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Discharge rate profiles of paratrigeminal nucleus neurons throughout a pressor event in non-anaesthetized rats☆

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ABSTRACT

Located in the lower brainstem, the paratrigeminal nucleus (Pa5) is related to cardiorespiratory autonomic reflex functions. To characterize the structures' role in blood pressure regulation and the cardiovascular reflex responses Pa5 unit activity was evaluated during a phenylephrine-produced pressor response in non-anaesthetized rats by means of simultaneous many-unit recording. Ninety five percent of the identified Pa5 responded to baroreceptor stimulation, 77% increasing and 23% decreasing firing rates. Cross-correlation analysis of neuron electrical behavior referenced to the heart beat event revealed that 65% of the featured cardiac cycle-locked rhythmic activity. The identification of neurons that change firing rates in response to increases of arterial pressure with cardiac cycle-locked rhythmic activity, further supports for a role for the nucleus in moment to moment control of blood pressure. The largest changes in firing rate occurred in the units with low resting firing rates in response to the ascending phase of the pressor event. Thus, the group displaying both cardiac cycle-locked and other rhythmic activities within the ranges of cardiac and respiratory rates or arterial pressure low frequencies, is probably the most influential regarding homoeostatic reflex responses. The findings advance the notion that the dynamic control of blood pressure involves lower brainstem integration of cardiac and respiratory reflexes.

1. Introduction

The nucleus of the solitary tract (NTS) and the paratrigeminal nucleus (Pa5) are the only two structures of the dorsal lower brain stem known to play a role in the maintenance of the cardiovascular tone and baroreflex function (Sousa and Lindsey, 2009), sharing common, but not entirely overlapping connectivity to the cardiovascular structures in the caudal and rostral ventral medulla (Dampney, 1981; Spyer, 1981; Caous et al., 2001). As the NTS, the paratrigeminal nucleus receives carotid both sinus (Blessing et al., 1999) aortic depressor nerve (Gieroba et al., 1991), baroreceptor inputs and holds a high baroreceptor-sensitive neurons (Zhang and Mifflin, 2000; Yu and Lindsey, 2003; Balan et al., 2004). Additionally, recent findings show that, similarly to NTS lesions, chemical ablation of the Pa5 leads to increased resting blood pressure levels and impairment of the baroreflex responses (Sousa and Lindsey, 2009). Earlier investigations determined a direct and functional connection between the Pa5 and the rostral ventrolateral medulla (RVLM) that hosts premotor sympathetic neurons that drive sympathetic tone (Calaresu and Yardley, 1988). Differently from the NTS the Pa5 has direct efferent projections to the RVLM in addition to its projections to the solitary tract in the NTS itself. Both structures project to the lateral reticular nucleus (LRt), and nucleus ambiguus (Caous et al., 2001). The NTS on the other hand projects to the caudal ventrolateral medulla (CVLM) which in turn connects to the RVLM making up the main link of the baroreflex arc (Dampney, 1981; Dampney, 1994). Furthermore the stimulation of the Pa5 with the neuropeptide and vasoactive agent bradykinin causes significative arterial blood pressure increases (Lindsey et al., 1997) and RVLM neuron firing rate changes preceding the blood pressure change (Caous et al., 2004). Understanding that the Pa5 is on the verge of being recognized is an active component of the lower brainstem baroreflex circuitry the aim of the present investigation was to determine the discharge rate profiles of Pa5 blood pressure responsive units during the different phases of drug-produced pressor events in order to provide a functional characterization of the paratrigeminal neurons in the reflex homoeostatic mechanisms.

Moreover the classification of the baroreceptor-sensitive units by response pattern, heart rate driven rhythmic activity or other slow wave relate rhythmic activities as well as resting firing rate levels the present investigation expects to provide ample support to earlier predictions (Lindsey et al., 1997; Couture and Lindsey, 2000; Yu and

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Lindsey, 2003; Balan et al., 2004; Caous et al., 2004) relating the Pa5 to the reflex regulation of cardiorespiratory moment to moment homoeostatic functions.

2. Methods

2.1. Animal care and use

All experiments were approved by an institutional ethical review committee and procedures ere carried out in compliance to the Guide for the Care and use of Laboratory Animals (National Institute of Health publication No. 86-23). All the experiments were conducted on adult, normotensive male Wistar rats (EPM-1 strain, Festing, 1980), 240–260 g at time of surgery. Animals were individually housed and maintained on a 14/10 h light/dark cycle. At the termination of experimental recording, animals were promptly anaesthetized and sacrificed as described below. The adequacy of anaesthesia throughout surgeries was verified by the absence of withdrawal response of a hind paw or tail pinch and vibrissa movement or tail tonus.

