Autonomic Neuroscience: Basic and Clinical 169 (2012) 87–94

Contents lists available at SciVerse ScienceDirect

Autonomic Neuroscience: Basic and Clinical

journal homepage: www.elsevier.com/locate/autneu

The rostral parvicellular reticular formation neurons mediate lingual nerve input to the rostral ventrolateral medulla

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ARTICLE INFO

Article history:
Received 30 August 2011
Received in revised form 6 April 2012
Accepted 3 May 2012

Keywords:
Rostral parvicellular reticular formation
Rostral ventrolateral medulla
Lingual nerve
Pulse-relative activity
Cardiovascular pathway
Antidromic spike
Rat

ABSTRACT

In rats that had been anesthetized by urethane–chloralose, we investigated whether neurons in the rostral part of the parvicellular reticular formation (rRFp) mediate lingual nerve input to the rostral ventrolateral medulla (RVLM), which is involved in somato-visceral sensory integration and in controlling the cardiovascular system. We determined the effect of the lingual nerve stimulation on activity of the rRFp neurons that were activated antidromically by stimulation of the RVLM. Stimulation of the lingual trigeminal afferent gave rise to excitatory effects (10/26, 39%), inhibitory effects (6/26, 22%) and no effect (10/26, 39%) on the RVLM-projecting rRFp neurons. About two-thirds of RVLM-projecting rRFp neurons exhibited spontaneous activity; the remaining one-third did not. A half (13/26) of RVLM-projecting rRFp neurons exhibited a pulse-related activity, suggesting that they receive a variety of peripheral and CNS inputs involved in cardiovascular function.

We conclude that the lingual trigeminal input exerts excitatory and/or inhibitory effects on a majority (61%) of the RVLM-projecting rRFp neurons, and their neuronal activity may be involved in the cardiovascular responses accompanied by the defense reaction.

1. Introduction

The rostral part of the parvicellular reticular formation (rRFp) has been implicated in oral sensory perception and in cardiovascular regulation. The location and neuronal connections of the rRFp, found in anatomical and physiological studies, suggest that it is a relay nucleus between sensory nerve inputs and the cardiovascular center of the medulla. The rRFp receives afferent projections from the orofacial and gustatory sensory nuclei (Shammah-Lagnado et al., 1992; Zerali-Mailly et al., 2001), and has efferent projections to rostral ventrolateral medulla (RVLM) (Ter Horst et al., 1991; Esser et al., 1998), which is involved in somato-visceral sensory integration (Sun and Spyer, 1991a; Siddall et al., 1994; Zagon, 2001a, 2001b) as well as control of the cardiovascular system (Guyenet, 1990). Stimulation of trigeminal nerves (Hanamori et al., 1996; Allen and Pronych, 1997) in the rat induces changes in arterial blood pressure and heart rate. It has been proposed that the RVLM is involved in trigeminal-induced reflex cardiovascular responses (Kumada et al., 1990; Allen and Pronych, 1997). A direct pathway from the rRFp to the RVLM has been suggested by an electrophysiological report (Oskutyte et al., 2006). Thus, neurons in the rRFp are hypothesized to mediate the orofacial sensory nerve input to the RVLM as part of the trigeminal-cardiac reflex pathway. To test this hypothesis, we tested the effect of stimulating the lingual trigeminal nerve on the RVLM-projecting rRFp neurons. We also investigated whether these neurons exhibit a pulse-relative activity.

2. Materials and methods

These experiments were carried out on 14 male Sprague–Dawley rats (300–450 g) under urethane–chloralose anesthesia (500 mg/kg and 50 mg/kg, respectively, i.p.). The trachea was intubated, and the femoral artery and vein were cannulated in order to monitor arterial blood pressure (ABP) and administer drugs. Arterial blood and tracheal pressures were measured with pressure-sensitive transducers and were amplified. A lead II electrocardiogram (ECG) was recorded, using leads attached to the limbs of the rats. Rectal temperature was monitored and maintained at 37 °C using a regulated heating pad. All animal procedures were performed in accordance with the Guiding Principles for Care and Use of Animals in the Field of Physiological Sciences provided by the Physiological Society of Japan, and were approved by the Laboratory Animal Committee of The Nippon Dental University School of Life Dentistry at Niigata.

