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The nociceptin receptor-mediated inhibition of the rat rostral ventrolateral medulla neurons in vitro

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Abstract

The recently available antagonist selective for novel nociceptin receptor, [Phe$^1\psi$(CH$_2$-NH)Gly^2]NC(1–13)NH$_2$, was utilized in this study to verify specificity of nociceptin receptor in mediating the nociceptin-induced inhibition of electrical activity of neurons in the rostral ventrolateral medulla of rat brain slices. Perfusion of nociceptin (10 nM) considerably reduced spontaneously firing frequency of the medullary neurons. Co-perfusion of [Phe$^1\psi$(CH$_2$–NH)Gly^2]NC(1–13)NH$_2$ (10 μM) completely blocked the nociceptin-induced depression of the neuronal activity. Blocking effect of [Phe$^1\psi$(CH$_2$–NH)Gly^2]NC(1–13)NH$_2$ was concentration-dependent. However, the nociceptin antagonist did not modify basal, and opioid peptide enkephalin-depressed, firing rates of the neurons. In contrast to [Phe$^1\psi$(CH$_2$-NH)Gly^2]NC(1–13)NH$_2$, the non-selective opioid receptor antagonist naloxone (10 μM) failed to affect the nociceptin inhibition even though naloxone at a lower concentration (1 μM) readily blocked enkephalin-induced depression of the neuronal activity. These data indicate that the nociceptin-induced inhibition of spontaneous discharge of the rostral ventrolateral medulla neurons is specifically mediated by [Phe$^1\psi$(CH$_2$–NH)Gly^2]NC(1–13)NH$_2$-sensitive nociceptin receptors distinct from typical naloxone-sensitive opioid receptors.

Keywords: Nociceptin; Orphanin FQ; Opioid; [Phe$^1\psi$(CH$_2$–NH)Gly^2]NC(1–13)NH$_2$; Electrophysiology; Cardiovascular activity; Rostral ventrolateral medulla

1. Introduction

Besides the three well-known traditional opioid receptors (μ, δ and κ), a possibly new member of opioid receptor family was cloned in 1994 by a number of laboratories, which is usually named as orphan opioid receptor or opioid receptor-like 1 (ORL1) receptor (Bunzow et al., 1994; Chen et al., 1994; Fukuda et al., 1994; Mollereau et al., 1994; Wang et al., 1994; Wick et al., 1994). Soon after cloning of the ORL1 receptor, a heptadecaptide named nociceptin or orphanin FQ was identified in 1995, which is believed to be a specific endogenous ligand for the novel ORL1 receptor according to its highly affinity for the ORL1 receptor and very poor affinity for all three traditional opioid receptors (Meunier et al., 1995; Reinscheid et al., 1995). Nociceptin and ORL1 receptors have been found to abundantly distribute in neurons throughout central nervous system (Mollereau et al., 1994; Lachowicz et al., 1995; Anton et al., 1996; Houtani et al., 1996; Nothacker et al., 1996; Schulz et al., 1996). Thus, nociceptin, like other conventional opioid peptides, might play important roles in modulating a variety of physiological activities. Indeed, available data from emerging functional studies in recent years indicate that nociceptin can serve as a biologically active neuropeptide participating in modulation of pain perception, behavioral activity, and many other physiological events, usually in an inhibitory fashion (for a review, see Henderson and McKnight, 1997).

