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Microglial number is related to the number of tyrosine hydroxylase neurons in SHR and normotensive rats

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ABSTRACT

Microglia are ubiquitously distributed throughout the central nervous system (CNS) and play a critical role in the maintenance of neuronal homeostasis. Recent advances have shown that microglia, never resting cells of the CNS, continuously monitor and influence neuronal/synaptic activity levels, by communicating with neurons with the aid of their dynamic processes. The brainstem contains many catecholaminergic nuclei that are key to many aspects of brain function. This includes C1 neurons of the ventrolateral medulla that are thought to play a critical role in control of the circulation. Despite the role of catecholaminergic brainstem neurons in normal physiology, the presence of microglia that surrounds them is poorly understood. Here, we investigate the spatial distribution and morphology of microglia in catecholaminergic nuclei of the brainstem in 3 strains of rat: Sprague-Dawley (SD), Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR). Our data reveal that microglia are heterogeneously distributed within and across different strains of rats. Interestingly, intra-strain comparison of tyrosine hydroxylase-immunoreactive (TH-ir) neuronal and microglial number reveals that microglial number varies with the TH-ir neuronal number in the brainstem. Even though microglial spatial distribution varies across brainstem nuclei, microglial morphology (% area covered, number of end point processes and branch length) does not differ significantly. This work provides the first evidence that even though microglia, in their surveilling state, do not vary appreciably in their morphology across brainstem areas, they do have a heterogeneous pattern of distribution that may be influenced by their local environment.

1. Introduction

Microglia are the tissue resident macrophages of the central nervous system (CNS). They are ubiquitously distributed, and comprise ≈10–15% of cell population in the CNS (Xavier et al., 2014). Microglia are of mesodermal origin and populate the CNS during early stages of development, and are self-renewing in case of depletion (Ajami et al., 2007). Since 2005, microglia are no longer known solely for their critical role as immune cells of the CNS (Nimmerjahn et al., 2005). Recent work about the function of microglia concerns their role in maintaining normal brain homeostasis. The ability of microglia to communicate, detect changes, and influence the activity of neurons (Pocock and Kettenmann, 2007) and astrocytes (Aloisi et al., 1997; Pyo et al., 2003), highlights their role as an integral component of the "Glial Family". Microglia, in their resting/surveilling state, are capable of performing activities, such as release of neurotrophic factors (Karperien et al., 2013; Ueno et al., 2013) or synaptic pruning (Kettenmann et al., 2013; Miyamoto et al., 2013); during injury, microglia transform into a more macrophage-like 'activated' state removing debris, and enhancing tissue repair (David and Kroner, 2011; Kroner et al., 2014; Windelborn and Mitchell, 2012). The role of microglia in non-injurious situations is less clear.

Microglia are ubiquitously, yet heterogeneously, distributed in many areas of the CNS, including the hippocampus, olfactory telencephalon, cerebellum, cortex and spinal cord (de Haas et al., 2008; Lawson et al., 1990; Mittelbronn et al., 2001; Nikodemova et al., 2014). Heterogeneous microglial distribution, chemical expression profiles and morphology are hypothesised to be strongly influenced by their local environment (de Haas et al., 2008; Kapoor et al., in press; Lawson et al., 1990; Mittelbronn et al., 2001; Nikodemova et al., 2014). However, exact factors responsible for the heterogeneity in microglial expression, in a given environment, are still unknown.

Catecholaminergic nuclei of the brainstem are involved in almost every aspect of cardiorespiratory control, ranging from the maintenance of tonic activity of the sympathetic nervous system (SNS) to somatosensory reflex responses (Pilowsky et al., 2009). Phenotypically, catecholaminergic nuclei are immunoreactive for tyrosine hydroxylase (TH), and extend from the caudal ventrolateral medullary region to the pons, including A1, A2, C1, C3, A5 and A6

Abbreviations: ANS, autonomic nervous system; CNS, central nervous system; DβH, dopamine β hydroxylase; PBS, phosphate buffered saline; SD, Sprague-Dawley; SHR, spontaneously hypertensive rats; TH, tyrosine hydroxylase; WKY, Wistar-Kyoto.

