Decreased Number and Affinity of Rat Atrial Natriuretic Peptide (6-33) Binding Sites in the Subfornical Organ of Spontaneously Hypertensive Rats

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SUMMARY. Binding sites for rat atrial natriuretic peptide (6-33) were quantified by incubation of brain sections with $(3-[^{125}I]iodotyrosyl^{28})$ rat atrial natriuretic peptide (6-33), followed by autoradiography with computerized microdensitometry. Spontaneously hypertensive rats present lower numbers and lower affinity of binding sites than normotensive controls, Wistar-Kyoto rats, in the subfornical organ (binding capacity 61.7 ± 8.9 and 124.3 ± 10.7 fmol/mg protein; affinity constant 4.25 ± 0.55 and $11.10 \pm 1.67 \times 10^9$ M^{-1}, respectively). In the choroid plexus, hypertensive rats have lower numbers of sites than normotensive rats (binding capacity 72.7 ± 10.5 and 173.6 ± 22.8 fmol/mg protein, respectively), but there was no difference in the binding affinity (affinity constant 6.28 ± 0.82 and $7.60 \pm 2.06 \times 10^9$ M^{-1}, respectively). Our results suggest that discretely localized brain binding sites for rat atrial natriuretic peptide (6-33) may have a physiological function in genetically hypertensive rats. (*Circ Res 58: 389–392, 1986*)

SEVERAL peptides have been identified in the mammalian cardiac atrium, including the atrial natriuretic factor (ANF) (Cantin and Genest, 1985). Some of these peptides have been proposed to play active roles in blood pressure and fluid homeostasis and to act as physiological antagonists of the renin-angiotensin system through receptor-mediated actions in the kidneys, adrenal glands and vasculature (Napier et al., 1984; Bianchi et al., 1985; Cantin and Genest, 1985).

ANF induces natriuretic and hypotensive responses in normal rats (de Bold et al., 1981), in several models of experimentally hypertensive rats (Garcia et al., 1985a), and in the spontaneously (genetic) hypertensive rat (SHR) (Garcia et al., 1985b; Seymour et al., 1985). Infusion of ANF could lower blood pressure in SHR through a direct vasodilator effect, since hypertension in this model is associated with increased peripheral resistance (Garcia et al., 1985b; Seymour et al., 1985). Altered metabolism and secretion of ANF have been suggested in SHR (Cantin and Genest, 1985).

Certain cardiovascular effects of ANF, however, could be centrally mediated. ANF modulates vasopressin secretion acting at the hypothalamic and pituitary levels (Samson, 1985; Cantin and Genest, 1985). In the brain, there is a widespread network of ANF-containing neurons (Jacobowitz et al., 1985; Saper et al., 1985). Binding sites for rat ANF (8-33) have been recently reported in specific brain areas (Quirion et al., 1984) such as the subfornical organ,

which is a circumventricular structure devoid of blood-brain barrier, exposed to blood-borne peptides, and involved in blood pressure regulation (Mangiapane and Simpson, 1980; Lind et al., 1983; Bruner et al., 1985) and vasopressin secretion (Simpson, 1981; Mangiapane et al., 1984).

We have utilized quantitative autoradiographic techniques coupled to computerized microdensitometry (Israel et al., 1984) to characterize binding sites for ^{125}I-labeled rat atrial natriuretic peptide (6-33) (rANP) in specific brain areas of SHR and their normotensive controls, Wistar-Kyoto rats (WKY). rANP closely resembles rat ANF (8-33) containing at the N terminus only two aminoacids more (Ser-Leu) than this peptide (Flynn et al., 1983).

Methods

Groups of six 19-week-old male SHR and WKY rats (Taconic Farms) were housed at a constant temperature with lights on from 6 a.m. to 6 p.m., and were given free access to food and water. Blood pressures were measured one day before they were killed, by an indirect tail cuff method using a Programmed Sphingomanometer (Narco Biosystems, Inc.); these pressures were 125 ± 10 mm Hg for WKY and 194 ± 12 mm Hg for SHR ($P < 0.01$). The rats were killed by decapitation between 9 a.m. and 11 a.m., and their brains were immediately removed and frozen by immersion in isopentane ($-30°C$). Within 24 hours of sacrifice, tissue sections (16 μm) were cut in a cryostat at $-14°C$, thaw-mounted onto subbed glass slides, and placed under vacuum at 4°C until incubation.

