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Cardiovascular and baroreceptor functions of the paratrigeminal nucleus for pressor effects 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 baroreflex response, both resting cardiovascular parameters and reflex responses were evaluated during phenylephrine-produced pressor responses in non-anaesthetized rats with or without bilateral chemical Pa5 ablation. The Pa5-ablated animals, in contrast to the Pa5-intact control animals, presented increased resting arterial pressure (115 ± 4 vs. 100 ± 3 mm Hg), decreased heart (293 ± 10 vs. 315 ± 7 bpm) and increase of the respiratory (104 ± 3 vs. 94 ± 5 rpm) rates, larger pressor responses and reduced baroreflex index (1.6 ± 0.2 vs. 2.8 ± 0.2, $p<0.05$). The cardiovascular changes, compatible to those produced by nucleus of the solitary tract (NTS) lesions in non-anaesthetized rats, indicate a reduction of both the sympathetic and cardiac components of the baroreflex response. Further analyses showed the Pa5 mediates reflex responses to smaller blood pressure increases, while the NTS would be predominantly active in surges over 40 mm Hg. Thus, the integrity of the Pa5 is important for resting blood pressure maintenance as for a full baroreceptor response.

1. Introduction

Accumulated evidence portrays a role for the paratrigeminal nucleus (Pa5) in cardiovascular and other visceral homoeostatic functions. Published observations strongly support a role for the medullary structure in blood pressure maintenance (Lindsey et al., 1997) or baroreflex function (Balan et al., 2004; Caous et al., 2004). Also, there is substantial evidence for a role of the nucleus in cardiovascular responses to nociceptive hind paw somatosensory stimulation (Koepp et al., 2005; Caous et al., 2008). The nucleus integrates spinal, glossopharyngeal (Housley et al., 1987), vagal and trigeminal nerve sensory or visceral inputs and relays these to other brain structures (Menétrey et al., 1987). The Pa5 area of the dorsal lateral medulla receives carotid sinus inputs (Ciriello et al., 1981). The connectivity pattern of Pa5 to lower brainstem or pontine structures indicates an integration to medullary cardiovascular control neuronal circuitry. Concurrently, the connections to the nucleus of the solitary tract (NTS), the ambiguus nucleus, the lateral reticular nucleus, the parabrachial nuclei and the rostral ventrolateral medulla (RVLM) define the Pa5 as anatomically integrated to key components of the medullary baroreflex circuitry as well as to structures related to other physiologic functions as respiration, thermoregulation and nociception (Caous et al., 2001). The RVLM harbors sympathetic premotor neurons that command the cardiovascular and other components that make up the sympathetic tone (Dampney, 1981). The ambiguus nucleus on the other hand mediates the cardiac components of the baroreflex response (Ciriello and Caralesu, 1980).

The baroreflex functions of the NTS were mainly brought to light by evaluating the effects of selective lesions applied to target medullary and pontine structures (Dampney, 1981; Spyer, 1981) on baroreflex function in response to drug produced blood pressure increases. On the other hand, the existence of baroreceptor sensitive units in the NTS was demonstrated by single (Rogers et al., 1993) and many unit (Balan et al., 2004) neuron recording during drug produced pressor events. In view of remaining baroreceptor activity (Wang and Edwards, 1997) or functional recovery (Sato et al., 2003) after extensive NTS lesions as well as other considerations, some researchers predicted the participation of other brain structures in the baroreceptor arc (Dampney, 1994). The interest in the Pa5 functions blood pressure control originated from the studies relating medullary mediate bradykinin-produced pressor responses in normotensive and hypertensive animals. Considering the ample evidence linking central bradykinin receptors, blood pressure, the paratrigeminal nucleus (Lindsey et al., 1997) sympathetic tone (Buñag and Takahashi, 1981) and the high blood pressure in the SHR (Buñag and Takahashi, 1981; Martins et al., 1991) and mainly the identification of a large number of baroreceptor-sensitive neurons in the Pa5 (Yu and Lindsey, 2003; Balan et al., 2004) the aims of the present investigation were to evaluate the importance

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of the Pa5 integrity on resting and dynamic cardiovascular parameters related to phenylephrine-produced increases of AP in awake and freely moving animals.

2. Methods

2.1. Animal care and use

All experiments were approved by an institutional ethics review committee and procedures were carried out in compliance to the Guide for the Care and use of Laboratory Animals (National Institute of Health publication No. 86-23). The experiments were conducted on 240–260 g adult, normotensive male Wistar rats individually housed and maintained on a 14/10 h light/dark cycle. The adequacy of anaesthesia throughout surgery 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 isoflurane-anaesthetized (1–1.5%) and artificially ventilated (70% oxygen) animals to expose the dorsal and dorsolateral surfaces of the caudal medulla, as earlier described (Caous et al., 2001). Bilateral microinjections of ibotenic acid (0.15 µl, 1 µg/0.1 µl), were performed in Pa5 using glass micropipettes (0.2 mm OD). The injection sites were +1.3 mm antero-posterior, ±2.5 mm lateral and −0.3 mm vertical referenced to stereotaxic zero (Paxinos and Watson, 1987).

2.3. Experimental protocol

Animals pertained to one of the 4 groups: Control I ($n=8$; L50, 52, 55, 61, 62, 113, 115, 131), naive animals that underwent no other surgical procedures other than that of catheter implantation; Control II ($n=9$, L51, 53, 82, 95, 96, 114, 125, 141, 143), sham-lesioned rats that underwent catheter implantation and craniotomy procedures including pipette lowering to target area; Pa5_X_B ($n=9$; L104, L111, 112, 133, 134, 145, 173, 225, 227), all of the procedures described above plus microinjections of ibotenic acid in the Pa5; Adj_X_B ($n=6$: L59, 135, 203, 229-31), the same as procedures as for the Pa5_X_B group except for the ibotenic acid injection site that targeted adjacent structures to the nucleus.

