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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.

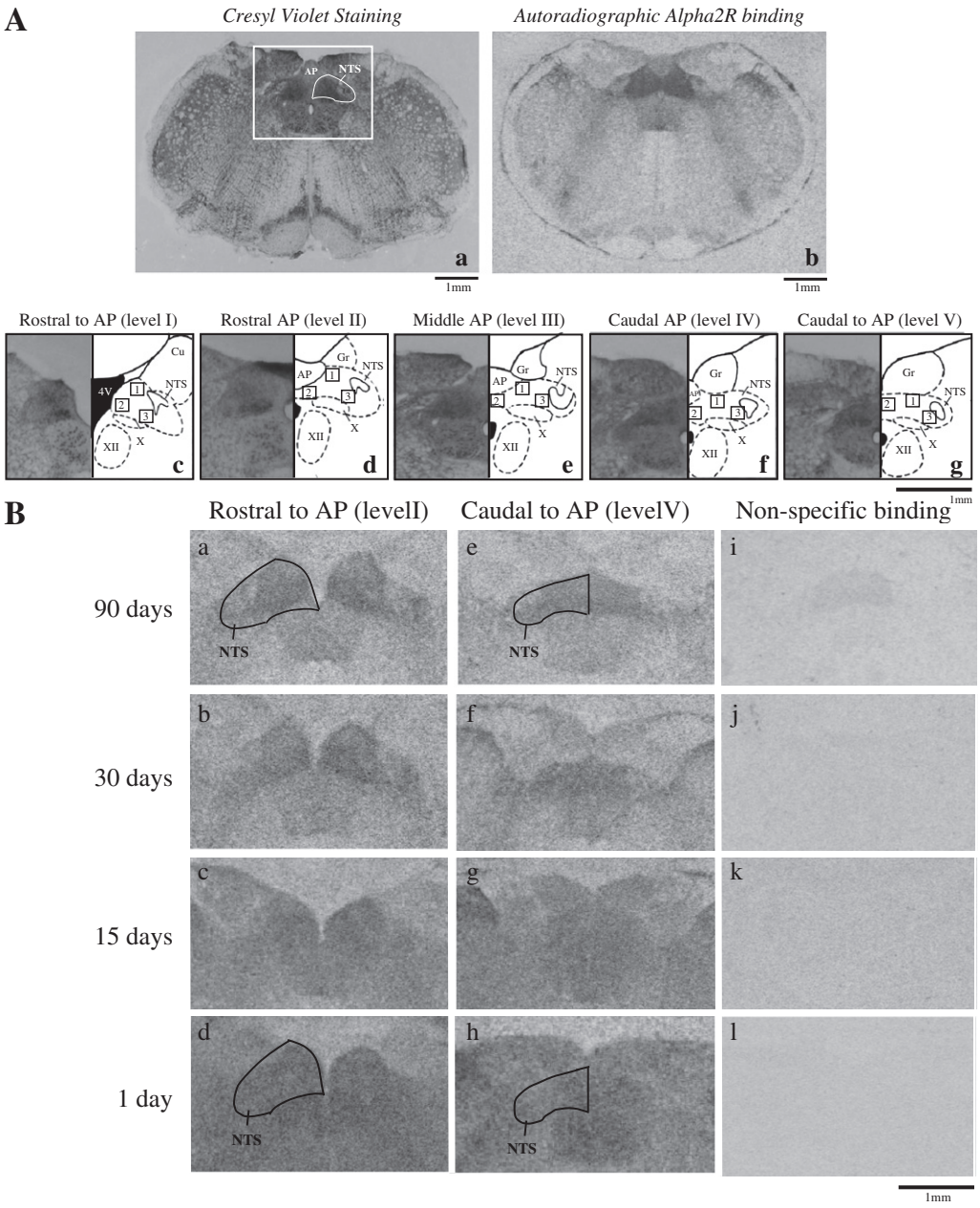


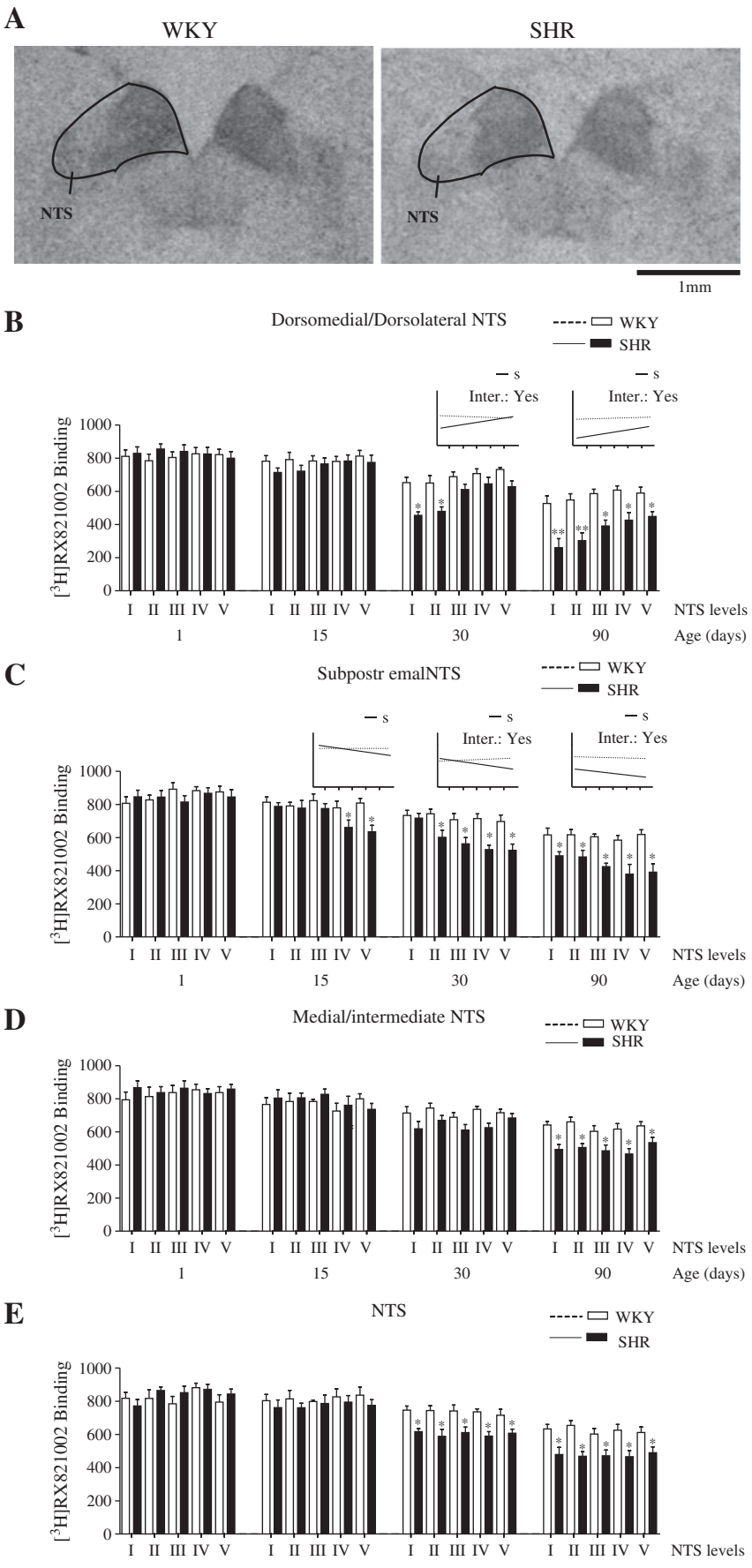
Fig. 1. Histological and autoradiographical representation of nucleus tractus solitarii (NTS) used to quantify the distribution and density of alpha$_2$-adrenoceptors in different levels, strain, age and subnuclei. A) Anatomical localization of NTS by Cresyl violet staining (a), by autoradiographic alpha$_2$-adrenoceptor binding (b) and by Cresyl violet staining from the five sections related to area postrema showing the three subnuclei analyzed: 1—dorsomedial/dorsolateral, 2—subpostremal and 3—medial/intermediate. Landmarkers: area postrema (AP), 4th ventricle (4V), hypoglossal nucleus (XII), cuneate nucleus (Cu), gracile nucleus (Gr), dorsal motor nucleus of vagus (X) (c–g). B) Autoradiographic alpha$_2$-adrenoceptor binding showing NTS in different ages, 1, 15, 30 and 90 day-old rats (a–h). Representative sections of non-specific binding according to age (i–l).

