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Colonic nociception via nucleus submedius is modulated by pontine centres in the rat

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Abstract

The rat thalamic nucleus submedius responds to noxious pressure stimuli in the colon. Some neurons in and near Barrington's nucleus, a pontine center related to bladder function, also respond to colon distension. We hypothesized that colonic nociception may be relayed via Barrington's nucleus to the nucleus submedius. Experiments were carried out in 22 urethane-anesthetized male rats. Noxious stimuli were applied to the toes using standardized clips and to the colon by inflation of the balloon to 80 mmHg for 30 s using a barostat. The brain was exposed to allow recording from the nucleus submedius with a monopolar tungsten electrode and the activity of rectus muscle was assessed via silver wire electrodes. A glass pipette was inserted into Barrington's nucleus for injection of 5 mM $CoCl_2$, a temporary neural blocker. The site of $CoCl_2$ injection was confirmed by the presence of FluoroGold which was incorporated into the $CoCl_2$ solution. We recorded 51 units in submedius that were excited by noxious toe pinch, 4 were inhibited. Colon distension to 80 mmHg produced visceromotor responses, excited 23 units in submedius and inhibited 13 units. Injection of $CoCl_2$ into the region of Barrington's nucleus blocked the response to colon distension in 10 of 12 Sm units tested, but had no influence on the accompanying visceromotor response. These data point to a previously unrecognized relationship between Barrington's nucleus and submedius that may subserve colon nociception.

Keywords: Colorectal distension; Visceral nociception; Thalamus; Barrington's nucleus; Locus ceruleus

Barrington's nucleus in the rat is a critical structure in the control of micturition as stimulation of the nucleus induces bladder contraction [9,12], and chemical or electrolytic disruption of the nucleus abolishes the micturition reflex [19]. However, some neurons in and near Barrington's nucleus respond to noxious colon distension [18]. Neuronal tracing experiments using pseudorabies virus have shown that the distal colon receives input from Barrington's nucleus via the lumbosacral spinal cord [22,25]. In addition, activation of Barrington's nucleus neurons produced increases in colonic intraluminal pressure [14]. It has been suggested that Barrington's nucleus projections to locus ceruleus might underlie central alerting responses to colon distension [23].

Neurons in the nucleus submedius (Sm) of the thalamus respond to cutaneous and deep somatic stimuli [2,4,10], and to colon distension at noxious levels [5]. Further evidence has supported involvement of Sm in a postulated descending modulatory pathway [26] that also may involve the periaqueductal gray (PAG) [29].

This study was designed to examine the involvement of Barrington's nucleus in relaying noxious input from the distal colon to higher areas of the brain, specifically the Sm. To address this hypothesis, cobalt chloride, a temporary neuronal blocker [9] was injected into Barrington's nucleus, and its effect on Sm neuronal response to colorectal distension was examined, along with changes in blood pressure or visceromotor responses.

Twenty-two male Wistar rats, 311–503 g body weight, were anesthetized with urethane (1.5 g/kg body weight, i.p.). A polyethylene cannula containing heparinized saline was inserted into the carotid artery for monitoring blood pressure. In some experiments, two silver Teflon®-coated wires (0.25 mm uncoated diameter) were inserted into the lateral

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abdominal muscle approximately 1 cm apart, as electromyographic (EMG) electrodes to monitor the visceromotor response.

The rats were positioned in a stereotaxic instrument using blunt ear bars. The skull was exposed, and a craniotomy was performed at two locations, one over the thalamic recording site and one over the pontine injection site. The coordinates for unit recording in the submedius were: 2.5–3.1 mm caudal to bregma, 0.5–0.9 mm lateral, and 6.0–6.9 mm below the brain surface [15]. During the surgery, body temperature was maintained at 37 °C with a temperature control unit.

Extracellular unit recordings were obtained with 10 MΩ monopolar tungsten electrodes, using the skin as an indifferent electrode. Unit activity in the Sm was amplified (10–20,000×), filtered (bandpass: 300 Hz–10 kHz), and monitored and stored using Spike2 software (Cambridge Electronic Design). Blood pressure, colon distension pressure and abdominal EMG activity (1000×, bandpass: 10 Hz–3 kHz) were stored in the same record.

A 50 mm flexible balloon (constructed from plastic tubing and a latex condom) was inserted into the distal colon through the anus and kept in position by taping the catheter to the base of the tail. Protocol Plus® software was used to control colon distension at various levels of pressure by activation of a Distender Series II Dual Drive Barostat (G&J Electronics, Mississauga, Ont., Canada). A sequence consisting of 30 s distensions at 20 mmHg (innocuous) and 80 mmHg (noxious) [11] with a 100 s separation between distensions was used as a search stimulus.

