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Stimulation of the nucleus locus coeruleus/subcoeruleus suppresses visceromotor responses to colorectal distention in the rat

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Abstract

The aim of the present study was to examine whether the nucleus locus coeruleus/subcoeruleus (LC/SC) modulates visceromotor function. In the present study, an electromyogram (EMG) of the external abdominal oblique muscle evoked by colorectal distention was measured as a visceromotor reflex response, and inhibitory effects of LC/SC stimulation were estimated by the decrease of EMG activity. Under halothane anesthesia (1% in air), graded colorectal distentions (30, 60 or 80 mmHg) were produced by inflating a balloon inside the descending colon and rectum. A bipolar EMG electrode was inserted into the left external abdominal oblique muscle to record the EMG response to colorectal distention. Colorectal distention at a pressure of 30 mmHg did not evoke any EMG activity in the external abdominal oblique muscle in all rats tested. Electrical stimulation of the LC/SC (30, 50 and 70 μA, 100 Hz, 0.1 ms pulses) reduced EMG responses evoked by colorectal distention to 60 and 80 mmHg. LC/SC stimulation was effective both ipsilaterally and contralaterally indicating a bilateral effect. EMG responses decreased with an increase of LC/SC stimulation intensity. Following recordings of the inhibitory effects of LC/SC stimulation, lesions of the LC/SC ipsilateral to the EMG recording site were induced; 1 h after lesions the inhibitory effects of LC/SC stimulation were examined again. LC/SC stimulation did not reduce the EMG responses when LC/SC stimulation was applied to the ipsilateral LC/SC, whereas EMG responses were observed by stimulation of the intact LC/SC contralateral to the EMG recording site. From lesion experiments, it could be considered that suppression of the visceromotor response to colorectal distention is due to activation of the LC/SC. The results suggest that the visceromotor function is under the control of the centrifugal pathways from the LC/SC.

Keywords: Locus coeruleus; Subcoeruleus; Visceromotor response; Colorectal distention; Suppression

The nucleus locus coeruleus (LC), the A6 cell group, is located in the dorsolateral pons and has been shown to send noradrenergic projections to the spinal cord [1,2,4,5,21,28]. The nucleus subcoeruleus (SC), located ventrolateral to the A6 cell group in the rostral pons, also provides noradrenergic innervation of the spinal cord [1,4,6,29]. These descending LC/SC neurons have been confirmed to be involved in modulation of nociceptive transmission. Activation of descending LC/SC neurons can produce profound antinociception [8,9,13,22,27] and can inhibit nociceptive activity of spinal dorsal horn neurons [7,10–12,15] and trigeminal subnucleus caudalis neurons [25].

It is known that acute abdominal diseases with irritation or inflammation of the peritoneal serosa induce a powerful somatic reflex within the abdominal wall, which is a spasm of the flexor muscles, in company with visceral pain [20]. This reflex is responsible for marked direct or rebound tenderness and severe rigidity of the abdominal musculature. Tonic contraction of flexor muscles evoked by noxious visceral stimuli provides an additional source of noxious stimulation. This phenomenon is important clinically, since the development of an additional source of noxious stimulation may result in the vicious circle of pain. Ness and Gebhart [17] have reported that clonidine, an α_2-adrenoceptor agonist, produces a dose-dependent suppression of the reflex spasm of abdominal muscles evoked by colorectal distention. An electrophysiological study has shown that LC neurons are activated by noxious visceral stimuli, such as distention of

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the distal colon [3]. These findings suggest that the LC/SC affects the visceromotor reflex evoked by noxious visceral stimuli because a neurochemical study has demonstrated that inhibition of nociceptive transmission in the dorsal horn by pathways descending from the LC/SC is mediated by an α_2-adrenoceptor [9]. However, no previous studies have explored such a possibility. The aim of the present study was to examine whether the LC/SC modulates reflex spasms of flexor muscles evoked by noxious visceral stimuli. In the present study, an electromyogram (EMG) of the external abdominal oblique muscle evoked by colorectal distention was measured as a visceromotor reflex response, and inhibitory effects of LC/SC stimulation were estimated by the decrease of EMG activity.

Experiments were performed on male Sprague–Dawley rats weighing 250–330 g. The animals were housed in groups of three or four in a cage containing sawdust bedding, with free access to food and water in a laboratory equipped with a 12-h light:12-h dark cycle. Room temperature and humidity were maintained at $23 \pm 0.5\,^{\circ}C$ and 60%, respectively. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Showa University and were in accordance with the International Association for the Study of Pain [30].