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(Dahlström and Fuxe, 1964). Studies using stimuli, such as hypotension, hypertension and glucoprivation, have shown that the neuronal activity of various catecholaminergic nuclei of the brainstem is affected by these homeostatic challenges (Dampney and Horiuchi, 2003; Graham et al., 1995; Springell et al., 2005). For example, acute hypotension, induced by intravenous injection of hydralazine or infusion of sodium nitroprusside, causes activation of neurons located in the A1 (Benarroch, 1998), C1 (Minson et al., 1996; Miyawaki et al., 2002), A5 (Dampney and Horiuchi, 2003) and A6 regions of the brainstem (Dampney and Horiuchi, 2003). On the other hand, induction of glucoprivation causes activation of A6 neurons (Ritter et al., 1998). The A2 catecholaminergic nucleus of brainstem not only responds to cardiorespiratory stimuli, but also responds to other diverse range of signals such as hormonal, gastrointestinal and inflammatory (Rinaman, 2011). The function of C3 group of neurons of the brainstem is the least studied, but they may be sympathoexcitatory and respond to stimuli affecting glucose homeostasis (Menuet et al., 2014). In fact, it may be that C3 neurons are simply anatomically displaced C1 or C2 neurons that did not reach their final destination in the ventral or dorsal medulla oblongata.

Recent work has shown that close lines of communication exist between microglia and the SNS (Bhandare et al., 2016; Bhandare et al., 2015; Kapoor et al., in press; Shi et al., 2010). As mentioned above, catecholaminergic nuclei in the brainstem are critical in the regulation of sympathetic nerve activity. The close link between microglia and the SNS, and previously proposed hypothesis that microglial distribution is influenced by their local environment (de Haas et al., 2008), formed the foundation of aims of the present study. Therefore, in this study, we aimed to identify if the microglial distribution is related to the neuronal heterogeneity in the SNS. For the purpose of this study, we used 3 different strains of rats: Sprague-Dawley (SD), the Wistar-Kyoto (WKY) and the spontaneously hypertensive rat (SHR).

2. Experimental procedures

2.1. Animals

Experiments were conducted in adult male rats from 3 different strains: SD, WKY (>18 weeks old) and SHR (>18 weeks old); n = 3 per group (300–420 g; Animal Resource Centre; Perth, Australia) in accordance with the Australian code of practice for the care and use of animals for scientific purposes. All procedures and protocols performed were approved by the Sydney Local Health District Animal Welfare Committee and the Macquarie University Animal Care and Ethics Committee, Sydney, Australia.

The blood pressure phenotype of SHR and WKY was determined by tail-cuff sphygmomanometery at >18 weeks of age, prior to perfusion (Farnham et al., 2012). Hypertension was defined as a systolic pressure of >180 mm Hg and normotension was defined as a systolic pressure of <150 mm Hg.

2.2. Experimental protocol

2.2.1. Perfusion and tissue processing

Animals were deeply anaesthetized with sodium pentobarbital (>72 mg/kg i.p.; Cenvet Australia) and were transcardially perfused with ≈400 ml of ice cold 0.1 M phosphate buffered saline (PBS) followed by fixation with ≈400 ml of ice cold 4% paraformaldehyde in 0.1 M PBS (pH 7.4). Extracted brainstem tissue was fixed overnight in 4% paraformaldehyde in 0.1 M PBS (pH 7.4) at 4 °C, while shaking continuously. Brainstems were then sectioned transversely, using a vibrating microtome (VT1200S, Leica), at 40 μm and were collected sequentially into 5 pots. These sections were stored in cryoprotectant solution (30% sucrose, 30% ethylene glycol, 2% polyvinylpyrrolidone in 0.1 M PBS) at −20 °C until processed for immunohistochemistry.

