]. There is notable difference between the ORL1 receptor and other opioid receptors; i.e., naloxone blocks the effects of mu, delta and kappa opioid receptor agonists [29] but not those of ORL1 receptor agonists [5,6]. A heptadecapeptide, named nociceptin [21,22] or Orphanin-FQ [27], is considered to be an endogenous ligand of ORL1 receptor because of its high and selective affinity for this receptor and a very poor affinity for other opioid receptors [11,20]. The presence of nociceptin immunoreactivity has also been demonstrated in different regions of the brain and spinal cord [1,3,15–17,23–26].

The majority of parasympathetic preganglionic neurons that provide vagal innervation to different regions of the heart are located in the nucleus ambiguus (NA) and the loose formation surrounding it [14,30–32]. The presence of ORL1 receptors [26] and nociceptin-immunoreactivity has been reported in the NA [25]. Preliminary results of our studies on the effects of nociceptin in NA, presented at a symposium held in Orlando, Florida, USA, have been published [28]. In the present paper, we report the cardiovascular effects of microinjections of nociceptin into the NA in more detail.

Experiments were done in adult male Wistar rats (Charles River Laboratories, Wilmington, MA, USA), weighing 300–350 g (n = 44). All animals were housed under controlled conditions with a 12-h light/dark cycle. Food and water were available to the animals ad libitum. The experimental procedures were performed in strict 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. The number of animals used was the minimum required for statistical

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analyses of the data and every effort was made to minimize any suffering to the animals.

Details of general procedures used in this study have been described by us previously [8]. Briefly, urethane (1.2–1.4 gm/kg; Sigma Chemicals, St. Louis, MO) was injected intravenously in 6–7 aliquots at 2-min intervals. The depth of anesthesia was established by pinching the hind paw of the rat; absence of a blood pressure (BP) response and/or withdrawal of the limb indicated that the rat was properly anesthetized. Pulsatile arterial pressure (PAP), mean arterial pressure (MAP) and heart rate (HR) were monitored by standard techniques. 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 supine position in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA, USA, model 1430), and a ventral approach was used to identify the NA. Four to five barreled glass micropipettes (tip size 20–40 μm) were used for microinjections; one barrel contained artificial cerebrospinal fluid (aCSF), second barrel contained L-Glu (5 mmol/L; Sigma Chemicals) and the remaining barrels were filled with other agents depending on the experiment. All of the solutions for the microinjections were freshly prepared in aCSF. Each barrel of the micropipettes was connected via polyethylene tubing to one of the channels on a picospritzer (General Valve Corp, Fairfield, NJ, USA) and the solutions were ejected (20–30 nL) by application of air-pressure. The ejected volume was visually confirmed by the displacement of fluid meniscus in the micropipette barrel under a binocular microscope with a graduated reticule in one eye-piece. The duration of microinjection was 10 s. Controls for microinjections consisted of aCSF (20–30 nL). The coordinates for the NA were: 0.5 caudal–0.9 mm rostral to the confluence of vertebral arteries, 1.5–1.9 mm lateral to the midline and 1.1–1.6 mm deep from the ventral medullary surface. NA was identified by microinjecting L-Glu (5 mmol/L); bradycardia with no concomitant change in BP indicated that the site of microinjection was in the NA. In order to test if leakage of nociceptin from the microinjection site in the NA into the peripheral circulation caused the observed responses, a maximally effective concentration of nociceptin (0.62 mmol/L) was also injected intravenously and cardiovascular responses were monitored.

Typical sites in the NA were marked by a microinjection (20–30 nL) 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].

The means and standard error of the means (SEM) were calculated for maximum changes in HR in response to microinjections. Comparisons of increases in HR elicited by different concentrations of nociceptin (Sigma Chemicals), were made by using a one-way analysis of variance followed by Tukey–Kramer multiple comparison test. Comparisons of the maximum increases in HR elicited by nociceptin before and after the microinjections of ORL1 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 $P < 0.05$.

Average resting values for MAP and HR, calculated from these values in different groups of urethane-anesthetized rats, were 101.3 ± 4.3 mm Hg and 455.2 ± 10.8 bpm, respectively (n = 39). The NA site was identified by unilateral microinjections of L-Glu (5 mmol/L); average value for decrease in HR elicited by microinjections of L-Glu (5 mmol/L) into the NA, calculated from these values obtained in different groups of rats (n = 39), was 87.3 ± 13.8 bpm. Only the sites from which bradycardic responses, accompanied by no change in BP, were selected for further studies. Microinjections of aCSF (20–30 nL) at these sites did not elicit any response. The interval between the microinjections of L-Glu and other agents was at least 5 min.

In one group of rats (n = 9), microinjections of nociceptin (0.31, 0.62, 1.25 and 2.25 mmol/L) into the NA elicited increases in HR (Fig. 1). A bell-shaped curve was observed for tachycardic responses when nociceptin was microinjected into the NA. Other authors have also reported similar concentration–response curves using other chemical agents. For example, Criscione et al. [15], using microinjections of carbachol and acetylcholine, and Ciriello and Zhang [10], using neurotensin microinjections into the mNTS, reported similar concentration–response curves. Although the reason for these observations is not readily apparent, one possible explanation may be that, at higher concentrations of the agonist, the receptors are desensitized. Maximal tachycardic responses were elicited by a 0.62 mmol/L concentration. Therefore, this concentration was selected for other studies in this paper. The onset and duration of responses to this concentration of nociceptin (0.62 mmol/L) were 22.8 ± 2.7 s and 9.4 ± 0.8 min,

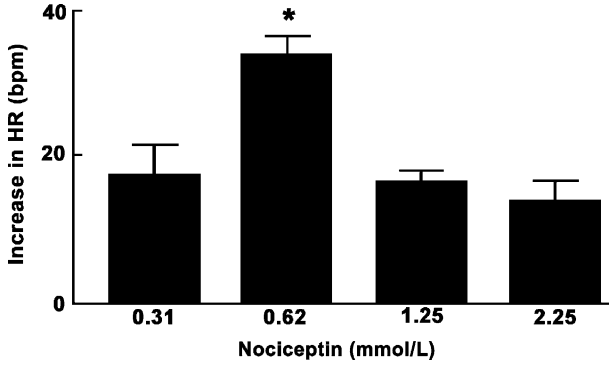

Fig. 1. Concentration–response for HR. Microinjections of different concentrations of nociceptin (0.31, 0.62, 1.25 and 2.25 mmol/L) into the NA elicited increases in HR (17.5 ± 4, 33.3 ± 2.9, 16.5 ± 1.5 and 13.9 ± 2.7 beats/min, respectively; n = 9). The responses to 0.62 mmol/L were significantly (*$P < 0.05$) greater compared to other concentrations.