

# Alterations in Angiotensin and Atrial Natriuretic Peptide Receptors in Brain Nuclei of Spontaneously Hypertensive Rats

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We studied angiotensin II (ANG II) and atrial natriuretic peptide (ANP) receptors in brain areas of young (4-week-old) and adult (14-week-old) spontaneously hypertensive rats (SHR) and their age-matched normotensive controls, Wistar-Kyoto rats (WKY), after incubation of brain sections with  $^{125}\text{I}$ -ligands and quantitative autoradiography. The number of ANP binding sites was decreased in the subfornical organ (SFO) and the area postrema of young and adult SHR and in the nucleus of the solitary tract (NTS) of adult SHR. Conversely, the number of ANG II binding sites was high in the SFO of young and adult hypertensive animals and in the NTS of young SHR.

Our results indicate a central role for ANP and ANG II in genetic hypertension and suggest that these peptides may act as mutual antagonists in brain areas related to the control of blood pressure and fluid regulation.

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**Keywords:** Atrial natriuretic factor, subfornical organ, area postrema, circumventricular organs, genetic hypertension, quantitative autoradiography.

## Introduction

After production in the cardiac atrium, ANP is released to the general circulation and acts at specific peripheral receptor sites to control blood pressure and fluid homeostasis, at least partially as an antagonist of the peripheral ANG II system [1-4]. Certain effects of ANP, however, may be centrally mediated, as binding sites for the peptide have been localized to the SFO [5-8]. In addition, the SFO contains large numbers of ANG II receptors [9-11], suggesting that, in addition to their interaction in the periphery, the two peptides could interact centrally also. The possibility of such a central relationship is supported by recent preliminary experiments indicating the existence of opposite alterations in the receptor number for ANG II and ANP in the SFO of the genetically hypertensive SHR rats [7,8].

We studied the number and affinity of ANP and ANG II receptors in several brain areas of young (4-week-old) and adult (14-week-old) SHR and age-matched WKY controls using modifications of recently developed quantitative autoradiographic techniques [8,10,11]. We report specific (and opposite) alterations in the ANP and ANG II receptor numbers in both young and adult genetically hypertensive rats.

## Methods

Groups of six male SHR and age-matched 4- and 14-week-old WKY (Taconic Farms, Germantown, New York) were maintained under normal laboratory conditions. Blood pressures were measured by tail plethysmography 1 day before they were killed and were, in mmHg:  $90 \pm 4$  and  $115 \pm 5$  in young rats and  $122 \pm 8$  and  $178 \pm 12$  in adult rats, WKY and SHR, respectively ( $P < 0.05$ ). The rats were killed by decapitation between 0900 and 1100 h and their brains were immediately removed and frozen by immersion in isopentane ( $-30^\circ\text{C}$ ). Within 24 h of killing, tissue sections ( $16 \mu\text{m}$ ) were cut in a cryostat at  $-14^\circ\text{C}$ , thaw-mounted on to gelatin-coated glass slides, and placed under vacuum at  $4^\circ\text{C}$  until incubation.

Atrial natriuretic peptide receptors were labelled *in vitro* by incubation of consecutive brain sections with  $^{125}\text{I}$ -labelled 3-iodotyrosyl $^{28}$  ANP, specific activity 1750 Ci/mmol (Amersham Corp., Arlington Heights, Illinois) in concentrations from 10 to 400 pmol/l [6,8]. To measure ANG II receptors, consecutive tissue sections were labelled by incubation with  $^{125}\text{I}$ -[Sar $^1$ ]ANG II, specific activity 1666 Ci/mmol (Meloy Labs, Springfield, Virginia) in concentrations from 80 pmol/l to 5 nmol/l [10,11]. After

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incubation, the slides were placed in X-ray cassettes and exposed to  $^3\text{H}$ -Ultrafilm (LKB Industries, Rockville, Maryland) [10, 11]. Atrial natriuretic peptide and ANG II receptors were measured in specific brain areas by computerized microdensitometry and comparison with  $^{125}\text{I}$ -standards [8,10,11]. Scatchard plots were calculated by linear regression. Values are the means  $\pm$  s.e.m. Statistical differences between groups were analysed using Student's *t*-test.

## Results

Both ANP and ANG II receptors were highly localized in the rat brain. The number of ANP receptors was decreased in the SFO of both young and adult SHR when compared with age-matched, normotensive controls (Table 1). The affinity constant, however, was decreased only in adult SHR;  $K_A$  ( $10^9$  mol/l) were  $8.5 \pm 1.3$  and  $6.9 \pm 1.5$  in young and  $11.1 \pm 1.6$  and  $4.2 \pm 0.5$  in adult ( $P < 0.05$ ) WKY and SHR, respectively.

**Table 1.** Maximal binding capacity (fmol/mg protein) of atrial natriuretic peptide (ANP) and angiotensin II (ANG II) receptors in brain nuclei of genetically hypertensive rats.

