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Research Report

The CRF_1 receptor antagonist, NBI-35965, abolished the activation of locus coeruleus neurons induced by colorectal distension and intracisternal CRF in rats

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Abstract

Corticotropin-releasing factor (CRF) receptors have been reported to play a role in tonic colorectal distension (CRD)-induced activation of locus coeruleus (LC) neurons. We examined the influence of repeated phasic CRDs and intracisternal (ic) CRF on the spontaneous discharge rate of LC neurons in chloral hydrate-anesthetized rats and the role of CRF receptors using the nonselective CRF_1/CRF_2 antagonist, astressin, and the water-soluble CRF_1 receptor antagonist, NBI-35965. Two consecutive phasic CRDs (43.7 ± 1.1 mm Hg, 30 s each) at a 10-min interval increased LC activity to 184.9 ± 15% and 171.9 ± 12.2%, respectively. There was no difference in magnitude, onset (within 1 s), and duration (5–7 min) of the LC responses between the 1st and 2nd CRDs. CRF (300 ng/rat, ic) injected 10 min after the 2nd CRD increased LC activity to 191.1 ± 11.2%. Astressin (3 μg, ic) completely blocked the 2nd CRD- and ic CRF-induced LC activation. Neither ic vehicle nor astressin influenced basal LC neuronal activity. NBI-35965 (10 mg/kg, iv) prevented the 2nd CRD- and ic CRF-induced LC neuronal activation, while at 5 mg significantly reduced the LC response to the 2nd CRD by 80%, but did not block that of ic CRF injected 30 min later. These findings indicate a primary role of brain CRF interacting with CRF_1 receptors in mediating the activation of LC neurons in response to a phasic CRD within the nociceptive range (>40 mm Hg). This activation may have relevance to irritable bowel syndrome characterized by lower pain threshold to CRD and hypervigilance to colonic input.

Theme: Neurotransmitters, modulators, transporters and receptors
Topic: Peptides: physiology

Keywords: Extracellular recording; LC discharge; Astressin; NBI-35965; Stress; Colorectal distension

1. Introduction

The pontine nucleus locus coeruleus (LC) is a distinct cluster of neurons that accounts for over 50% of norepinephrine cell bodies in the brain [16]. The LC provides the major source of norepinephrine to the forebrain through its divergent projections and receives convergent inputs from several brain nuclei [2,17,28,60]. Activation of LC activity by corticotropin-releasing factor (CRF) induced increased vigilance and attention to novel or fear-provoking stimuli, as well as an anxiogenic behavior [7,24,59]. Anatomical and

Abbreviations: CRD, colorectal distension; CRF, corticotropin-releasing factor; IBS, irritable bowel syndrome; ic, intracisternal; icv, intracerebroventricular; im, intramuscular; iv, intravenous; LC, locus coeruleus; r/hCRF, rat/human CRF

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functional experimental data clearly support a role of endogenous CRF in the anxiogenic response associated with stress exposure through the increase in neuronal activity of the noradrenergic LC neurons [7,10,26,38,47].

The LC complex is also involved in brain–gut interactions. In an early study, Mönnikes et al. reported that microinfusion of CRF into the LC complex stimulates colonic transit without influencing gastric emptying [33]. Conversely, electrophysiological studies showed that LC neurons are activated by colorectal distension (CRD) [13,25]. In addition, Rouzade-Dominguez et al. [43] identified the Barrington's nucleus as the source of CRF that activates LC neurons in response to a tonic CRD at a low distension volume in anesthetized rats.

CRF interacts with two CRF receptor subtypes, CRF_1 and CRF_2, which are encoded by two distinct genes [20]. Both receptors display different morphological distributions and pharmacological characteristics [41]. CRF has preferential affinity for CRF_1 and lesser affinity for CRF_2 receptors [40,41]. The increased LC activity induced by the intracoerulear or intracerebroventricular (icv) administration of CRF was inhibited by the nonselective CRF_1 and CRF_2 receptor antagonist, α-helical CRF_{9-41}, or [D-Phe^{12,38}, C$^{\alpha}$MeLeu^{37}]r/hCRF_{12-41} [10] and selective CRF_1 antagonists [27,37,44], suggesting a role of CRF_1 receptor in central CRF-induced excitatory neurotransmission in LC. [D-Phe^{12,38},C$^{\alpha}$MeLeu^{37}]r/hCRF_{12-41} injected icv or into the LC has been reported to attenuate tonic CRD at low volume-induced activation of LC in anesthetized rats [25]. Patients with irritable bowel syndrome (IBS) have a lower tolerance to CRD and hypervigilance to rectal stimuli [6,34], suggesting a possible relevance of LC circuitry in such a disorder [49,58]. In addition, hypersensitivity in patients with IBS is mainly seen with the use of phasic distension isobaric protocols [42].