3. Results

The present study analyzes the distribution and density of alpha$_{2R}$ binding within the NTS considering four dimensions of comparison: age, intraNTS, different NTS levels and strain in order to point out regionally selective developmental differences between strains that might have special pathogenetical importance for hypertension development.

Mean arterial pressure (MAP) and heart rate (HR) of WKY and SHR were measured according to age (Table 1). MAP is increased in SHR of 60 and 90 days-old rats as compared to WKY. The HR did not show any differences between strains in different ages (Table 1).

Fig. 1 shows histological and anatomical representation of nucleus tractus solitarii (NTS) by cresyl violet and autoradiographic staining used to quantify the distribution and density of alpha$_2$-adrenoceptors within the NTS at different levels, strain, age and subnuclei. Fig. 1A shows: a) Cresyl violet staining showing NTS, b) Autoradiographic alpha$_2$-adrenoceptors binding showing NTS. Note a dense radioactive binding of alpha$_2$-adrenoceptors within the NTS. c–g) Cresyl violet staining from five NTS sections related to area postrema showing the three subnuclei analyzed: 1—dorsomedial/dorsolateral, 2—subpostremal and 3—medial/intermediate. Landmarkers: area postrema (AP), 4th ventricle (4V), hypoglossal nucleus (XII), cuneate nucleus (Cu), gracile nucleus (Gr), dorsal motor nucleus of vagus (X). Fig. 1B shows autoradiographic alpha$_2$-adrenoceptor staining illustrating two NTS sections (level I and level V) out of five used to quantify the distribution and density of alpha$_2$-adrenoceptors of both strains according to age (1, 15, 30 and 90 day-old rats).


A
WKY
SHR
NTS
NTS
1mm
B
Dorsomedial/Dorsolateral NTS
WKY
SHR
s
Inter.: Yes
s
Inter.: Yes
[3H]RX821002 Binding
1000
800
600
400
200
0
I II III IV V I II III IV V I II III IV V I II III IV V
NTS levels
1 15 30 90
Age (days)
C
Subpostr emalNTS
WKY
SHR
s
s
Inter.: Yes
s
Inter.: Yes
[3H]RX821002 Binding
1000
800
600
400
200
0
I II III IV V I II III IV V I II III IV V I II III IV V
NTS levels
1 15 30 90
Age (days)
D
Medial/intermediate NTS
WKY
SHR
[3H]RX821002 Binding
1000
800
600
400
200
0
I II III IV V I II III IV V I II III IV V I II III IV V
NTS levels
1 15 30 90
Age (days)
E
NTS
WKY
SHR
[3H]RX821002 Binding
1000
800
600
400
200
0
I II III IV V I II III IV V I II III IV V I II III IV V
NTS levels
1 15 30 90
Age (days)

