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FORMALIN AS A PERIPHERAL NOXIOUS STIMULUS CAUSES A BIPHASIC RESPONSE IN NUCLEUS PARAGIGANTOCELLULARIS NEURONS

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ABSTRACT

The effects of formalin as a peripheral noxious stimulus on the activity of lateral paragigantocellularis nucleus (LPGi) neurons were examined. Spontaneous activity of LPGi neurons was recorded after confirmation of their responsiveness to acute pain, and thereafter formalin (50 μL, 2.5%) was injected in the contralateral hindpaw. The response of the LPGi neurons was monitored for 60 min. A biphasic response with a peak lasting 3 to 5 min post-injection, and a second more prolonged tonic excitatory response were obtained which corresponds to the nature and time course of behavioral studies. It is concluded that LPGi neurons may be involved in the processing of nociceptive information related to formalin as a noxious stimulus.

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Keywords: Nucleus paragigantocellularis, Formalin test, Multiple unit recording, Rat.

INTRODUCTION

The lateral paragigantocellularis nucleus (LPGi) is located in the rostral ventrolateral medulla. This region was first defined in the human brain,[6] and was subsequently described in other species[4,16,24,30,31,32] Anatomical and physiological studies have implicated the LPGi in many autonomic processes. These functions include: 1) control of resting arterial pressure, 2) cardio-pulmonary reflexes, 3) respiration and 4) parasympathetic function.[10,13,33] In addition, many LPGi neurons respond to noxious, but not to non-noxious, cutaneous stimulation.[22] Iontophoretically-applied morphine or its analogs [2,5,17,20,28] can alter spontaneous and noxious-evoked activities of these neurons. Such microinjections could activate inhibitory reticulospinal systems originating in LPGi, which has projections to the dorsal horn via the dorsolateral funiculus and the ventral quadrant, and can be blocked with the nanoinjection of tetracaine in the periaqueductal gray (PAG).[27] It has been shown that stimulation of LPGi inhibits evoked activity of dorsal horn neurons.[18] Electrical stimulation and glutamate injection into the PGi cause marked antinociception in phasic pain and moderate antinociception in tonic pain.[3] PGi lesions resulted in significant hyperalgesia.[7] These findings indicate the putative existence of a tonic descending analgesia system in the brain of which PAG is one component, and the putative existence of an opiate-analgesia system involving PAG and LPGi working together in an organized tandem, together with the bulbar nucleus raphe magnus.[27]

As indicated above, it is shown that several areas in the

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brainstem are involved in morphine analgesia and they may be distinguished by being active in different types of pain.[1] The most striking feature of the data is that no lesion sites in these regions produce the same effects in both formalin and tail-flick tests. This implies that the neural substrate of morphine analgesia in a test that involves a rapid response to threshold-level pain (the tail-flick test) differs from that in a test that involves continuous pain generated in injured tissue (the formalin test). These observations raise the possibility that this brain structure might have a special role in the nociception and/or descending inhibition of these two kinds of pain. The effects of formalin as a peripheral noxious stimulus on the activity of LPGi neurons were examined in this study.

MATERIAL AND METHODS

Animal preparation

The experiments were carried out on male NMRI rats (250-350 g). They were maintained in group cages of 2 or 3 in the colony room. Food and water were available *ad libitum*. The animals were initially anesthetized with sodium thiopental (40 mg/kg i.p.); anesthesia was maintained with injections of 4 mg/kg thiopental supplemented as necessary (approximately every 45 - 60 min). Body temperature was maintained at 36±1°C with a feedback controlled heating pad.

Following tracheotomy, the animals were mounted in a stereotaxic frame. A single midline incision was made and the scalp retracted. Based on atlas coordinates,[23] a hole was drilled at 2.8 mm caudal to the interaural line and 1.5 mm to the right of the midline. The dura covering the caudal cerebellum was removed.

Recording procedures

Multiple unit activity was recorded extracellularly in LPGi using classic electrophysiologic techniques. Briefly, we used glass micropipettes filled with 0.5 M sodium acetate and 2% pontamine sky blue that served as an electrolyte and also to mark recording sites. The impedance of these electrodes (measured at 1000 Hz) was between 3-8 M. The electrodes were lowered 8.4 mm below the dura to reach LPGi. Spike amplitude and waveforms were continuously monitored using an oscilloscope and audio monitor. Multiple unit activity was recorded on a tape recorder (Honeywell) for off-line analysis. Following the introduction of the data to a computer, using an A/D board, the rate of multiple unit activities was analyzed by homemade software. The program was capable of filtering unwanted noise, by means of a manually controlled threshold in the software.