Rat atrial natriuretic peptide (6-33) (rANP)-binding sites were labeled in vitro by incubation with $(3-[^{125}I]$-iodotyrosyl[28]) rANP (specific activity 1750 Ci/mmol, Amersham Corporation). Consecutive tissue sections were preincubated at 20°C for 15 minutes in 50 mM Tris-HCl buffer, pH 7.4, and were incubated for 60 minutes at room temperature in 50 mM Tris-HCl buffer, pH 7.4, containing 100 mM NaCl, 5 mM $MgCl_2$, 0.5% bovine serum albumin, 40 μg/ml bacitracin, 4 μg/ml leupeptin, 2 μg/ml chymostatin, 0.5 μg/ml phenylmethylsulfonyl-fluoride (PMSF) (Quirion et al., 1984), and ^{125}I-rANP in concentrations ranging from 10 to 400 pM. Nonspecific binding was determined in consecutive sections in the presence of unlabeled rANP (atrial peptide, rat, 28 aminoacids, Peninsula Laboratories, Inc.), in concentrations ranging from 0.025 to 1 μM. After incubation, the slides were washed three times (2 minutes each) in Tris-HCl buffer at 4°C and dried under a cold stream of air. Quantification of rANP-binding sites was performed by autoradiography with $[^3H]$-Ultrofilm (LKB Industries) computerized microdensitometry, and comparison to ^{125}I standards (Israel et al., 1984).

Binding data were calculated and Scatchard plots were produced with the LIGAND program (Munson, 1983). All data were presented as the mean ± SEM. Statistical differences between groups were analyzed by Student's *t*-test.

Results

Saturable, single-class binding sites for ^{125}I-rANP were localized in the subfornical organ and in the choroid plexus (Table 1; Figs. 1 and 2). Nonspecific binding was less than 30% of total binding (Fig. 1). The addition of unlabeled rANP or ANF (8-33) resulted in a displacement of more than 80% of the ^{125}I-rANP binding (results not shown).

Scatchard analyses were performed, using consecutive sections from individual brains. The results obtained for maximum binding capacity (B_{max}) and affinity constant (K_a) are summarized in Table 1 and

FIGURE 1. *Autoradiographic localization of ^{125}I-rANP-binding sites in coronal sections at the level of the rat subfornical organ. The sections were incubated in the presence of 0.3 nM ^{125}I-rANP and exposed for 2.5 days to tritium-labeled Ultrofilm. Arrows point to the subfornical organ and to the choroid plexus. Upper level: a section from a Wistar-Kyoto rat. Middle level: a section from a SHR. Lower level: nonspecific binding determined in a section from a Wistar-Kyoto rat incubated as above, plus addition of 0.75 μM unlabeled rANP.*

TABLE 1
Rat Atrial Natriuretic Peptide (6-33)-Binding Sites in Spontaneously Hypertensive Rats

Area	Binding capacity (B_{max}) (fmol/mg protein)	Binding affinity (K_a) ($\times 10^9$ M^{-1})
Subfornical organ		
WKY (7)	124.3 ± 10.7	11.10 ± 1.67
SHR (6)	61.7 ± 8.9*	4.25 ± 0.55*
Choroid plexus		
WKY (7)	173.6 ± 22.8	7.60 ± 2.06
SHR (5)	72.8 ± 10.5*	6.28 ± 0.82
Olfactory bulb		
WKY (6)	81.5 ± 9.2	5.02 ± 0.60
SHR (6)	87.0 ± 8.7	6.13 ± 0.91
Paraventricular nucleus		
WKY (5)	25.0 ± 3.4	11.20 ± 1.65
SHR (5)	28.2 ± 5.9	9.64 ± 1.23