Several initial experiments performed in this laboratory and others indicate an involvement of nociceptin in peripheral and central regulation of cardiovascular activity. Intravenous injection of nociceptin markedly reduced arterial blood pressure in anesthetized rats (Champion and Kadowitz, 1997). The decrease in blood pressure was not blocked by the non-selective opioid receptor antagonist naloxone at the dose that blocked enkephalin-induced depressor responses (Champion and Kadowitz, 1997), indicating a non-traditional opioid receptor mechanism mediat-

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ing this peripheral cardiovascular effect of nociceptin. Since nociceptin receptors (ORL1 receptors) have been shown to densely distribute in the rostral ventrolateral medulla, a key site and integrated center in central nervous system for regulation of cardiovascular activity (Sun, 1996), nociceptin receptor might exert a profound influence on cardiovascular functions through influencing the rostral ventrolateral medulla neurons. Indeed, in rat brain slices, perfusion of nociceptin substantially inhibited spontaneous discharges of the neurons recorded in the rostral ventrolateral medulla region (Chu et al., 1998). Direct injection of nociceptin into the rostral ventrolateral medulla in the anesthetized rats decreased blood pressure and heart rate (Chu et al., 1998). Since inhibitory effects of nociceptin in vitro and in vivo were strongly resistant to naloxone (Chu et al., 1998), nociceptin effects are less likely processed by typical opioid receptors. However, due to lack of selective nociceptin receptor antagonist, mediating role of nociceptin receptor in the nociceptin-induced changes was unable to be clarified in the most functional studies performed so far.

In the present study, we therefore utilized recently available antagonist selective for nociceptin receptor (Guerrini et al., 1998), [Phe$^1\psi$(CH$_2$–NH)Gly^2]NC(1–13)NH$_2$ (NC–NH$_2$), to verify if the nociceptin-induced inhibition of electrical activity of the rostral ventrolateral medulla neurons in rat brain slices is mediated through the nociceptin receptor. Moreover, specificity of NC–NH$_2$ in interacting with nociceptin receptor was evaluated by comparing effects of NC–NH$_2$ and naloxone on nociceptin- and [met^5]enkephalin-induced inhibition of the medullary neurons.

2. Materials and methods

Recordings of unit discharges in brain slices containing the rostral ventrolateral medulla were carried out according to standard procedures in this laboratory (Chu et al., 1997; Chu et al., 1998). Briefly, male Sprague–Dawley rats weighing 100–150 g were decapitated under deep ether anesthesia, and 500 μm transverse slices containing the rostral ventrolateral medulla were prepared with a vibratome (ZQP-86, Zhejiang, China). Slices were incubated in a well-oxygenated artificial cerebrospinal fluid (ACSF in mM: NaCl 124, KCl 5, KH_2PO_4 1.24, $MgSO_4$ 1.3, $CaCl_2$ 2.1, $NaHCO_3$ 26 and glucose 10) for 1–2 h at 21–22°C, and were then placed on a piece of nylon netting in a recording chamber and perfused with ACSF equilibrated with 95% O_2–5% CO_2 at a rate of 4-5 ml/min. Fluid level was maintained at level of upper surface of the slice, and temperature of chamber was maintained at 33 ± 0.5°C by a thermostatically controlled heating element. Single unit recording was obtained with glass micropipette filled with solution of 0.5 M sodium acetate/2% Pontamine Sky Blue (resistance = 10–30 MΩ). Electrical activity was amplified and displayed on an oscilloscope. Spikes were counted by an IBM-486 computer and unit activity was displayed with spike frequency histograms. Recording electrode was positioned in the rostral ventrolateral medulla under visualization through a dissecting microscope. This area lies lateral to the rostral tip of the inferior olive, medial to the compact portion of the nucleus ambiguus, within 700-800 μm of the ventral surface of the medulla, and centered 1.7 mm lateral to the midline. After recording, the brain slices were labeled by iontophoretic deposition of Pontamine Sky Blue (60 μA, 1 min) and Riboni-staining was used to identify recording level of the rostral ventrolateral medulla.