Noxious somatic stimulation consisted of the contralateral application of hindlimb toe pinch for 30 s. Somatic stimulation was applied after colorectal distension, as tactile stimuli reportedly inhibit neuronal responses to colorectal distension in ventroposterior lateral (VPL) nucleus of the thalamus [28].

A glass pipette containing a 5 mM $CoCl_2$/2% FluoroGold in artificial cerebrospinal fluid (composition (mM): 2.5 potassium chloride, 2.0 magnesium chloride, 1.26 calcium chloride, 1.3 sodium phosphate (dibasic), and 125 sodium chloride in distilled water) was stereotaxically guided to Barrington's nucleus (AP: −12.42; ML: 1.1; H: −7.66) at an angle of 20° (tip rostral). When Sm units were stable, with responses to at least two consecutive 20 mmHg/80 mmHg colon distension sequences, 72–200 nl of the $CoCl_2$/FluoroGold solution was pressure injected (PV830 Pneumatic PicoPump, WPI) into Barrington's nucleus ipsilateral (in 1 case contralateral) to the Sm electrode. The injection volume was determined by the movement of the meniscus in the pipette using a calibrated eyepiece graticule in the operation microscope. Injection sites were marked by the FluoroGold tracer present in the $CoCl_2$ solution. Unit activity in Sm was monitored immediately after injection into Barrington's nucleus in order to detect changes in basal activity. The 20 mmHg/80 mmHg colon distension sequence was conducted for at least 1 and up to 3 h post-injection to monitor the return of unit activity.

At the end of the experiment, animals were killed by anesthetic overdose and the head was placed in 10% formaldehyde solution. The brain was then removed and placed in formaldehyde for another 2 days, and then transferred to a buffered 30% sucrose solution for approximately 24 h. Thirty-micrometer sections were cut in the coronal plane, and slides were stained with 0.1% thionin. Electrode tracks in Sm were identified under light microscopy, injection sites by fluorescence microscopy, and both were mapped using a stereotaxic atlas [15].

Although the focus of the present experiments was Sm units responsive to noxious colon distension, responses to somatic noxious stimulation were also assessed in order to confirm location. Our tracks yielded 51 units excited by noxious toe pinch and 4 units inhibited by this stimulus in 14 experiments. Based on micrometer measurements, some somatic responsive units were encountered at levels more dorsal than the upper boundary of Sm. In 21 units that were evaluated further, the discharge usually had an onset that coincided with the application of the stimulus (18/21) and usually was maintained at least for the 30 s duration of the stimulus (16/21). Two units had shorter durations of activity (13 and 23 s) and 2 others showed short (3 s) bursts of activity at the onset and offset of the pinch. An afterdischarge lasting at least 10 s beyond the cessation of the stimulus was observed in 11/21 units.

Units recorded in Sm often had a high rate of background discharge. In 18 experiments, 23 Sm units were excited by colon distension to 80 mmHg and 13 units were inhibited by distension. The noxious character of colon distension to 80 mmHg has been reported previously [11] and was confirmed here by the fact that it caused a discharge of abdominal muscle EMG activity (6 of 6 experiments) (Fig. 1). Colon pressures in the innocuous range (20 mmHg) usually did not elicit an EMG response (1/6). Units recorded in Sm that responded to noxious colon distension were considered to have an immediate onset if they began or ceased firing or abruptly accelerated or decelerated firing within 6 s after the beginning of the pressure pulse (ramp time to 80 mmHg = 4.5 s). Among the units evaluated, 6/11 units excited at 80 mmHg had an immediate onset. The others showed delays of 7–28 s. Of the units inhibited with the noxious stimulus, 4/6 evaluated units had an immediate onset and the other 2 showed delays of 8 and 14 s. Most units excited by colon distension exhibited discharges that outlasted the stimulus by 20 to >200 s (7/11). In half of the units inhibited by colon distension, the period of decreased firing coincided with the stimulus period (3/6) (Fig. 2), and only 1 unit exhibited a response that outlasted the stimulus. In the other 2 units inhibition did not extend through the full period of colon distension.

$CoCl_2$ microinjected into the region of Barrington's nucleus reduced the ongoing background activity in Sm units in 7/12 tests (Figs. 1 and 2). For 10 of 12 Sm units tested, $CoCl_2$ microinjection aimed at Barrington's nucleus blocked the response to 80 mmHg colon distension (Figs. 1 and 2). All but one of the Sm recording sites in the $CoCl_2$ injection experiments lay near or within the boundary of Sm (Fig. 3A). Both dorsal and ventral parts of Sm were represented (Fig. 3A). In