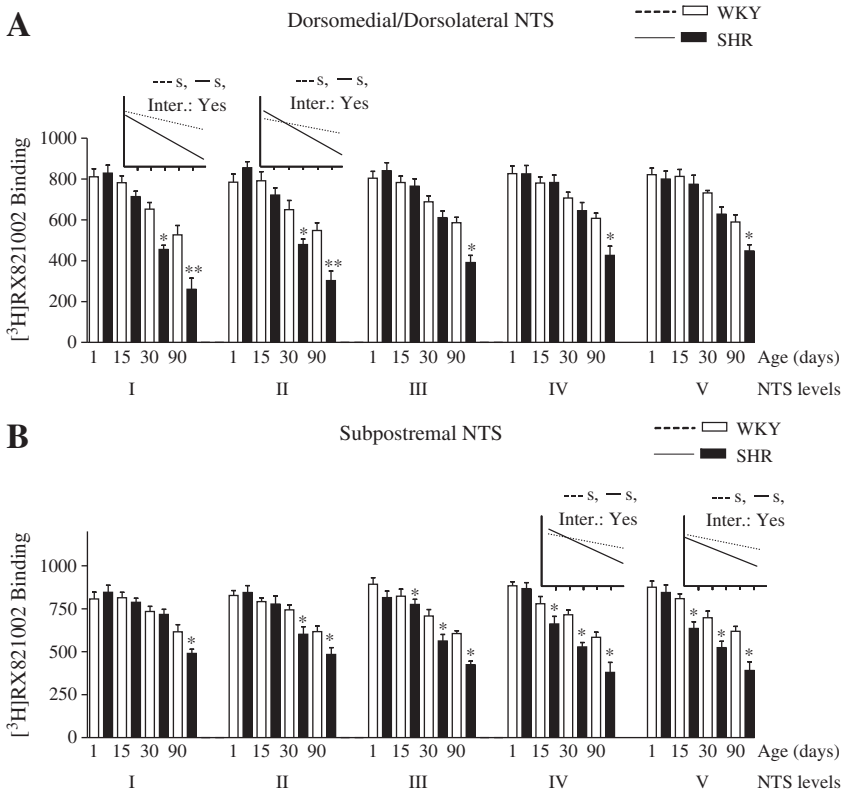


Fig. 3. In order to analyze statistically the changes in alpha$_{2R}$ binding in different strain (WKY/SHR) according to age, the data presented in Fig. 2 were reorganized. A) Adrenoceptors from 1 to 90 day-old in levels I and II (—--,— s, see insets). The result indicated that dorsomedial/dorsolateral NTS subnuclei of WKY and SHR showed a decreased alpha$_2$-adrenoceptor binding and that there are differences between strains in the same levels (I and II) (Inter.: Yes, see insets). B) Subpostremal NTS subnucleus of WKY and SHR showed a decrease in alpha$_2$-adrenoceptor density from 1 to 90 day-old in levels IV and V (—--,— s, see insets). The result also indicated that there are differences between strains in the same levels (IV and V) (Inter.: Yes, see insets). Values are shown as means ± S.E.M, n = 6 animals.

Representative sections of non-specific binding are shown in i–l. The NTS (delimited by continuous line) is illustrated in 4 out of 8 images (Fig. 1B). The NTS shows heterogeneous distribution of alpha$_{2R}$ binding in dorsomedial/dorsolateral, subpostremal and medial/intermediate NTS subnuclei in different levels and ages. Alpha$_{2R}$ binding decreases from 1 to 90 day-old rat (Fig. 1B).

In order to analyze and quantify the changes in [^{3}H]RX821002 binding in the different strain (WKY/SHR) according NTS levels, the autoradiograms were carefully analyzed and presented in Fig. 2. Fig. 2A shows representative images illustrating the differences in alpha$_{2R}$ binding density within the NTS between strains. SHR shows decreased density of alpha$_{2R}$ binding as compared with WKY rats. Dorsomedial/dorsolateral NTS subnuclei of SHR showed increased alpha$_{2R}$ binding density from level I to level V in 30 and 90 days (above the bar graph in the insets, s, Fig. 2B). Dorsomedial/dorsolateral NTS subnuclei of WKY rats did not show any changes from level I to level V at any age (Fig. 2B). The statistical analysis also indicated that there are differences between strains in 30 and 90 day-old rats (above the bar graph in the insets, Inter.: Yes, Fig. 2B). In addition to that, SHR rats show a decreased alpha$_{2R}$ binding density as compared with WKY in the 30 (* level I and II) and 90 (** level I, II, * levels III, IV and V) day-old rats (Fig. 2B, bar graph).

Subpostremal NTS subnucleus of SHR showed a decreased alpha$_{2R}$ binding density from level I to level V in 15, 30 and 90 day-old (Fig. 2C). Subpostremal NTS subnucleus of WKY rats did not show any changes from level I to level V at any age (Fig. 2C). The statistical analysis also indicated that there are differences between strains in 30 and 90 day-old rats (above the bar graph in the insets, Inter.: Yes, Fig. 2C). SHR showed a decreased alpha$_{2R}$ binding density as compared with WKY in 15 (* at levels IV and V), 30 (* at levels II, III, IV and V) and 90 (* at levels I, II, III, IV and V) day-old rats (Fig. 2C).

Although there are changes in alpha$_{2R}$ binding density in dorsomedial/dorsolateral and subpostremal subnuclei within the NTS from level I to level V in SHR, medial/intermediate NTS subnuclei did not show any changes according levels or age (Fig. 2D). Medial/intermediate NTS subnuclei of the SHR showed decreased alpha$_{2R}$ binding density only in 90 day-old rats in all level as compared with WKY (Fig. 2D). Furthermore, the analysis of the whole NTS did not show any changes from level I to level V at any age (Fig. 2E). The NTS of SHR showed a decreased alpha$_{2R}$ binding density as compared with WKY in 30 (* levels I, II, III, IV and V) and 90 (* levels I, II, III, IV and V) day-old rats (Fig. 2E).