Stable extracellular recordings from LPGi neurons were obtained and the receptive field properties of the neurons were determined using both innocuous (brush) and noxious (pinch) stimulations, applied to the dorsal body surface including both hind limbs, and the tail. After recording the spontaneous activity, and also the response to phasic mechanical pain, 50 µL of 2.5% formalin was injected subcutaneously to the plantar surface of the animals' right hind paw and the response to this chemical stimuli was monitored and recorded for 60 minutes.

Histology

At the end of each experiment, micropippete penetrations were marked by iontophoretic ejection of pontamine sky blue dye using a negative current, 10 A for 10 min. Animals were given an overdose of sodium thiopental and perfused intracardially with 0.9% saline, followed by a 10% formalin solution. The brain was removed and stored in formalin for a minimun of 3 days. 50 µ sections of the brain were cut using a vibrotome (Campden Instruments), and studied for histological verification. Only those animals with correct recording placement were included in the analysis (Fig.1).

Statistical methods

Statistical comparisons were performed with one-tailed Student's t-test.

RESULTS

The spontaneous activity of LPGi neurons was 1-20 spike/sec. This activity shows an oscillatory behavior. Figure 2 show the basal spontaneous activity of LPGi neurons in one of the recordings with a mean of 1.7 spike/sec. The majority of neurons had negative waveform, with an amplitude of less than 0.5 mV. Some of the neurons exhibited respiratory rhythms.

After the application of mechanical stimulation to the animals' limbs, back and tail, three distinct neuronal groups were seen in LPGi: 1) A group of neurons which did not respond to noxious, mechanical stimuli, 2) Another group, which showed a decrease in their firing rate, following noxious stimuli, 3) And the third group, with an elevation in the rate of their spontaneous activity, after inducing mechanical stimuli. Only when the neurons in the third group were found did the nociceptive test proceed. These neurons were recorded for 5 minutes in order to gain a steady baseline. The mean firing rate obtained was 3.62 ± 0.09 spike/sec. Then by pinching the animals' limbs and body back, we recorded the neuronal response (Table I). Baseline neuronal firing showed a significant difference with the firing rate seen during pinching of both limbs ($p<0.01$), and body back ($p<0.03$). It is also shown that the right LPGi neurons are responsive to noxious mechanical stimuli applied to both right and left parts of the body, but the most responsive was related to the left parts of the body ($p<0.05$).

Figure 3 shows the mean LPGi neuronal activity during

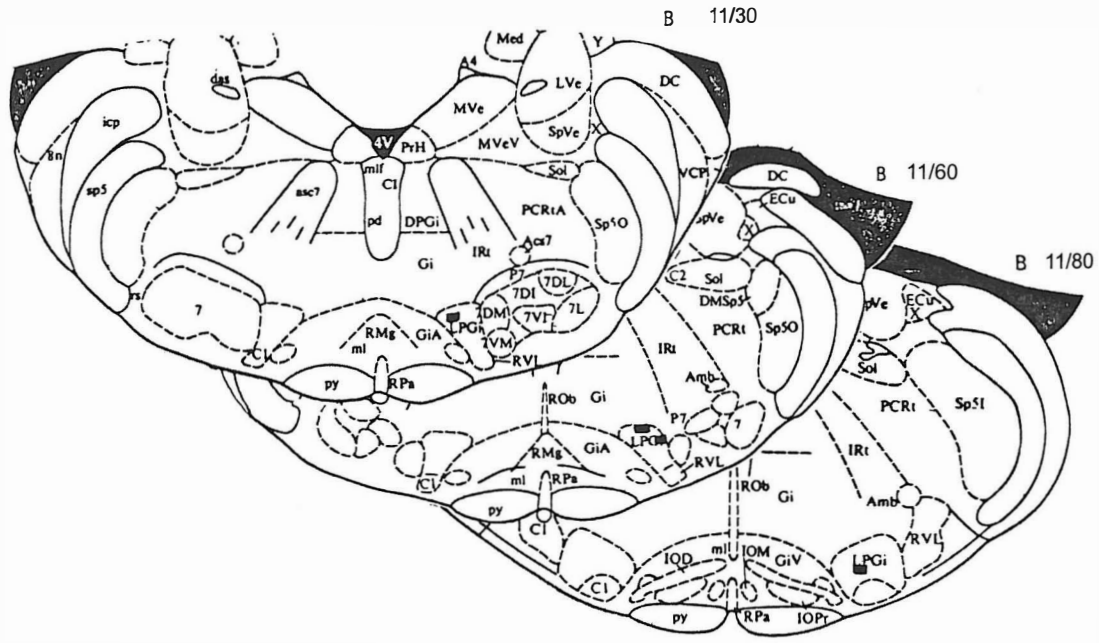

Fig. 1. Diagramatic representations of the LPGi at three rostral- caudal levels relative to the bregma, showing the locations of LPGi where recorded, according to the atlas of Paxinos.[23]