The animals were initially anesthetized with 4% halothane in air. After tracheotomy, the head was fixed rigidly in a stereotaxic frame. Animals were artificially ventilated to maintain halothane anesthesia. The core body temperature was regulated at approximately $37.5\,^{\circ}C$ using a thermostatically controlled heating blanket. The skull was exposed and two holes (bilateral) were drilled for stereotaxic placement of a stimulating electrode into the LC/SC. Two monopolar Teflon-coated stainless-steel electrodes (0.1 mm in diameter), insulated except for 0.5 mm at the tip, were inserted bilaterally into the LC/SC, respectively. Stereotaxic coordinates have been defined by Paxinos and Watson [19] (9.6 mm caudal to bregma; 1.15 mm lateral to the midline; 2.5 mm above the interaural axis). Electrical stimulation (30, 50 and 70 μA, 100 Hz, 0.1 ms pulses) of the LC/SC either ipsilateral or contralateral to the site of EMG recordings was initiated 5 s prior to and remained throughout the application of colorectal distention. At this stimulus intensity, there was no trouble with electrical artifact in relation to EMG measures during LC/SC stimulation. In addition, LC/SC stimulation never produced contraction of the masseter muscle associated with stimulation of the mesencephalic trigeminal nucleus, located just lateral to the LC/SC, suggesting that LC/SC stimulation may not have spread to nuclei or axons outside the LC/SC.

Graded colorectal distentions were produced by inflating a balloon inside the descending colon and rectum. The balloon was made from the finger of a latex glove with a length approximately 5 cm and attached to polyethylene tubing. The balloon was inserted into the descending colon and was held in place by taping the tubing to the tail. At the end of surgical procedures, a bipolar EMG electrode made of enamel-coated stainless steel wire was inserted into the left external abdominal oblique muscle to record EMG responses to colorectal distention. Anesthesia was decreased to 1% halothane and the animals were left for at least 30 min to recover. After EMG response to colorectal distention at a pressure of 60 mmHg was observed, the experiment was initiated. Under 1% halothane anesthesia, no spontaneous movement was observed in the animals, but flexor reflexes could be evoked by stimulating the skin on the hindpaws.

After the effect of LC/SC stimulation on EMG response was examined, the rats received electrolytic lesions of the LC/SC ipsilateral to the EMG recording sites to confirm that LC/SC stimulation may not have spread to nuclei or axons outside the LC/SC. At the time of the LC/SC lesions the anesthesia was increased to 2% halothane, and lesions were induced with a cathodal current (1 mA, 20 s). After the lesions were made, the anesthetic level was reduced to 1% halothane, and effects of LC/SC stimulation on the EMG response to colorectal distention were recorded 1 h later.

The descending colon was distended by inflating the balloon to the desired pressures (30, 60 or 80 mmHg). The pressures were delivered for intervals of 10 s with a 30 s interval between the distentions. The distentions were repeated three times during each experimental situation, with a 5 min interval between each series. Stimuli of intensity >40 mmHg were considered noxious [17,18]. The EMG signal was amplified, displayed on an oscilloscope, fed into an A/D converter (CED 1401+) and recorded using Spike2 software. The EMG signal was rectified off-line for analysis. The magnitude of muscle activity was represented by the area of integrated EMG for 1 s. Baseline activity was subtracted and the area of integrated EMG was divided by the duration of the colorectal distention. The values obtained at each intensity of distention were averaged in each animal for each experimental situation. The results were then averaged across the animals tested. Data are represented as the mean ± S.E.M. Statistical analysis was carried out using analysis of variance (ANOVA), and Duncan's new multiple-range test was used for post hoc analysis of the difference between the responses of various experimental conditions. A difference was accepted as significant when $P < 0.05$.

At the end of each experiment, cathodal electrolytic lesions (500 μA DC for 3–5 s) were made to mark the site of electrical stimulation in the intact LC/SC, and then the animals were deeply anesthetized and perfused intracardially with a 10% formalin solution. Brains were removed, sectioned at a thickness of 50 μm, and stained with cresyl violet for histological verification of electrode placement and reconstruction of the lesion. In the lesioned LC/SC, the sites of electrode stimulation were determined from the center of the lesioned area.

A total of seven rats were selected on the basis of histological results which showed the location of the stimulating electrode in the brainstem. The tip of the stimulating electrode in these adopted rats was located within the LC/SC (Fig. 1A). The rostrocaudal extension of the stimulation sites was 9.6 ± 0.1 mm caudal to the bregma. Colorectal distention