Recording of electrical activity on individual unit continued throughout 0.5 min perfusion of nociceptin (1–17) (RBI) or [met^5]enkephalin (Sigma) to detect change in firing frequency following drug perfusion. To examine effects of pharmacology blockade of nociceptin receptor or conventional opioid receptor on nociceptin- or [met^5]enkephalin-induced inhibition of the rostral ventrolateral medulla neuronal activity, NC–NH$_2$ (Tocris) or naloxone (Sigma) was perfused 0.5 min prior to, remained during, and continued for 2 min after cessation of, 0.5 min nociceptin or [met^5]enkephalin perfusion. In a concentration-dependent experiment, co-perfusion of four different concentrations of NC–NH$_2$ (0.3, 1, 3, 10 μM) with 10 nM nociceptin were performed in a random sequence. Repeated perfusions were given to the units with stable recording. Interval between perfusions was kept at 15–20 min, which allows a full recovery of alteration in unit activity, if any, in response to previous perfusion. All drugs were freshly prepared in ACSF.

Data are presented as the mean ± S.E.M. Statistical analysis was performed by using the paired *t*-test and the criterion for statistical significance was $P < 0.05$.

3. Results

In the study examining effect of NC–NH$_2$ on nociceptin-induced depression of the medullary neuronal activity, a total of 67 units with spontaneous discharge ranging from 2 to 26 Hz (mean ± S.E.M., 7.1 ± 1.5 Hz) was recorded in the rostral ventrolateral medulla region. In agreement with our previous observation (Chu et al., 1998), 61% (41 out of 67) of these neurons exhibited marked inhibition of their firing in response to perfusion of nociceptin at a concentration of 10 nM. According to histological examination, these inhibited neurons are distributed within the defined the rostral ventrolateral medulla region (see methods). Among these inhibited units, experiments on 18 units were continued to test effects of NC–NH$_2$ or naloxone on basal, or nociceptin-inhibited, firing rates of these neurons. As can be seen from Fig. 1 (for representative recordings) and Fig. 2, co-perfusion of NC–NH$_2$ (10 μM) with 10 nM nociceptin almost completely prevented

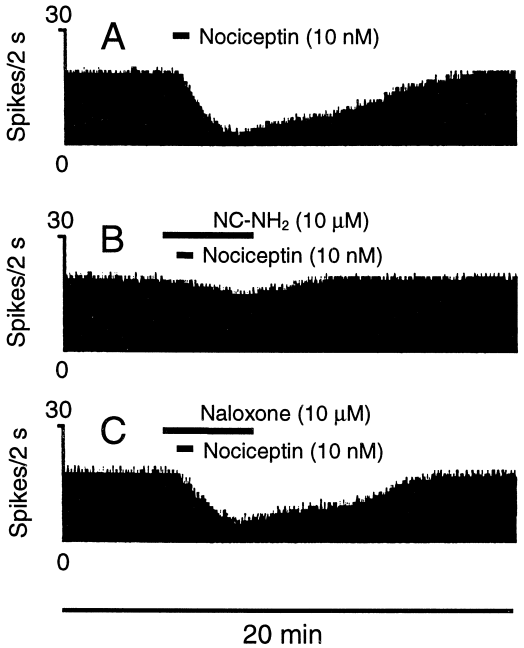

Fig. 1. Representative histograms showing inhibition of spontaneous discharge of a rostral ventrolateral medulla neuron in response to perfusion of nociceptin at a concentration of 10 nM (A). The nociceptin-induced inhibition of activity of this same neuron was prevented by co-perfusion of 10 μM NC–NH_2 (B), but not 10 μM naloxone (C).

the nociceptin-induced inhibition of the rostral ventrolateral medulla neuronal activity. On the contrary, co-perfusion of naloxone (10 μM) did not alter the reduction of the medullary neuronal activity induced by nociceptin. In addition, perfusion of NC–NH_2 or naloxone alone did not affect spontaneous firing of the rostral ventrolateral medulla neurons (Fig. 2).