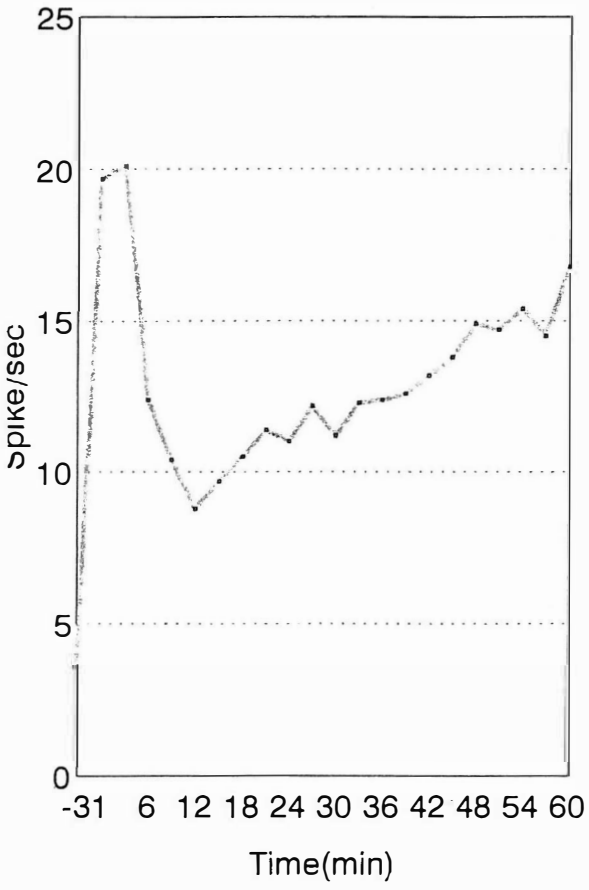

Fig. 2. An example of the baseline spontaneous activity of LPGi neurons.

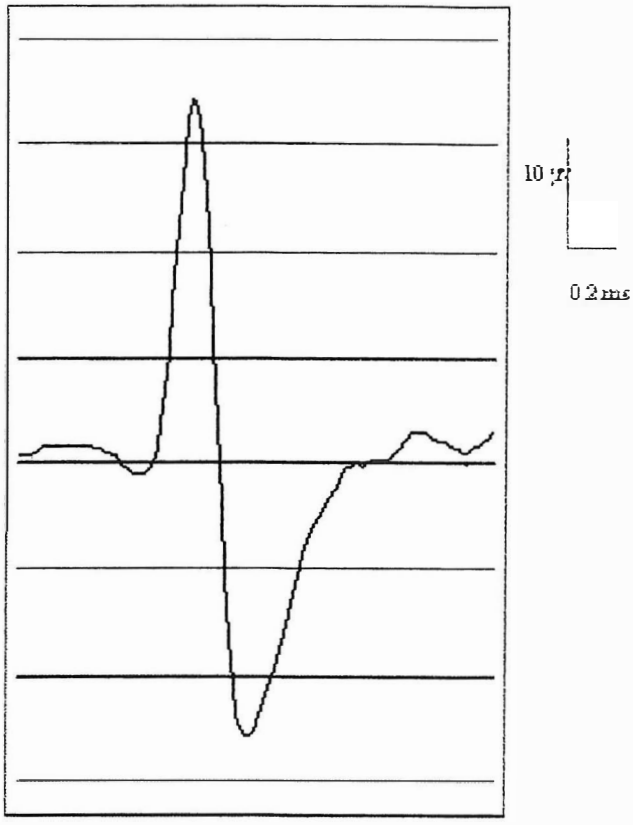

Fig. 3. Mean neuronal activity in LPGi during 60 minutes post formalin injection (n=4).

60 minutes post formalin injection (n=4). As shown in this figure, the activity of the LPGi neurons exhibit a first peak,

Table I. Firing rate of LPGi neurons during baseline, mechanical pinch, and early and late phase of formalin test. Significant difference with baseline firing is shown by * ($p < 0.01$) & ** ($p < 0.03$).

Baseline	Left Leg	Right Leg	Back (left)	Back (right)	1st formalin peak	2nd formalin peak
3.62±0.09	37.25±5.86**	22.58±1.6**	40.8±6.6*	34.1±5.95*	32.5±3.7**	19.6±4.25*

lasting for about 10 minutes ($p<0.01$), followed by a decrease in the firings ($p>0.05$), and then again a gradual increase, building the second phase ($p<0.02$). This scheme resembles the behavioral manner seen in the formalin test.