Brain area	4-week-old		14-week-old	
	WKY	SHR	WKY	SHR
SFO				
ANP	85 $\pm$ 12	38 $\pm$ 4*	124 $\pm$ 11	73 $\pm$ 10*
ANG II	444 $\pm$ 85	735 $\pm$ 91*	848 $\pm$ 136	1390 $\pm$ 64*
Area postrema				
ANP	109 $\pm$ 9	54 $\pm$ 6*	206 $\pm$ 15†	104 $\pm$ 14*†
ANG II	638 $\pm$ 49	561 $\pm$ 54	1129 $\pm$ 355	1198 $\pm$ 133
NTS				
ANP	—	—	51 $\pm$ 3†	6 $\pm$ 2*†
ANG II	405 $\pm$ 25	651 $\pm$ 81*	1717 $\pm$ 369	1191 $\pm$ 643

WKY, Wistar-Kyoto rat; SHR, spontaneously hypertensive rat; SFO, subfornical organ; NTS, nucleus of the solitary tract. Means  $\pm$  s.e.m. \* $P < 0.05$ ; †tissues incubated with a single concentration (0.03 nmol/l) of  $^{125}\text{I}$ -ANP; —, not determined.

Differences in ANG II receptors were evident in the SFO of young and adult animals, but these differences were the reverse of those found for ANP receptors. SHR had a higher concentration of ANG II receptors than the normotensive rats at both ages.  $K_A$  ( $10^9$  mol/l), however, were lower in young and adult SHR than in WKY rats:  $0.7 \pm 0.1$  and  $0.3 \pm 0.06$  for young and  $1.9 \pm 0.3$  and  $0.8 \pm 0.05$  for adult ( $P < 0.05$ ) WKY and SHR, respectively.

Another circumventricular organ studied, the area postrema, showed a decreased number of ANP receptors in both young and adult SHR when compared with age-matched WKY (Table 1). Technical difficulties, however, did not allow us to perform complete Scatchard analysis in adult animals (Table 1). The  $K_A$  for ANP receptors was not different in young SHR and WKY:  $20.98 \pm 4$  and  $18.0 \pm 0.4 \times 10^9$  mol/l for WKY and SHR, respectively. No alterations in the number (Table 1) or the affinity for ANG II occurred in the area postrema of SHR. The  $K_A$  for ANG II were  $0.3 \pm 0.03$  and  $0.4 \pm 0.03$  for

young and  $0.4 \pm 0.08$  and  $0.4 \pm 0.05 \times 10^9$  mol/l for adult WKY and SHR, respectively.

The number of ANP receptors was low in the NTS. Only adult animals showed significant binding, and that of SHR was lower than that of age-matched normotensive WKY rats (Table 1). Technical difficulties prevented us from determining complete Scatchard analysis of the binding data. In contrast, SHR showed a higher number of ANG II receptors than WKY rats, but only in young SHR when compared with age-matched controls (Table 1).  $K_A$  were not significantly different in young rats ( $1.3 \pm 0.2$  and  $0.9 \pm 0.3 \times 10^9$  mol/l for WKY and SHR, respectively) but the  $K_A$  was higher in adult SHR when compared with adult WKY ( $0.2 \pm 0.05$  and  $0.5 \pm 0.1 \times 10^9$  mol/l respectively,  $P < 0.05$ ).

Other brain areas, such as the olfactory tract, which are unrelated to blood pressure control showed no changes in ANP or ANG II receptors (results not shown).

## Discussion

Our results demonstrate alterations in ANP and ANG II receptors in brain areas of SHR related to cardiovascular regulation. The SFO plays a crucial role in the central effects of circulating ANG II [12,13] and is part of a neural circuitry rich in ANG II receptors which also includes the anteroventral-third ventricle area [14,15]. The area postrema was implicated in the central control of blood pressure, and, like the SFO, is exposed to variations in the concentration of circulating peptides [16]. Both the SFO and the area postrema have been proposed as sites of interaction between the peripheral and central ANG II systems [15,16]. Angiotensin II receptors in the NTS can be considered as part of the central ANG II system [9,17]. Similarly, these areas can be considered as brain sites for the central effects of circulating ANP and as links between peripheral and central ANP systems [6,8].

Our results provide further evidence for the proposed role of ANG II and ANP in blood pressure control and in genetic hypertension [18–21] and indicate that some of their effects can be central in origin [7,8].

The alterations in ANP and ANG II receptors described here are present in young, early hypertensive SHR, and are not likely to be the result of chronic hypertension. In addition, most of the changes are maintained in adult, chronically hypertensive rats. These results indicate a possible role of central ANP and ANG II in both the developmental and the chronic phases of genetic hypertension in the rat.

The present data support the hypothesis of a hyperactive central ANG II system [8,20,21] and of a deficient central ANP system in SHR [7,8] and strongly suggest that the peptides could act as mutual antagonists in the brain as well as in the periphery [3].

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