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Alpha$_2$-adrenergic receptor distribution and density within the nucleus tractus solitarii of normotensive and hypertensive rats during development ☆,☆☆

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ABSTRACT

The nucleus tractus solitarii (NTS), located in the brainstem, is one of the main nuclei responsible for integrating different signals in order to originate a specific and orchestrated autonomic response. Antihypertensive drugs are well known to stimulate alpha$_2$-adrenoceptor (alpha$_{2R}$) in brainstem cardiovascular regions to induce reduction in blood pressure. Because alpha$_{2R}$ impairment is present in several models of hypertension, the aim of the present study was to investigate the distribution and density of alpha$_{2R}$ binding within the NTS of Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats during development (1,15,30 and 90 day-old) by an in vitro autoradiographical study. The NTS shows heterogeneous distribution of alpha$_{2R}$ in dorsomedial/dorsolateral, subpostremal and medial/intermediate subnuclei. Alpha$_{2R}$ increased from rostral to caudal dorsomedial/dorsolateral subnuclei in 30 and 90 day-old SHR but not in WKY. Alpha$_{2R}$ decreased from rostral to caudal subpostremal subnucleus in 15, 30 and 90 day-old SHR but not in WKY. Medial/intermediate subnuclei did not show any changes in alpha$_{2R}$ according to NTS levels. Furthermore, alpha$_{2R}$ are decreased in SHR as compared with WKY in all NTS subnuclei and in different ages. Surprisingly, alpha$_{2R}$ impairment was also found in pre-hypertensive stages, specifically in subpostremal subnucleus of 15 day-old rats. Finally, alpha$_{2R}$ decrease from 1 to 90 day-old rats in all subnuclei analyzed. This decrease is different between strains in rostral dorsomedial/dorsolateral and caudal subpostremal subnuclei within the NTS. In summary, our results highlight the importance of alpha$_{2R}$ distribution within the NTS regarding the neural control of blood pressure and the development of hypertension.

1. Introduction

The nucleus tractus solitarii (NTS), located in the brainstem, is one of the main nuclei responsible for integrating signals from different brain areas (St Lambert et al., 1996) and from afferent fibers of arterial baro and chemoreceptors (Finlay and Katz, 1992; Cirrielo et al., 1994) in order to originate a specific and orchestrated autonomic response (Guyenet, 2006). The NTS is a complex nucleus that contains several neurotransmitters/neuroreceptors distributed in different subnuclei; its integrity is paramount for normal blood pressure (BP) regulation (Guyenet, 2006). Alpha$_2$-adrenoceptors (alpha$_{2R}$), present in brainstem cardiovascular areas, are well known targets of antihypertensive drugs inducing peripheral sympathoinhibition and BP reduction (Van Zwieten et al., 1984; Reid, 1986). These substances have lost much of their clinical interest because of their adverse side-effects (Reid, 1986). Hypertension is a chronic increase of BP, and the disease is known as neurogenic if the cause is an abnormal function of the central nervous system (CNS) rather than a primary vascular or renal defect (Guyenet, 2006). This abnormality might be originated within the NTS circuitry. In the spontaneously hypertensive (SHR) rats and other hypertension rat models, a decrease in norepinephrine content and alpha$_{2R}$ density within the NTS has been reported (Yamada et al., 1989). The high BP in this strain appears to be related to an increased sympathetic activity (Judy et al., 1976). According to functional studies, SHR have reduced baroreflex control (Hayward et al., 2002) and an increased sensitivity to alpha$_{2R}$ modulation (Hayashi et al., 1993) when compared to WKY, that suggests that interstrain differences are relevant for baroreflex performance and BP regulation. It has also been reported that the portion of the NTS that receives baroreceptor afferences also receives a dense noradrenergic input (Palkovits and Jacobowitz, 1974) with a high expression levels of alpha$_{2R}$ (Young and Kuhar, 1979; Feldman and Moises, 1988; MacLean et al., 1990). Interestingly, destruction of noradrenergic neurons within the NTS result in marked instability of BP (Granata

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et al., 1983). These data suggest an important role of this system in hypertension development.

Although there is considerable interest in understanding the catecholaminergic system within the NTS of mature animals, the role of alpha$_{2R}$ during development of normotensive and hypertensive rats has received less attention. It has been observed that a decrease in alpha$_{2R}$ from birth to adulthood indicating that the brainstem catecholaminergic system is significantly involved in the transition of newborn to adult life (Mansouri et al., 2001).

The BP in SHR strain is shifted gradually to a high level (systolic pressure greater than 140 mm Hg) approximately 5 weeks after birth (Okamoto et al., 1972; Dickhout and Lee, 1998; Ferrari and Fior-Chadi, 2005). Thus, the period between birth and the development of hypertension in the first five weeks, is relevant for understanding the mechanism associated with the onset of high blood pressure. Alpha$_{2R}$ might regulate the pressure response in a particular way within the complex NTS processing according to age. The specific distribution and density of alpha$_{2R}$ within this nucleus are not well characterized and the hemodynamic response profile originated by NTS processing to noradrenaline is still not completely known. Although several studies reported a differential functional role of alpha$_{2R}$ in the NTS, there are no data showing the distribution and density of alpha$_{2R}$ within this nucleus.

By knowing that alpha$_{2R}$ receptor stimulation promotes a depressor response and that this system is altered in the SHR, the purpose of the present study was to quantify the distribution and density of alpha$_{2R}$ binding within different NTS subnuclei of SHR as compared with the normotensive WKY from birth to adulthood by an in vitro autoradiography study. We found a heterogeneous distribution of alpha$_{2R}$ binding within the NTS and an interstrain difference within specific subnuclei during rat development in pre- and pos-hypertensive stages. Our results highlight the importance of alpha$_{2R}$ binding distribution within the NTS regarding the neural control of blood pressure and hypertension development.