In order to analyze statistically the changes in alpha$_{2R}$ binding in different strain (WKY/SHR) according to age, the data presented

Fig. 2. Quantification of alpha$_2$-adrenoceptors binding within the NTS in different levels, strain, age and subnuclei. A) Autoradiographic images illustrating the distribution and density of alpha$_2$-adrenoceptors within the NTS of 90 day-old SHR and WKY rats. Alpha$_2$-adrenoceptors are decreased in SHR as compared with WKY. B) Dorsomedial/dorsolateral NTS subnuclei showed an increased alpha$_2$-adrenoceptors density from level I to level V in 30 and 90 day-old SHR ← s, see insets). The result indicates differences between strains in 30 and 90 day-old rats (Inter.: Yes, see insets). SHR showed a decreased alpha$_2$-adrenoceptors density as compared with WKY in 30 and 90 day-old rats (*, ** bar graph). C) Subpostremal NTS subnucleus showed a decreased alpha$_2$-adrenoceptors from level I to level V in 15, 30 and 90 day-old SHR ← s, see insets). The results indicate differences between strains in 30 and 90 day-old rats (Inter.: Yes, see insets). SHR showed decreased alpha$_2$-adrenoceptor binding compared with WKY in 15, 30 and 90 day-old rats (*, bar graph). D) Medial/intermediate NTS showed no changes in alpha$_2$-adrenoceptors from level I to level V at any age. SHR showed decreased alpha$_2$-adrenoceptors binding as compared with WKY in 90 day-old rat in all level analyzed. E) The whole NTS showed no changes in alpha$_2$-adrenoceptors from level I to level V at any age. SHR showed decreased alpha$_2$-adrenoceptors density as compared with WKY rat in 30 and 90 day-old rats (*, bar graph). Values are shown as means ± S.E.M, n = 6 animals.

Table 1
Mean arterial pressure (MAP) and heart rate (HR) of Wistar Kyoto (WKY) and Spontaneously Hypertensive (SHR) rats of 15, 30, 60 and 90 days-old.

Age	MAP (mm Hg)		HR (bpm)	
	WKY	SHR	WKY	SHR
15 days	94.9 ± 5.6	96.8 ± 2.3	445.9 ± 7.3	450.3 ± 6.0
30 days	104.0 ± 2.7	106.5 ± 2.7	423.2 ± 11.0	440.4 ± 9.2
60 days	109.0 ± 5.6	139.4 ± 4.8*	394.3 ± 8.6	375.9 ± 7.1
90 days	110.5 ± 6.0	151.0 ± 5.5**	411.0 ± 6.6	401.9 ± 10.7

Values are shown as mean ± SEM. *$p<0.05$ and **$p<0.01$ as compared with agematched WKY according to the Student's *t* test, n = 6.

in Fig. 2 were carefully reorganized and presented in Fig. 3. Dorsomedial/dorsolateral and subpostremal subnuclei of WKY and SHR show a decrease in alpha$_{2R}$ binding density from birth (1 day-old) to adult rats (Figs. 1 and 3). In addition to that, although there is a decrease in alpha$_{2R}$ binding density according to age, the statistical analysis also indicated a significant difference between strains in the levels I and II of dorsomedial/dorsolateral and in the levels IV and V of subpostremal subnuclei (above the bar graph in the insets, Inter.: Yes, Fig. 3A and B, respectively) that was not present in medial/intermediate subnucleus suggesting the importance of these regions.

4. Discussion

The catecholaminergic system, especially the alpha$_2$-adrenoceptor (alpha$_{2R}$), plays a key role in mediating various autonomic functions in different brainstem regions, e.g., the nucleus tractus solitarii (NTS). The complex hemodynamic output of the NTS is highly dependent of its internal organization which is not yet completely understood. In light of this, the most important findings in the present study were: (1) the heterogeneous distribution of alpha$_{2R}$ binding within the NTS of SHR as compared with WKY rats, (2) differences between strains observed in pre-hypertensive stages (3) The importance of subpostremal NTS subnucleus, (4) the gradual decrease in alpha$_{2R}$ binding according to age is different between strains in specific NTS levels of dorsomedial/dorsolateral and subpostremal NTS subnuclei.