The general surgical procedures and experimental protocols were similar to those described previously (Oskutyte et al., 2004, 2006). In summary, animals were fixed in a stereotaxic apparatus, and were artificially ventilated with room air containing 20–35% O_2 following neuromuscular blockade with pancuronium bromide (1 mg/kg, i.v.). A craniotomy was performed, and the cerebellum was removed by suction to expose the dorsal surface of the caudal brainstem. The depth of anesthesia was assessed by monitoring the stability of the arterial pressure and heart rate to noxious pinching of the hind paw

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doi:10.1016/j.autneu.2012.05.001

at regular 1 hour intervals, and if necessary anesthesia was supplemented by injections of urethane–chloralose (50 and 5 mg/kg/h; i.v.). The right lingual nerve (LN) was carefully exposed, and was separated from the surrounding connective tissues. An electrode was introduced for stimulation and was insulated from the surrounding tissues by silicone elastomer (Kwik-Cast, World Precision Instruments, USA). The chorda tympani nerve in the tympanic cavity was cut in order to activate only lingual somatic afferent fibers by stimulation of the lingual trigeminal nerve. A bipolar concentric electrode (tip diameter 0.05 mm, polar distance 0.5 mm, outer shaft diameter 0.2 mm, IMB-9008, Inter Medical Co., Japan) was stereotaxically inserted into the right side of the RVLM at an angle of 40° (located 1.6–2.5 mm rostral from *calamus scriptorius*, 1.5–2.0 mm lateral from the midline, and 2.8–3.1 mm ventral from the surface of the brainstem).

Extracellular recordings of single unit activity were made using a single microelectrode from the right side of the rRFp at an angle of 30° (located 3.0–4.0 mm rostral from *calamus scriptorius*, 1.5–2.2 mm lateral from the midline, and 1.8–3.0 mm below the dorsal surface of the medulla). The single microelectrode was filled with 2% pontamine sky blue in 0.5 M sodium acetate.

Searching stimuli (1 pulse with 0.2 ms duration, 20–40 μA, 1 Hz) for antidromic activity of rRFp neurons were delivered to the RVLM. Stimulation of the RVLM at a current intensity of less than 40 μA was expected to activate neuronal structures within the RVLM, as described previously (Oskutyte et al., 2006). The antidromic activity evoked by RVLM stimulation was identified by standard criteria (Lipski, 1981; Barman and Gebber, 1987, 1997; Oskutyte et al., 2004, 2006). The onset latency of each antidromic spike was determined in 10–20 trials, using commercially available software (Signal for Windows). Test stimuli (mainly 1 pulse with 0.2 ms duration at 10 Hz for 10 s, in some cases 1–3 pulses with 0.2 ms duration at intervals of 2 ms, 1 Hz) were applied to the lingual nerve in order to determine whether the identified neurons alter their spontaneous firing activity and/or evoke orthodromic spikes.

If RVLM-projecting rRFp neurons, exhibiting antidromic activity evoked by the RVLM stimulation, proved to be spontaneously active, then the spontaneous activity was recorded for 30–120 s.

Ratemeter histograms (bin width 1 s) and ECG/ABP-triggered correlation histograms (bin width 4 ms, 150 bins, pre-trigger time 0.2 s) of the spontaneous activity for 20–40s were displayed using commercially available software (Spike 2 for Windows). In the ECG/ABP-triggered correlation histogram, the maximum and minimum numbers of phase-locked counts, averaged over three cardiac cycles, were taken as the peak and background counts, respectively. A neuron was classified as having pulse-related activity if the coefficient of peak counts per background counts (cardiac coefficient) was more than 2.0 (Barman and Gebber, 1998; Barman et al., 2002; Oskutyte et al., 2006).

All data are presented as mean ± standard deviation (SD) except where indicated, and the means were compared using Student's *t* test. Differences between means were taken as significant when $p<0.05$.

The recording sites were marked by electrophoretic injection of the pontamine sky blue. Electrolytic lesions were made by passing negative direct current (20 μA for 30 s) through the RVLM stimulating electrode. The animals were given additional anesthetic of urethane–chloralose (50 and 5 mg/kg; i.v.) and perfusion was undertaken through the left cardiac ventricle with 0.9% of saline, followed by 10% of buffered formalin solution (pH 7.4). Their brains were removed and fixed in 10% formalin. Serial coronal/sagittal sections of the brain (50 μm thick) were cut, and were stained with Cresyl Violet. The recording and stimulating sites were visualized and mapped onto standard sections of the brain (Paxinos and Watson, 2005). In a case where more than two neurons were recorded in the same animal the unmarked recording site was determined by the relative locations from marked sites on the atlas.