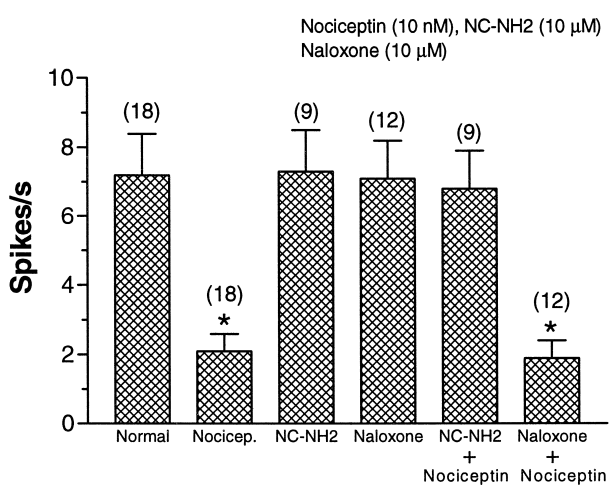

Fig. 2. Effects of NC–NH_2 and naloxone on basal, and nociceptin-suppressed, firing rates of the rostral ventrolateral medulla neurons. Maximal alterations in neuronal firing frequency were measured 0.5–2 min following single drug perfusion or following nociceptin perfusion in combination with NC–NH_2 or naloxone. The values are expressed as mean ± S.E.M. The numbers in parentheses represent the number of units tested. $* P < 0.05$ as compared with normal control.

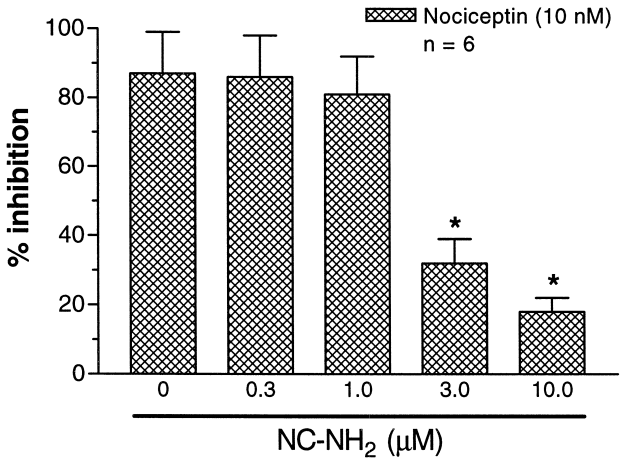

Fig. 3. Concentration-dependent blockade of the nociceptin-induced inhibition of the rostral ventrolateral medulla neuronal activity by NC–NH_2. Maximal alterations in neuronal firing frequency were measured 0.5–2 min following nociceptin perfusion. The values are calculated according to the formula: [(firing frequency prior to perfusion–firing frequency after perfusion)/firing frequency prior to perfusion] × 100%, and expressed as mean ± S.E.M. $* P < 0.05$ as compared with group treated with nociceptin in absence of NC–NH_2.

In separate experiments, 6 medullary neurons which exhibited reduction of spontaneous activity following nociceptin perfusion were surveyed to evaluate concentration-dependent blockade of nociceptin inhibition by NC–NH_2. At the two lower concentrations (0.3 and 1 μM) tested, NC–NH_2 perfusion was unable to alter percentage of the inhibition of the neurons induced by nociceptin (Fig. 3). However, with the presence of NC–NH_2 perfusion at the