Values represent mean ± SEM; number of animals given in parentheses. Complete Scatchard plots were determined using consecutive sections from each animal (see Methods).
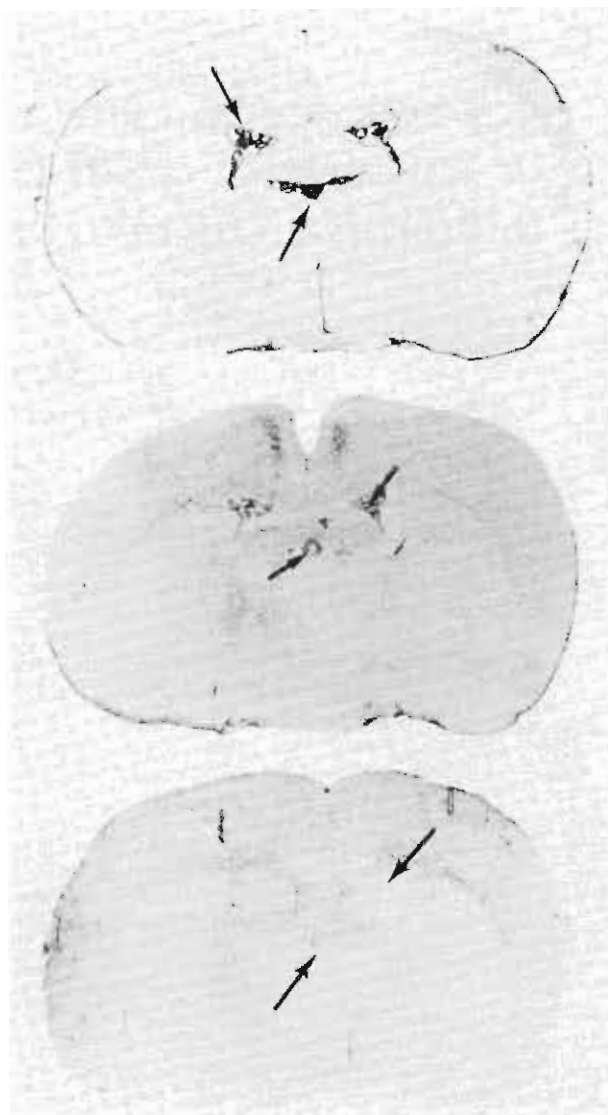
* $P < 0.01$ (Student's *t* test).

Figure 2. In the subfornical organ, SHR had a much lower concentration of binding sites than their normotensive controls. The affinity of the binding sites for ^{125}I-rANP was also lower in the subfornical organ of SHR. In the choroid plexus, SHR had much lower concentrations of rANP-binding sites than WKY rats, but the binding affinities were not significantly different. In contrast, no differences in concentration or affinity of rANP-binding sites between SHR and WKY were found in the olfactory bulb or in the hypothalamic paraventricular nucleus (Table 1).
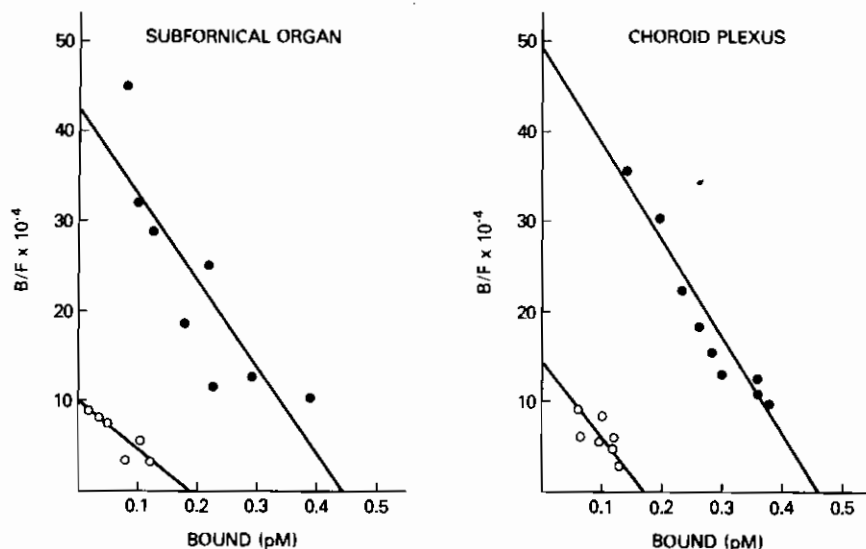
FIGURE 2. *Scatchard analysis of specific ^{125}I-rANP binding to rat subfornical organ and choroid plexus. Tissue sections from single rats were incubated as described in Methods. Data represent a typical experiment which was replicated five to seven times per group (see Table 1). Closed circles: WKY rats. Open circles: SHR. Correlation coefficients (r) are: subfornical organ, WKY: 0.869, SHR: 0.854; choroid plexus: WKY: 0.959, SHR: 0.729.*