2.4. Baroreflex sensitivity

Three days after lesion surgeries, the baroreflex responses were assessed by HR changes in function of AP alterations caused by intravenous phenylephrine administration using the steady-state method (Farah et al., 1999). Phenylephrine (100 µg/ml solution at the rates of 25 to 300 µl/min) were delivered at every 5 min after return to resting values at a constant infusion rate and discontinued as steady-state effect was achieved. The baroreflex parameters were determined by analyses of the HR/AP sigmoidal plots (McDowall and Dampney, 2006) for Control and Pa5-ablated animals. Baroreflex sensitivity was determined by calculating the slope from the linear ΔHR/ΔAP plots (Farah et al., 1999). Alternatively, ΔHR/ΔAP ratios for each drug dose were averaged (Cerutti et al., 1995). Analysis of variance (ANOVA) and post hock Bonferroni test were applied in the case of multiple comparisons, the level of significance was taken at $p<0.05$.

2.5. Post surgical procedures

After the end of the experimental procedures, rats were anaesthetized and intra-cardially perfused with saline and 4% paraformaldehyde. The brainstems were removed and placed in 30% sucrose in paraformaldehyde for 24 h. Forty µm thick transverse slices of the medulla were cut using a HM-500 cryostat (Microm, Waldorf, Germany). Light microscopy analyses of cresyl-violet-stained sections for site verification.

3. Results

3.1. Histological verification of ibotenic acid lesions

Ibotenic acid-caused lesions of the Pa5 were characterized in medulla sections, under light microscopy, by an intense accumulation

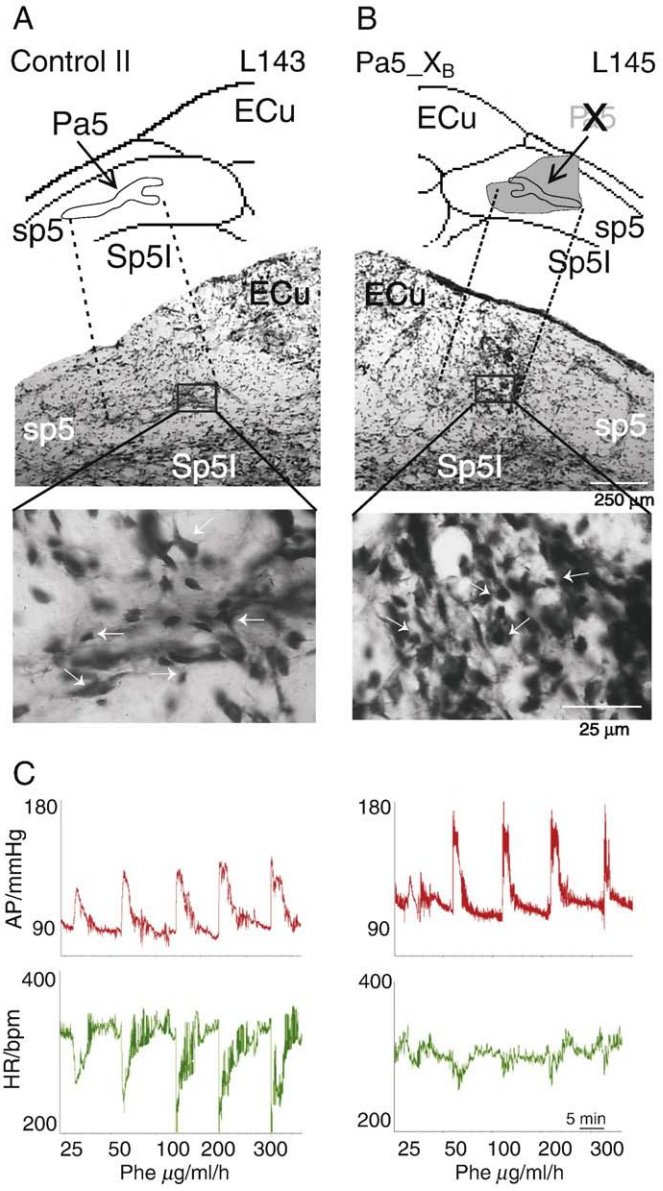

Fig. 1. Cardiovascular effects of paratrigeminal nucleus (Pa5) lesions. A. Photomicrograph of rat medulla section (animal L143) showing an intact Pa5 and surrounding structures as the spinal trigeminal tract (sp5), the interpolar spinal trigeminal nucleus (Sp5I) and the external cuneate nucleus (ECu). The drawings of the dorsal–lateral medulla sections were adapted from Paxinos and Watson (1987). The enlarged micrograph shows healthy neuron soma (diameter 7.8 ± 0.6 µm; white arrows) and occasionally the smaller sized glial cells (diameter 3.9 ± 0.5 µm; grey arrows). B. Medulla section (animal L145) of ibotenic acid-lesioned Pa5 (0.15 µl, 1 µg/0.1 µl). The lesioned area is depicted in the drawing above the section. The enlarged area, bellow the section, shows profuse glial cells (grey arrows) and absence of neuron cell bodies. C. Line tracings of arterial blood pressure (AP) and heart rate (HR) responses to different doses of intra-arterially applied phenylephrine to animals L143 (Controle II) and L145 (Pa5_X_B).