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Noxious stimulation excites serotonergic neurons: A comparison between the lateral paragigantocellular reticular and the raphe magnus nuclei

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ABSTRACT

The present study was designed to record electrophysiological responses to graded noxious thermal stimuli of serotonergic and nonserotonergic neurons in the lateral paragigantocellular reticular (LPGi) and the raphe magnus (RMg) nuclei in rats. All of the neurons recorded were juxtacellularly filled with neurobiotin and identified with double immunofluorescent labeling for both neurobiotin and serotonin. Under halothane anesthesia (0.75%), noxious thermal stimuli ⩾48°C activated almost all (88%) of the serotonergic neurons located within the LPGi ($n = 16$). The increase in firing was clear (3.4 ± 0.3 spike/s: mean of responses above the population median) and sustained during the whole application of strong thermal noxious stimuli, with a high mean threshold (48.3 ± 0.3°C) and large receptive fields. Recording of serotonergic neurons in the RMg ($n = 21$) demonstrated that the proportion of strongly activated (>2 spike/s) neurons (19% vs 59% for the LPGi) as well as the magnitude of the activation (2.1 ± 0.4 spike/s: mean of responses above the population median) to thermal noxious stimuli were significantly lower than in the LPGi ($P < .05$). Within the boundaries of both the LPGi and the RMg (B3 group), nonserotonergic neurons were also predominantly excited (75%) by noxious stimuli, and the resulting activation (7.9 ± 1.2 spike/s) was even greater than that of serotonergic neurons. Thermal noxious stimuli–induced activation of LPGi serotonergic cells probably plays a key role in serotonin-mediated modulations of cardiac baroreflex and transmission of nociceptive messages occurring under such intense noxious conditions.

1. Introduction

Serotonergic neurons located within the lateral paragigantocellular reticular nucleus (LPGi) and the raphe magnus nucleus (RMg) are well known to be involved in descending control of the transmission of nociceptive messages [7,11,28,31]. In particular, strong antinociceptive effects can be induced by electrical stimulation of the LPGi [57] or the RMg [23,65,73]. In line with these behavioral observations, spinal neuronal response to noxious stimuli is decreased by direct stimulation of the LPGi [42] or the RMg [29,59,80] through serotonin receptor activation at the spinal level [6,44,68]. Importantly, LPGi and RMg serotonergic neurons together constitute the B3 group [83], the major source of serotonin release within spinal superficial laminae [50–52,72,82].

Gau et al. [38] demonstrated that serotonergic neurons of the LPGi (located laterally to RMg wings) are also responsible for the inhibition of cardiac baroreflex induced by strong thermal noxious stimuli (⩾48°C). Importantly, the latter stimuli increased c-Fos expression in serotonergic neurons, markedly within the LPGi, and moderately within the RMg, which shed new light on the physiological role of the serotonergic B3 group. Indeed, these data support the idea that most serotonergic nociceptive neurons are grouped within the LPGi, whereas previous electrophysiological studies, although conflicting, showed that the serotonergic neurons which respond (slightly) to noxious stimuli are located within the RMg [3,19,35,64,78,95,96].

However, our inference about the functional implication of the LPGi was based on a c-Fos technique that provides only an indirect view of neuronal response [38]. Thus, it was essential to support and extend these data with single-unit electrophysiological recordings. Indeed, to our knowledge, neuronal recordings have been so far centered on the RMg, and no electrophysiological studies have yet been dedicated to the responses of LPGi serotonergic neurons to noxious stimuli.

The first aim of the present study was therefore to evaluate the intensity and the nature of the electrophysiological responses of LPGi serotonergic neurons to nonnoxious and noxious thermal

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stimuli of different intensities. In order to make a comparison, RMg serotonergic neurons were also recorded under the same conditions. Within the boundaries of B3 serotonergic region, we have also recorded and identified nonserotonergic neurons whose importance in descending controls of nociception and their involvement in the analgesic action of morphine were early recognized [30–32,56,88]. We took advantage of the electrophysiological juxtacellular technique to fill each recorded neuron with neurobiotin, to determine its precise location and evaluate whether or not it had a serotonergic phenotype (double immunofluorescent labeling: neurobiotin + serotonin).

2. Materials and methods

2.1. Animal preparation

Electrophysiological experiments were performed on 84 Sprague–Dawley male rats weighing 250–300 g. Animals were kept under controlled environmental conditions (ambient temperature 21 ± 1°C, 60% relative humidity, unrestricted access to food and water, alternate 12 h light/12 h dark cycles) for at least 1 week after receipt from the breeding center (CER Janvier, Le Genest-St Isle, France). Procedures involving animals and their care were conducted in conformity with the institutional guidelines in compliance with the Council Directive No. 87-848 of the Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale (permissions No. 75-148 to JFB, No. 75-116 to MH, and No. 75-855 to CSC).