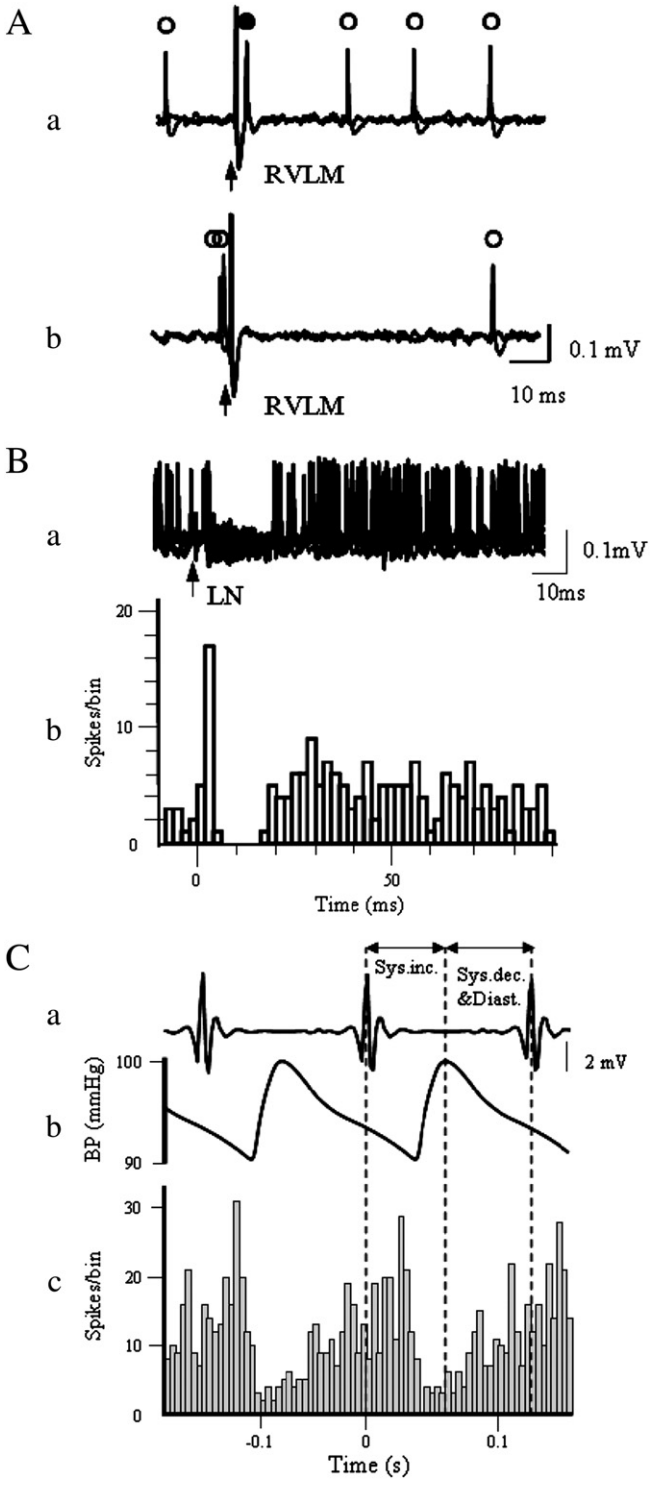

Fig. 1. Example of an RVLM-projecting rRFp neuron with a complex excitatory–inhibitory–excitatory response to LN stimulation and a pulse-related activity. Neuronal activities in (A), (B) and (C) were recorded from the same rRFp neuron. (A) Traces showing characteristics of rRFp neuron activated antidromically by RVLM stimulation (1 Hz, 0.2 ms duration, 30 μA). Two superimposed sweeps showing antidromic spikes (●) with constant latency (a), and occasional preceding spontaneous spikes (○) that eliminated antidromic spikes (b). An upward arrow indicates the onset of stimuli. The voltage calibration and time base in (b) apply to (a). (B) Twenty superimposed sweeps showing that LN stimulation (0.48 mA, 10 Hz for 10 s) elicits an early excitation followed by a period of silenced firing, and late excitations. An upward arrow indicates the onset of stimuli. Peri-stimulus time histogram (b) of spike discharge with lingual nerve stimulation (0.48 mA), constructed from 100 consecutive stimulus trials at 10 Hz. (C) Averaged wave form of ECG (a) and blood pressure (b); and correlation histogram (c) triggered by the R-wave of ECG.