2.2.2. Immunohistochemistry

Immunohistochemistry was conducted as described previously (Kapoor et al., in press; Nedoboy et al., 2016). Briefly, free floating 40 μm brainstem sections were washed in 0.1 M PBS with 0.3% Triton X-100, 3 times for 30 min each, at room temperature. After washing, sections were incubated for >48 h in TTPBSm (10 mM Tris-HCl, 0.1 M PBS, 0.9% NaCl, 0.3% Triton X-100 and 0.1% merthiolate at pH 7.4), 10% normal donkey serum with primary antibodies raised against TH (mouse monoclonal (IgG1κ), Avanti antibodies, #AV1 (Nedoboy et al., 2016); 1:100) and Iba1 (rabbit polyclonal, Wako Pure Chemical Industries Ltd., 019-19741; 1:2000) at 4 °C while shaking. Sections were then washed 3 times for 30 min each in TPBS (10 mM Tris-HCl, 0.1 M PBS and 0.9% NaCl at pH 7.4) and incubated overnight with secondary antibodies raised in donkey: donkey anti mouse Cy5 (Jacksons Immunoresearch 715-175-151; 1:500) and donkey anti rabbit Cy3 (Jacksons Immunoresearch 711-166-152; 1:500) in TPBSm (10 mM Tris-HCl,

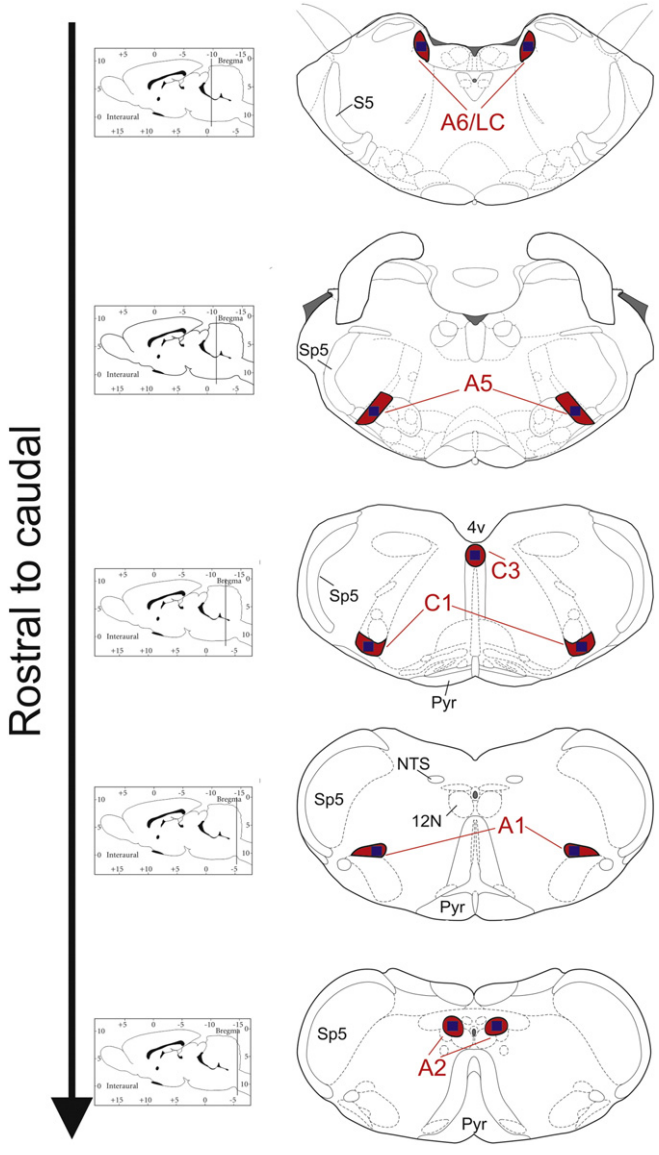

Fig. 1. Location of various catecholaminergic nuclei in the brainstem of a rat (SD, WKY or SHR). 6 catecholaminergic nuclei of interest (A1/A2/C1/C3/A5/A6) are highlighted in red. The area of interest imaged was the same between catecholaminergic nuclei (blue). S5, sensory root of the trigeminal nerve; Sp5, spinal trigeminal tract; 4v, 4th ventricle; Pyr, pyramidal tracts; NTS, nucleus of the solitary tract; 12N, 12th nucleus. The figure is adapted from Paxinos and Watson (2007) (see Experimental procedures for details).