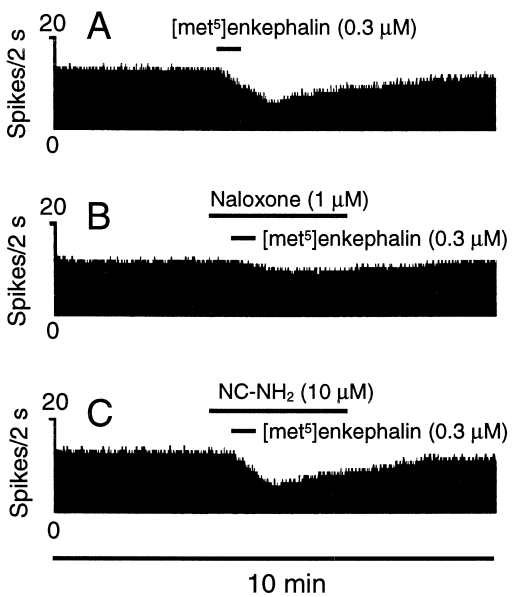

Fig. 4. Representative histograms showing inhibition of spontaneous discharge of a rostral ventrolateral medulla neuron in response to [met^5]enkephalin perfusion at a concentration of 0.3 μM (A), and the prevention of the [met^5]enkephalin inhibition by co-perfusion of 1 μM naloxone (B), but not 10 μM NC–NH_2 (C).

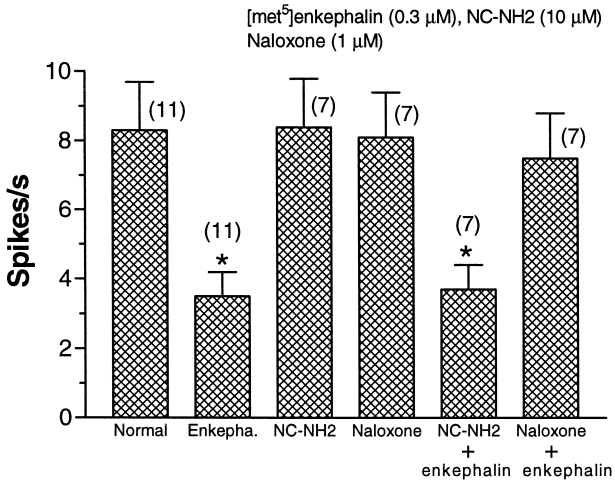

Fig. 5. Effects of NC–NH_2 and naloxone on basal, and [met^5]enkephaline-depressed, firing rates of the rostral ventrolateral medulla neurons. Maximal alterations in neuronal firing frequency were measured 0.5–2 min following single drug perfusion or following [met^5]enkephalin perfusion in combination with NC–NH_2 or naloxone. The values are expressed as mean ± S.E.M. The numbers in parentheses represent the number of units tested. $* P < 0.05$ as compared with normal control.

two higher concentrations (3 and 10 μM), nociceptin produced suppression of neuronal activity at a much less percentage (Fig. 3).

To evaluate specificity of antagonists used in this study, especially NC–NH_2, effects of NC–NH_2 and naloxone on [met^5]enkephalin-induced suppression of neuronal activity were detected in 7 to 11 units. Like nociceptin, [met^5]enkephalin perfusion (0.3 μM) substantially decreased spontaneous firing of the neurons (Fig. 4A and Fig. 5). This inhibition was prevented by co-perfusion of naloxone (Fig. 4B and Fig. 5) at the concentration of 1 μM which was ten times lower than the concentration (10 μM) ineffective in blocking the nociceptin-induced inhibition as described earlier. As opposed to naloxone, NC–NH_2 (10 μM) failed to affect the [met^5]enkephalin inhibition of the medullary neuronal activity (Fig. 4C and Fig. 5).

4. Discussion

Our previous work has demonstrated that spontaneous activity of the rostral ventrolateral medulla neurons in vitro can be readily inhibited by nociceptin perfusion (Chu et al., 1998). This inhibition was insensitive to pharmacological blockade of traditional opioid receptor by naloxone (Chu et al., 1998). Thus, the nociceptin-induced inhibition is not mediated through conventional opioid receptor. However, whether the nociceptin inhibition is mediated through the ORL1 receptor, which exogenous nociceptin is supposed to interact with, is still unclear. As a matter of fact, experimentally clarifying this issue has been obstructed by lack of pharmacological antagonist selective for nociceptin receptor. With the newly-developed nociceptin receptor antagonist, NC–NH_2 (Guerrini et al., 1998), a prompt attempt was made in the current study to determine role of ORL1 receptor in mediating the nociceptin effect. We found that NC–NH_2 concentration-dependently blocked the nociceptin-induced depression of neuronal activity. This indicates that the inhibitory effect of nociceptin is mediated through the nociceptin receptor.