The NTS showed high density of alpha$_{2R}$ binding as compared with other nuclei within the brainstem (Fig. 1A, b) supporting the previous findings of several authors (Young and Kuhar, 1979; Feldman and Moises, 1988; MacLean et al., 1990). In adult rats the NTS showed heterogeneous distribution of alpha$_{2R}$ binding in three different subnuclei: dorsomedial/dorsolateral, subpostremal and medial/intermediate (Fig. 2). The NTS is a complex nucleus integrating visceral and neural information, so that each action mediated by this nucleus is under a fine-tuning modulation. This highlights that the analysis of alpha$_{2R}$ binding distribution should be carefully evaluated, considering different NTS subnuclei. Support for this assumption comes also from the study of McRitchie and Tork (1993) showing the complex internal organization of the NTS.

The NTS of the one day-old rat showed a homogeneous distribution (Fig. 2) and high density of alpha$_{2R}$ binding as compared with the 90 day-old rat (Fig. 3). A decrease in the alpha$_{2R}$ binding in the brainstem of humans has been reported from birth to adult (Mansouri et al., 2001), indicating that the brainstem catecholaminergic system is related to the transition of newborn to adult life. It might be involved in trophic processes early in development, declining once completed (Mansouri et al., 2001). We can also speculate that the gradual decrease of alpha$_{2R}$ binding density from 1 to 90 day-old rats (Fig. 3) may be important for the fine-tuning organization of alpha$_{2R}$ system within the NTS in order to establish a mature and specific autonomic response in adult life. Moreover, our data showed that 1 day-old SHR and WKY rats presented no differences in density or distribution of alpha$_{2R}$ binding according NTS levels (Fig. 2), suggesting that the functional defects in catecholaminergic system associated with the development of hypertension in those animals proposed by Nomura et al. (1985) might occur postnatally.

The subpostremal subnucleus of 15 day-old SHR rat showed heterogeneous distribution of alpha$_{2R}$ binding (Fig. 2C). This result point out that the period between 1 and 15 days of life might be crucial to establish a difference in receptor distribution between strains which might be related to neurogenic hypertension development. Indeed, the major finding of the present study that decreasing alpha$_{2R}$ binding starts early in life (15 day-old rats) within a specific NTS subnucleus (subpostremal subnucleus) (Fig. 2C). Moreover, this interstrain difference also appears in dorsomedial/dorsolateral NTS subnuclei of 30 day-old rat and finally in all subnuclei analyzed in the 90 day-old rat. It is important to point out that the decrease of alpha$_{2R}$ binding in the older animals (90 days old) might be a function of the increased blood pressure since hypertension is already established (Table 1).

Our data agree with previous studies reporting a decreased alpha$_{2R}$ binding density within the NTS in SHR as compared with WKY rats (Fig. 2) suggesting an important role of this system in the development of hypertension (Yamada et al., 1984, 1989). Support for this assumption comes also from the study of Nomura et al. (1985). The authors showed a reduced K_D and B_{max} of the alpha$_{2R}$ in SHR as compared to WKY animals, even at 4 weeks after birth when hypertension was not yet established, suggesting that some dysfunction in the alpha$_2$-adrenergic transmission system may be involved in the development of hypertension in the SHR rats.

It is important to point out that hypertension is not apparent in 15 or 30 day-old SHR rats (Table 1). This result was also previously reported by our group (Ferrari and Fior-Chadi, 2005) and by others (Nomura et al., 1985). Thus, the changes in alpha$_{2R}$ binding between strains in 15 day-old rats observed in the present study agree with previous finding that pre-hypertensive changes in alpha$_{2R}$ binding density might be relevant for the development of hypertension (Nomura et al., 1985). More notable is the fact that the subpostremal subnucleus within the NTS seems to be specifically important and might be related with the development of hypertension in SHR lineage, since alteration in catecholaminergic systems starts in specific NTS levels of this subnucleus in 15 day-old SHR rats (Fig. 2C). However such statement should be carefully tested.