Discussion

Our results provide a precise quantification and characterization of rANP-binding sites in the rat brain. The subfornical organ, the choroid plexus, and the olfactory bulb were the only forebrain areas to exhibit large numbers of rANP-binding sites. This distribution is in agreement with previous observations of high levels of binding sites for a related peptide, (synthetic ANF 8-33) in the same brain areas (Quirion et al., 1984). rANP-binding sites were also found in the area of postrema, as reported earlier (Quirion et al., 1984). However, technical limitations prevented us from obtaining reliable estimates of binding site kinetics in this area. In addition, low concentrations of rANP-binding sites were found in the hypothalamic paraventricular nucleus, an area where ANF 8-33-binding sites have not been reported previously (Quirion et al., 1984).

Both the subfornical organ and the choroid plexus contain a single class of saturable, high affinity, specific rANP-binding sites. The subfornical organ lacks a blood-brain barrier, and its rANP-binding sites are exposed to fluctuations in blood levels of the peptide. Presently, it is not known if these binding sites are physiologically active receptors. If they are, the subfornical organ could represent a target site for blood-borne rANP.

The subfornical organ sends projections to the hypothalamus, and especially to the anteroventral 3rd ventricle (AV3V) region, an area critical for the development and maintenance of experimental hypertension, as well as fluid and electrolyte balance (Hartle and Brody, 1984). This area contains the largest accumulation of ANF-positive cells in the brain (Jacobowitz et al., 1985; Saper et al., 1985). Thus, rANP-binding sites in the subfornical organ could represent the anatomical link between the peripheral and central ANF systems.

Decreased number and affinity of rANP-binding sites could be related to the increased blood pressure in SHR. ANF has been reported to decrease vasopressin release through a central mechanism (Samson, 1985) and decreased brain rANP-binding sites could be related to the increased blood vasopressin levels reported in SHR (Crofton et al., 1978). In addition, a decreased number of rANP-binding sites in the subfornical organ could modify the function of angiotensin in this area. The subfornical organ contains large numbers of angiotensin II receptors (Israel et al., 1984; Mendelsohn et al., 1984), and stimulation of these sites results in increased blood pressure (Phillips, 1978; Simpson, 1981). A physiological antagonism between ANF and the angiotensin system in peripheral tissues has been proposed (Cantin and Genest, 1985).

The presence of ^{125}I-rANP-binding sites in the choroid plexus suggests a possible role of this or other ANF-related peptides in the control of ion fluxes across the brain-cerebrospinal fluid barrier. The alterations in rANP binding observed in SHR indicate the possibility for a role for ANF-related peptides at the choroid plexus level in the maintenance of genetic hypertension.

The present data provide the first demonstration of differences in the concentration of rANP-binding sites in brain areas of normotensive and genetically hypertensive rats. Our results support the hypothesis of an altered ANF system in this model of genetic hypertension. SHR present a decreased cardiac atrial content of ANF (Sonnenberg et al., 1985) and increased blood levels of the peptide (Cantin and Genest, 1985). Our findings of decreased brain rANP-binding sites could be interpreted as a down-regulation of central binding sites or as a primary alteration leading to a secondary increased release of the peptide into the circulation.

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