We are aware of importance of specificity of NC–NH_2 in interacting with ORL1 receptor when this drug is used in pharmacological study such as this to evaluate contribution of ORL1 receptor to inhibition of firing activity of the neurons. Conventional opioid peptide enkephalin has been demonstrated to be a powerful inhibitor of the neurons (Sun et al., 1996). It is, therefore, possible that nociceptin might produce inhibition of the neurons through activation of traditional opioid receptor (δ). NC–NH_2 might also block the nociceptin-induced inhibition by affecting traditional opioid receptor. To test this possibility, effect of NC–NH_2 on enkephalin-induced inhibition of the neurons was then examined in the same experimental conditions. It was found that NC–NH_2 at the dose that blocked the nociceptin-induced inhibition had no effect on the inhibition of the neurons induced by enkephalin. Thus, the blocking effect of NC–NH_2 was not due to blockade of traditional opioid receptor. In other words, the inhibitory effect of nociceptin was most likely mediated by activation of nociceptin receptor rather than traditional opioid receptor. To further assure the specificity of nociceptin receptor in mediating the nociceptin inhibition, we investigated the effect of traditional opioid receptor antagonist naloxone on the nociceptin inhibition. It was found that naloxone prevented inhibition of the neurons induced by [met^5]enkephalin, but not by nociceptin.

Although the neuron recorded in the rostral ventrolateral medulla in vitro may not necessarily be the reticulospinal vasomotor neurons, a principal population of neurons in this area controlling cardiovascular activity (Sun, 1996), direct injection of nociceptin into the rostral ventrolateral medulla was found to decrease blood pressure in our previous in vivo study (Chu et al., 1998). Decrease in blood pressure should be a reflection of inhibition of the vasomotor neurons in the rostral ventrolateral medulla. Moreover, depressor response to nociceptin administration was also blocked by only NC–NH_2, but not naloxone (Chu et al., unpublished observation). Thus, the units recorded in the rostral ventrolateral medulla in vitro most likely represent the functional group of vasomotor neurons involving central regulation of peripheral hemodynamics.

It is noticeable that pharmacological blockade of nociceptin receptor with NC–NH_2 had no effect on spontaneous firing frequency of the rostral ventrolateral medulla neurons. Parallel with this, injection of NC–NH_2 into the rostral ventrolateral medulla did not alter baseline of blood pressure (Chu et al., unpublished observation). Thus, the inhibitory modulation of the neuronal activity in the rostral ventrolateral medulla by endogenous nociceptinergic sys-

tem seems not active in normal physiological conditions. Further study should investigate whether endogenous nociceptinergic system functions in modulating responses of the neurons to extracellular stimulation.

In summary, using a new selective nociceptin receptor antagonist, $[Phe^1\psi(CH_2-NH)Gly^2]NC(1-13)NH_2$, this study investigated whether the nociceptin-induced inhibition of the rostral ventrolateral medulla neurons is specifically mediated by nociceptin receptors. The results showed that the nociceptin-induced inhibition of the rostral ventrolateral medulla neuronal activity that was not sensitive to naloxone was concentration-dependently blocked by the nociceptin receptor antagonist. In contrast, $[met^5]$enkephalin-induced suppression of the medullary neuronal activity was blocked by naloxone, but not by the nociceptin receptor antagonist. Neither naloxone nor the nociceptin receptor antagonist alone had significant effect on basal firing of the neurons. These data confirm (1) specific role of nociceptin receptors in mediating the nociceptin inhibition of the medullary neurons, and (2) convincing selectivity of $[Phe^1\psi(CH_2-NH)Gly^2]NC(1-13)NH_2$ in interacting with the nociceptin receptor in in vitro experimental conditions.

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