2.2. General surgical procedures

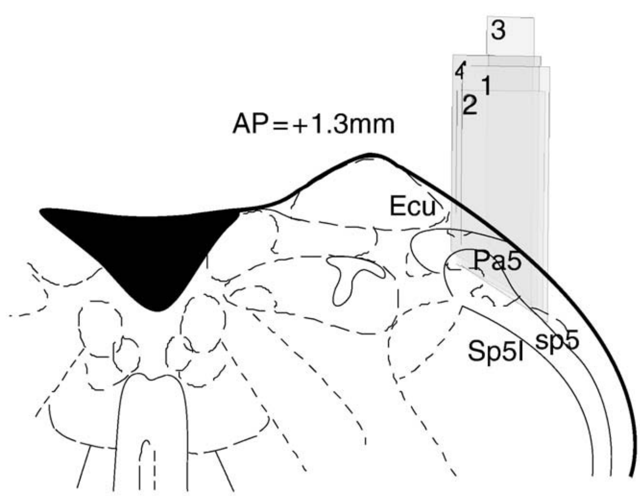
Surgical procedures were performed on 4 isoflurane-anaesthetized (1–1.5%) and artificially ventilated (70% oxygen) animals. For electrode placement, the anaesthetized animals were fixed to a stereotaxic apparatus and the dorsal medulla exposed for electrode placing in the Pa5, coordinates: + 1.3 mm antero-posterior, ± 2.5 mm lateral and − 0.3 mm vertical referenced to stereotaxic zero (Paxinos and Watson, 1987), as described elsewhere (Yu and Lindsey, 2003). Bundle or array configurations of sixteen Teflon-coated stainless steel microwire (50 μm diameter, 100–200 kΩ measured at 1 kHz; NB Labs, Denison, TX, USA) electrode were slowly lowered (100 μm min^{-1}) on to the target and fixed to the skull as detailed elsewhere (Nicolelis and Chapin, 1994; Yu and Lindsey, 2003). On day 14 post surgery, indwelling heparin (1:50 v/v) filled catheters (PE-10 connected to PE-50, Clay Adams, Parsippany, NJ, USA) were placed in the abdominal aorta and vena cava through the femoral artery and vein and exposed at the neck for drug injection and recording of cardiovascular parameters.

2.3. Computer acquisition of spike-timing data for multiple neurons

Neuron activity data was collected with a Multichannel Neuron Acquisition Processor (MNAP, Plexon Inc., Dalas, TX, USA) running a realtime signal discrimination application package RASPUTIN (Real-time Acquisition System Programs for Unit Timing In Neuroscience, Plexon Inc., Dalas). A neurophysiological, time domain, data analysis program NexExplorer, (Nex Technology Inc., Dallas, TX, US), connected as a client to RASPUTIN, provided graphical display quantitative statistical analysis of data that was used for offline or online analysis.

2.4. Acquisition neuron time stamp and cardiovascular data

Arterial blood pressure and heart rate were monitored and recorded online using a Cambridge Electronics Design (CED, UK) interface running a Spike2 data acquisition program (CED) following



	Rat	Electrode	Neuron Count	Digital signal processor (dsp)	Resting Firing Rate (spikes.s^{-1})
1	L540	16 array	15	1(3), 2(1), 3 (2), 6(1), 8 (2), 9(2), 12(2), 16(2)	1.9 ± 0.2
2	L541	16 array	32	1(3), 2(4), 3(2), 5(2), 6(3), 7(1), 8(1), 9(2), 10 (2),11(4), 12(3), 13(2), 14(1), 15(1), 16(1)	4.5 ± 0.3
3	L542	8 array	16	1(4), 2(2), 3(1), 4(2), 5(2), 6(2), 7(1), 8(2)	6.8 ± 0.4
4	L544	16 array	39	1(2), 2(2), 3(1), 4(2), 5(2), 6(4), 7(2), 8(2), 9(3), 10(2), 11(4), 12(2), 13(1), 14(2), 15(4), 16(4)	30.7 ± 0.2

Fig. 1. Electrode placement sites, electrode type and numbers of discriminated neurons in each animal. The upper panel shows a representation of the rat dorsal lateral medulla (adapted from Paxinos & Watson, 1987). The grey bars depict electrode placements in the 4 animals. In the lower panel identification of the electrode type used in each animal, and the number of identified units per wire. In the far most column to the right, the average firing rate (mean ± sd) of identified units in each animal. AP, antero-posterior; Ecu, external cuneate nucleus; Pa5, paratrigeminal nucleus; sp5, spinal trigeminal tract; Sp5i, spinal trigeminal nucleus, interpolar.