In the present study, we first examined the influence of two repeated phasic CRDs on LC spontaneous discharge rate recorded from chloral hydrate-anesthetized rats and whether these neurons, which received colonic input, are also responsive to intracisternal (ic) injection of CRF. We then assessed the role of CRF receptors in mediating LC response to a phasic CRD and ic CRF using the potent CRF_1/CRF_2 antagonist, astressin [19], and intravenous (iv) injection of the newly developed water-soluble selective CRF_1 antagonist, NBI-35965, that crosses the blood–brain barrier [32].

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Harlan Laboratories, San Diego, CA, USA) weighing 280–320 g were maintained on Purina Laboratory Chow and tap water under conditions of controlled temperature (21 ± 2 °C) and illumination (6:00 am to 6:00 pm). Experiments were performed in rats deprived of food for 24 h, but allowed to drink water up to the beginning of the study. All experimental procedures were approved by the VA GLAHS and UCLA Animal Care and Use Committees.

2.2. Surgeries

All surgeries and experiments were performed in rats anesthetized by an intramuscular (im) injection of chloral hydrate (400 mg/kg, Sigma Chemical Co., St. Louis, MO, USA), and placed on a heated isothermal pad. An additional injection of chloral hydrate (0.2–0.4 ml, im) was performed when needed during the surgery to provide a stable level of anesthesia (as verified by the absence of reflex response to ear pressure during, and at the end of, the experiment). After the tracheostomy, polyethylene tubing (PE-240, 1.67 mm ID, 2.42 mm OD, 6 cm length, Intramedics, Clay Adams, Parsippany, NJ, USA) was inserted into the trachea (~2 cm) to ensure a patent airway. Then, the left jugular vein was cannulated by inserting a PE-20 catheter (0.38 mm ID, 1.09 mm OD, Intramedics) connected to an injection port tubing by a short length of stainless steel 26 gauge. The perfusion of saline into the left jugular vein was performed at a rate of 0.4 ml/h and throughout the study to prevent dehydration. Thereafter, animals were positioned in a stereotaxic instrument (Model 1404, David Kopf Instruments, Tujanga, CA, USA) and the head oriented at a 45° angle (nose down) to the horizontal plane. In one control group of animals, the EKG electrodes were connected to rats for heart rate monitoring during time of ic cannulation, stereotaxic fixation of animal head, follow-up surgery, and recording from LC. A catheter (PE-10 polyethylene tubing, 0.28 mm ID, 0.61 mm OD, and 8 cm length, Intramedics) was positioned into the cisterna magna as previously detailed [62] and secured to the occipital brain membrane with instant Krazy® glue. After a 20- to 30-min period, a lubricated compliant latex balloon (4 cm × 1 cm) connected to a PE-240 catheter, was inserted through the anus–rectum and positioned such that the base of the balloon was 1 cm past the anus. The catheter was connected to a 5-ml syringe for inflation with air and the pressure within the balloon was monitored via the pressure transducer. The balloon was secured in place by taping the catheter to the tail.

For brain surgery and LC activity recording, animals were repositioned in a stereotaxic instrument and the incisor bar was adjusted until the height of lambda and bregma skull points were equal [39]. Then, the skull was exposed and a 3-mm diameter hole was opened over the cerebellum to approach the left LC. The dura and pia mater over the cerebellum were removed by fine forceps and scissors.

2.3. Recording of LC neurons

Conventional extracellular single-unit recordings were made using borosilicate, thin wall filament-filled glass capillary tubing with a tip diameter of ~1 to 2 μm (5–10