2. Material and methods

2.1. Animal and blood pressure recording

Male normotensive Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats aged 1, 15, 30 and 90 day-old from the Institute of Biosciences, University of São Paulo (São Paulo, Brazil) were used in the present study. Six rats (n = 6) of similar weight were used for each aged groups (1, 15, 30 or 90 day-old). The rats were kept in individual cages under a regular light–dark cycle (light on at 7:00 a.m. and off at 7:00 p.m.) in temperature and humidity-controlled rooms receiving food and water ad libitum. All procedures and protocols used were in accordance with Institutional and International Guidelines for Animal Experimentation.

Mean arterial pressure (MAP) and heart rate (HR) were measured in conscious rats by tail-cuff method with a computerized blood pressure monitor (IITC model 31, USA). Rats were warmed at 30 °C for 5 min and then three stable measurements of blood pressure were averaged. MAP and HR measurement were performed by a single investigator in a blinded fashion. Experiments were done at the same time of day in order to avoid the influence of the circadian rhythm and an appropriate cuff was selected according to animal size. During three days before the experiments, rats were habituated to the restraint, warming and exposed to tail-cuff inflation in order to minimize stress.

2.2. Quantitative receptor autoradiography

The procedure for quantitative receptor autoradiography has been described elsewhere (Peretti-Renucci et al., 1991; Fior et al., 1994). The rats were euthanized by decapitation and their brains were rapidly removed from the skull and frozen in dry ice cooled isopentane (−35 °C). Coronal sections (20 μm thick) of the medulla oblongata were made in a Leica cryostat (CM3050) according to the atlases of Paxinos and Watson (1986) and Altman and Bayer (1995).

Five sections of the medulla oblongata were obtained from WKY (n = 6) and SHR (n = 6) at various ages (1, 15, 30 and 90 day-old). Those sections were obtained based on relative location to area postrema (AP) and others landmarks and are represented as: rostral to AP (level I), rostral AP (level II), middle AP (level III), caudal AP (level IV) and caudal to AP (level V) in all figures. The sections were thaw-mounted on gelatin-coated slides for the analysis of total alpha$_{2R}$ binding using [^{3}H]RX821002 (specific activity 62 Ci/mmol, Amersham, Buckinghamshire, UK; Nowadays GE Healthcare) ligand. Phentolamine (Sigma, St. Louis, USA), a non-selective alpha adrenoceptor antagonist (Gotoh et al., 2011), was used for non-specific binding in addition to [^{3}H]RX821002, using adjacent sections to those assaying total alpha$_{2R}$ binding of 1, 15, 30 and 90 days old SHR and WKY rats.

Slide-mounted brainstem sections were incubated in phosphate buffer 0.01 M, pH 7.4, containing 50 nM KCl and 10 nM of $MgCl_2$ with 3 nM of [^{3}H]RX821002 for 60 min at room temperature. The [^{3}H]RX821002 concentration used in the present study was around K_D values previously obtained by our group in saturation experiments using adults (Carrettiero et al., 2008) and newborn SHR and WKY rats (Carrettiero et al., 2009). Non-specific binding was performed using 10 μM of Phentolamine. Following the incubation, the slices were washed two times in phosphate buffer for 5 min each, rinsed twice in distilled water (0–4 °C) and dried in a stream of cold air. Sections were then exposed to a tritium-sensitive film ([^{3}H]Hyperfilm) in the presence of standard [^{3}H] microscales (Amersham, Buckinghamshire, UK; Nowadays GE Healthcare) for 7 weeks.

2.3. Data analysis

The autoradiograms were quantified using a computer assisted image analyzer and software developed by Imaging Research (Brock University, Canada). Optical density values were determined from the gray images generated from the radioactive ligand in the tritium-sensitive film. A square (0.20 × 0.20 mm) was used as a sample field and the area of analysis was kept constant in all NTS levels in the specific region (dorsomedial/dorsolateral, subpostremal and medial/intermediate NTS subnuclei) except for the whole NTS which was delimited (continuous line as shown in Fig. 1A, a and B). A curve calculated from prefabricated [^{3}H] labeled polymer strip with eight known activities (Microscale - Amersham, Buckinghamshire, UK; Nowadays GE Healthcare) was used as a standard to convert gray to units of binding (arbitrary units).

2.4. Statistical analysis

Statistical differences between SHR and WKY blood pressure (MAP) and heart rate (HR) in several ages were evaluated by Student's *t*-test. Two-way ANOVA followed by Bonferroni post-test to analyze the changes in [^{3}H]RX821002 binding in the two strains (WKY/SHR) according to NTS levels (Fig. 2), or age (Fig. 3). "S" means that changes in [^{3}H]RX821002 binding according NTS levels or age are significantly non-zero (slope ≠ 0), S $p<0.05$. The two-way ANOVA also shows if changes in [^{3}H]RX821002 binding according NTS levels or age are differently affected by strains—the test call it "interaction"; "Inter.: Yes" means that the interaction between strain and NTS levels or age is significant, Yes $p<0.05$. Statistical analysis comparing WKY and SHR rats section in the same NTS level or age was automatically performed by Bonferroni Post-test (* $p<0.05$) (compare black bar with white bars in Figs. 2 and 3). Values are shown as mean ± standard error of the mean (S.E.M), n = 6 animals.