Before anesthesia induction, rats received a dose of atropine (0.75 mg/kg, i.p.) to decrease respiratory secretion. Anesthesia was induced with 2% halothane in a 67% N_2O–33% O_2 mixture. A tracheal cannula was inserted by endotracheal intubation [94]. The animals were mechanically ventilated at a rate of 48 breaths/min with a Palmer pump for the whole duration of the experiment. The expiratory CO_2 was monitored continuously with a Capnomac II (Datex Instruments, Helsinki, Finland). The end-tidal CO_2 was maintained at 4%. The end-tidal levels of halothane, O_2, and N_2O were also checked systematically during the whole experimental period. The core temperature was maintained at 37.0 ± 0.5°C by means of a homeothermic blanket system.

The animals were then paralyzed with an infusion of gallamine triethiodide (48 mg/kg/h; Sigma-Aldrich, St. Quentin-Fallavier, France) via an intraperitoneal catheter and mounted in a stereotaxic frame with the incisors bar placed 18 mm below the interaural horizontal plane (ie, 14.7 mm below the horizontal plane defined in the stereotaxic atlas of Paxinos and Watson [75]). This represents an approximate 30° head tilt from the reference position in the atlas of Paxinos and Watson [75]. The dorsal surface of the brainstem was then exposed by a small incision of the atlanto-occipital ligament and the dura matter underneath to allow electrode descent.

After surgery, the halothane level was reduced to 0.7–0.8% in a mixture of 50% N_2O–50% O_2. A 15–30-min stable anesthetic condition was allowed before starting recording. This level of anesthesia, which did not excessively depress neuronal responses to noxious stimuli (see discussion in Bester et al. [12]), met the IASP ethical recommendations for animal experiments [97]: it was sufficient to reach the minimum alveolar concentration of inhaled anesthetic agent required to prevent a direct response to a noxious stimulus in 50% of animals [20]. In previous experiments, it was also sufficient to prevent electrocortical signs of arousal during strong noxious stimulation [43].

2.2. Recordings

Single extracellular recordings were made with glass micropipette electrodes (12–20 MΩ) filled with an aqueous solution of 1.5% (w/v) neurobiotin (Vector Laboratories, Burlingame, CA, USA) and 2% (w/v) NaCl. The micropipettes were inserted via the cisterna magna into the dorsal surface of the brainstem at a level close to the medullocerebellar angle. The micropipettes were positioned in a parasagittal plane at 45° angle with respect to horizontal plane. Coordinates of the rostral tip were: 0.2 mm caudally to the obex, 0.2–0.4 or 0.8–1.2 mm laterally to the midline to record neurons in the RMg or the LPGi, respectively. The recording depth was 4.2–5.2 mm below the surface of the brainstem, along the micropipette track.

After initial recording amplification (Axoclamp 2B; Axon Instruments, Union City, CA, USA), complementary amplification with minimal filtration and removal of 50 Hz component without filtration (Hum Bug electronic device; Quest Scientific, Vancouver, Canada), the signal was observed on an oscilloscope to allow on-line monitoring and digitized at 24 kHz frequency with a data acquisition system (CED 1401 with Spike 2 software; Cambridge Electronic Design, Cambridge, UK). The action potentials were also fed directly into a window discriminator and monitored with a second oscilloscope. Storage of the full recording signal in a computer allowed further controls and analysis (online as well as off-line).

Each time a spontaneously active unit was found, its baseline activity was recorded for a minimal time around 5 min in the absence of stimulation and at a steady-state concentration of halothane. This sampling method ignored silent neurons but had the advantage of minimizing the disruption caused by repeated noxious stimuli.

2.3. Innocuous and noxious stimulation

Thermal and mechanical noxious stimuli usually lasted 20 s. A pause of at least 3 min between successive noxious stimuli allowed the return of the unit activity to its background level during interstimuli intervals. First, innocuous mechanical stimuli (touch, brushing, rubbing, light pressure) were applied to a paw and the tail. Then thermal noxious stimulation consisted of immersing consecutively each paw and occasionally the tail of the animal in a 50°C waterbath during 20 s. This method allowed us to estimate the size of the receptive field of the recorded unit.

Finally, additional tests were made in the portion of the receptive field that gave the most intense response. The adequate reproducibility of this response was controlled; then graded temperatures of 44, 46, 48, 50, and 52°C were systematically applied to determine the threshold temperature and the encoding properties of the unit. Importantly, a pause of 5 min was respected after 50 and 52°C noxious stimuli. Furthermore, the 52°C temperature was used as sparingly as possible to avoid sensitization/desensitization phenomena. For most neurons, a mechanical noxious stimulation (pinch) was applied once with calibrated forceps (16–32 N/cm^2), at least to the most sensitive portion of the receptive field.

2.4. Juxtacellular filling

Once examination of the responses of an adequately isolated neuron was completed (as described above), neurobiotin was injected by juxtacellular iontophoresis with continuous control of neuronal firing to ensure that the same unit was recorded during the whole procedure [76]. Using the bridge circuit of the recording amplifier (Axoclamp 2B), the tracer was injected with 200 ms ON–200 ms OFF pulses of DC current at gradually increasing intensity (1–12 nA, anode in the pipette). After a delay of a few seconds to several minutes, the electrical background noise increased before the occurrence of a spike firing driven by current pulses, which indicated that the microelectrode tip was in juxtacellular position. A clear-cut increase in firing during the 200 ms ON period of the current pulse indicated that the neuron had been filled efficiently