Dorsomedial/dorsolateral NTS subnuclei of SHR showed increase in alpha$_{2R}$ binding from level I to level V (Fig. 2B). On the other hand, subpostremal NTS subnucleus of SHR showed decrease in alpha$_{2R}$ binding from level I to level V (Fig. 2C). Moreover, WKY rats showed no difference from level I to level V in 30 and 90 day-old rats suggesting that those subnuclei seems to be specifically important and might be related with the development of hypertension in SHR lineage. Again, this concept should be carefully tested. Therefore, although there are differences in the distribution of alpha$_{2R}$ binding in dorsomedial/dorsolateral and subpostremal subnuclei from level I to level V in 30 and 90 day-old SHR rat, medial/intermediate NTS subnuclei of both strains did not show any difference in the distribution of [^{3}H]RX821002 binding in all ages according to NTS levels (Fig. 2D). These observations suggest that the changes in alpha$_{2R}$ binding within the NTS reported in the present study and by others might be specific for dorsomedial/dorsolateral and subpostremal subnuclei (Matias et al., 1993; Carrettiero et al., 2008).

Hayward et al. (2002) demonstrated, for the first time, the effect of alpha$_{2R}$ modulation on the baroreflex function in the NTS of SHR. The group observed a reduced baroreflex control of mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) after aortic depressor nerve (ADN) stimulation followed by alpha$_{2R}$ blockade in the NTS of SHR but not in WKY. The study also demonstrated a disproportionately greater influence on baroreflex control of MAP than on RSNA in SHR suggesting that the reflex regulation of sympathetic outflow to the kidney is less influenced by the altered

alpha$_{2R}$. In light of this we can speculate that MAP processing might be involved directly with dorsomedial/dorsolateral and/or subpostremal subnuclei, since the most pronounced interstrain differences in alpha$_{2R}$ binding density were observed in these subnuclei (Fig. 2).

Hayashi et al. (1993) have observed that central baroreflex processing in SHR may have an increased sensitivity to alpha$_{2R}$ modulation. Systemic administration of the alpha$_{2R}$ agonist clonidine significantly increased baroreflex control of MAP and splanchnic sympathetic nerve activity in SHR but had no effect on baroreflex control in WKY (Hayashi et al., 1993). The authors also showed that the effect of clonidine administration in the SHR eliminates the difference on baroreflex function between the two strains. This result is interesting since alpha$_{2R}$ binding is decreased in specific NTS areas of the SHR strain as compared to WKY. We can speculate that the homeostatic hemodynamic mechanisms within the NTS might be well preserved in WKY but not in SHR lineage; as a result, baroreflex function is normally blunted and shifted to a higher pressure in SHR.

Another interesting point is that the fine-tuning modulation of NTS systems also depends on receptor–receptor interaction. It has been extensively studied for alpha$_{2R}$ specially interacting with Angiotensine-II (Fior et al., 1995; Jackson et al., 2005), endocannabinoids (Pertwee, 2005), Adenosine (Carrettiero et al., 2008, 2009) and Bradykinin (Fior and Fuxe, 1995). It has also been describe that NTS astrocytes expressing alpha$_{2R}$ play a special role in modulating the excitability of NTS neurons (Bhuiyan et al., 2009; Yao, 2009). Such studies point to a continued plasticity of receptor dynamic during life and might have potential interest for neuropharmacology of several disorders such as hypertension. Especial attention has been focused on separated populations of adenosine receptors within the NTS (Scislo and O'Leary, 2002; Carrettiero and Fior-Chadi, 2004, 2008) modulating cardiovascular control (Scislo and O'Leary, 2002, 2005). Interestingly, the dorsolateral/dorsomedial and subpostremal NTS subnuclei also presented high density of A1 adenosine receptor and the interstrain differences also starts early in life exactly in the subpostremal subnucleus (Carrettiero et al., 2008). Surprisingly, A1 adenosine receptors have been specially reported to differentially modulate alpha$_{2R}$ binding in SHR as compared with WKY rats (Carrettiero et al., 2008, 2009), suggesting that alpha$_{2R}$ binding might be, in association with others neuroreceptors, important for the development of hypertension.

In conclusion, the present work provides a significant contribution to the understanding of the neural control of blood pressure and the development of neurogenic hypertension within the NTS, especially through alpha$_{2R}$, and highlights the importance of a careful analysis of receptor distribution within this nucleus. More important than that, our study reports changes in the distribution and density of alpha$_{2R}$ binding within the NTS of hypertensive rat in the pre-hypertensive stage, suggesting a specific early defect in alpha$_{2R}$, which might be related to the development of hypertension.

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