DISCUSSION

In the past decade, considerable advances have been made towards understanding the pathways and mechanisms, which underly the role of different brain structures involved in nociception. It is well established that the descending inhibition in the nociceptive control system consists of the periaqueductal gray (PAG) in the mid brain, the ventrolateral and ventromedial medulla, and finally the spinal dorsal horn neurons.

The analgesic action of the PAG stimulation is eliminated only when the outputs of both the midline raphe (NRM) and the lateral medulla are blocked with local anesthetics.[27] It has been shown that lesions of the LPGi block the antinociceptive effect evoked by stimulation of PAG.[32] It is shown that the ventromedial medulla (nuclei raphe magnus, paragigantocellularis, magnocellularis pars alpha), plays a critical role in stimulation-produced analgesia and the descending control system of nociceptive information at the spinal level.[8,9,12,14,16,34]

Combined behavioral and neuropharmacological studies have shown that morphine microinjected into the LPGi produces strong analgesia.[2,5,17,20,28] The mechanism by which microinjection of morphine into LPGi results in increased nociceptive thresholds is not yet known. Such microinjections could activate inhibitory reticulospinal systems originating in LPGi, which has projections to the dorsal horn via the dorsolateral funiculus and the ventral quadrant. It has been shown that stimulation of LPGi inhibits evoked activity of dorsal horn neurons. Alternatively, the antinociceptive effects of morphine microinjected into LPGi may be mediated through NRM, since LPGi neurons project to NRM, and since electrolytic lesions of NRM block the antinociceptive effect of morphine microinjected into LPGi.[12] In addition, it is reported that electrical stimulation of the LPGi inhibits the firing of spinal cord dorsal horn neurons[27] and can elicit opioid withdrawal-like behaviors.[21,26]

The nociceptive activity of LPGi neurons have usually been studied using phasic pain stimuli such as the tail flick test[22] but it is established that the tonic pain pathway and its mechanisms may differ from that of phasic pain. In one study, contralateral increases of [^{14}C] 2 - deoxyglucose uptake in LPGi has been shown following subcutaneous formalin injection in the forepaw of the rat.[25] Biochemical studies have provided evidence for enhanced [Met] enkephalin release from LPGi after formalin injection.[15,19,29]

In this study, we assessed the response of LPGi neurons to formalin induced as a peripheral noxious stimulus. These neurons were heterogenous in terms of impulse waveforms, but the majority exhibited negative waveforms in unfiltered records with amplitudes less than 0.5 mV. Spontaneous discharge was also variable, ranging from 1 to 20 spike/sec. Some cells exhibited rhythmic discharge related to respiration. These results are in accordance with the findings presented by Aston-Jones et al.[13]

Electrophysiologically, by using single unit recording, two distinct populations of cells in LPGi are shown to send projections to the Locus Coeruleus. 1) Non - spontaneous, relatively fast conducting cells with small negative spikes and 2) slow conducting, spontaneously active cells with larger positive spike.[31] In our study, according to the activity of LPGi neurons in response to mechanical phasic stimuli, we divided the LPGi neurons into three types: 1) One group of neurons which did not respond to noxious mechanical stimuli, 2) The other group whose firing rate increased following this kind of stimuli, 3) A group whose firing rate decreased following this kind of stimuli. The difference between baseline neuronal firing and the firing rate seen during body pinching showed that LPGi neurons responded to phasic pinch in the right and left body, but the difference of the firing rate between right and left paws showed that right LPGi neurons responded to contralateral phasic pain more than ipsilateral. These findings were in accordance with the findings presented by Aston-Jones.[13] The activity of the LPGi neurons in response to formalin exhibit a biphasic shape which consisted of the first peak (0-10 min post-formalin injection) and implies that LPGi neurons are involved in the processing of phasic nociceptive mechanical stimului. The second peak (25-60 min after formalin injection) showed that pain-related neurons in LPGi are implicated in the processing of tonic nociception. The biphasic pattern of the neural activity in response to formalin injection is in accordance with [^{14}C] 2-deoxyglucose uptake studies undertaken by Porro in 1991.[25]

The biphasic shape of the LPGi neuronal response to formalin was similar to that recorded in the behavioral response following the injection of this chemical (Fig. 3). This

confirms the involvement of this nucleus in the nociceptive pathway. The same pattern was reported in the spinal cord dorsal horn neurons.[11] The activity of single dorsal horn nociceptive neurons was recorded in the spinal cord of halothane anesthetized rats. Subcutaneous injection of a 5% formalin solution into the receptive field of these neurons resulted in two peaks of neuronal firing over a period of 60 minutes.

It is concluded that LPGi neurons may be involved in the processing of nociceptive information related to formalin as a noxious stimulus.

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