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ATRIAL NATRIURETIC PEPTIDE RECEPTORS IN SYMPATHETIC GANGLIA: BIOCHEMICAL RESPONSE AND ALTERATIONS IN GENETICALLY HYPERTENSIVE RATS

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Summary. High concentration of atrial natriuretic peptide (99-126) (ANP) receptors were localized by quantitative autoradiography in superior cervical and stellate ganglia from young and adult Wistar Kyoto (WKY) rats. ANP increased cyclic GMP formation in stellate ganglia from adult rats. Both young and adult spontaneously hypertensive rats (SHR) had a much lower number of ANP receptors in the sympathetic ganglia. In spite of low receptor concentration, the cyclic GMP response to ANP in SHR was unchanged. These results suggest the existence of physiologically active ANP receptors in the rat sympathetic ganglia. These receptors may also be involved in the pathophysiology of spontaneous hypertension.

Atrial natriuretic peptides (ANP), synthesized in mammalian cardiomyocytes and released from the atria to the systemic circulation, are involved in the regulation of blood pressure and fluid homeostasis through the activation of specific ANP receptors in kidney, adrenal gland, and vasculature (1-3). Part of these receptors are thought to be coupled to particulate guanylate cyclase activation (4-9). In addition, circulating ANP may influence the central nervous system by activating specific receptors in circumventricular structures (10,11).

Recently, ANP-like immunoreactivity was found in rat sympathetic and parasympathetic ganglia (12-14). ANP was shown to inhibit carbachol-stimulated catecholamine synthesis in rat superior cervical ganglia (15); in addition, a peripheral effect of ANP on sympathetic nerves has been involved in the cardiosuppressive action of the peptide (16). These findings suggest that ANP may be involved in sympathetic ganglion function.

Spontaneously hypertensive rats (SHR) have increased peripheral sympathetic activity (17,18) concomitant with alterations in brain ANP receptors (19) and in ANP metabolism (20-22). In the present study, we investigated the presence of receptors for the circulating form of ANP, ANP(99-126), in rat sympathetic ganglia, and we analyzed whether these receptors had been altered in SHR.

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METHODS

Young (4-week-old) and adult (14-week-old) male SHR and WKY rats were obtained from Taconic Farms (Germantown, NY). Blood pressure was measured in all animals one day before sacrifice with an electrosphygmomanometer (Narco Bio-Systems Inc., Houston, TX) and photoelectric sensors (IITC Inc., Landing NJ). Blood pressure was 94 ± 5 mmHg and 89 ± 8 mmHg in young SHR and WKY rats, respectively ($p > 0.10$) and 170 ± 10 mmHg and 99 ± 5 mmHg in adult SHR and WKY rats, respectively ($p < 0.05$; groups of 8 animals).

Rats were killed by decapitation. The left superior cervical and stellate ganglia were removed immediately and frozen in isopentane at -30°C. Tissue sections, 16 µm-thick, were cut in a cryostat at -14°C, thaw-mounted on gelatin-coated glass slides, and stored overnight under vacuum at 4°C. ANP binding sites were labeled in vitro by incubation with 3-[^{125}I]-iodotyrosyl^{28} rat ANP (specific activity 2050 Ci/mmol, Amersham Corp., Arlington Heights, IL), as previously described (11), in concentrations ranging from 40 to 500 pM. Binding sites were quantified by autoradiography and computerized microdensitometry; results were compared with ^{125}I-standards (23). Nonspecific binding was determined by incubating adjacent sections with 1 µM unlabeled ANP (rat atrial peptide, 28 amino acids, Peninsula Laboratories, Inc., Belmont, CA). Adjacent sections were stained with Hematoxylin and Eosin. Scatchard plots of individual ganglia were produced with the computer program LIGAND (24).

ANP-stimulated cyclic GMP accumulation was studied in stellate ganglia from adult SHR and WKY rats. After decapitation, the left stellate ganglia were removed immediately and desheathed under microscope. During the procedure, ganglia were bathed with Locke's solution, pH 7.2, containing 136 mM NaCl, 5.6 mM KCl, 20 mM Na_2HCO_3, 1.2 mM NaH_2PO_4, 2.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 5.5 mM glucose, and were bubbled with 95% O_2-5% CO_2 at room temperature. Ganglia were preincubated for 30 min in the same fresh buffer with 0.5 mM isobutylmethylxantine and 0.1% bacitracin. Ganglia were then incubated at 37°C for 20 min with or without increasing concentrations of unlabeled ANP(99-126). The reaction was stopped by boiling the mixture for 5 min. Tissue was then homogenized by Polytron for 1 min. After centrifugation at 12,000 g for 5 min at 4°C, the supernatants were lyophilized and stored at -20°C until assayed. The samples were reconstituted in 50 mM sodium acetate buffer, pH 6.2, and the cyclic GMP content was measured by radioimmunoassay (New England Nuclear, Boston, MA). Cyclic GMP content was assayed in groups of 4-6 ganglia from adult SHR and WKY rats for each ANP concentration. Each ganglion was assayed in duplicate. In preliminary experiments ANP 10^{-6}M increased cyclic GMP content in ganglia linearly for 30-40 min of incubation time (data not shown).

Results are expressed as means ± S.E.M. Single comparisons were done by Student's t-test; $p < 0.05$ was considered significant.

RESULTS

Specific ANP binding sites were localized in tissue sections from superior cervical and stellate sympathetic ganglia of WKY rats (Fig. 1B, 2). Nonspecific binding was less than 5% of total binding (Fig. 1D, 2). Autoradiographic images showed a relatively diffuse distribution of silver grains (Fig. 1B), which corresponded to the diffuse distribution of principal ganglion cells in adjacent stained sections (Fig. 1A). Binding to preganglionic and postganglionic nerve fibers and binding to surrounding connective tissue was undetectable. Binding kinetic analysis indicates the existence of a single class of saturable, high affinity ANP binding sites in both superior cervical and stellate ganglia from young and adult WKY rats

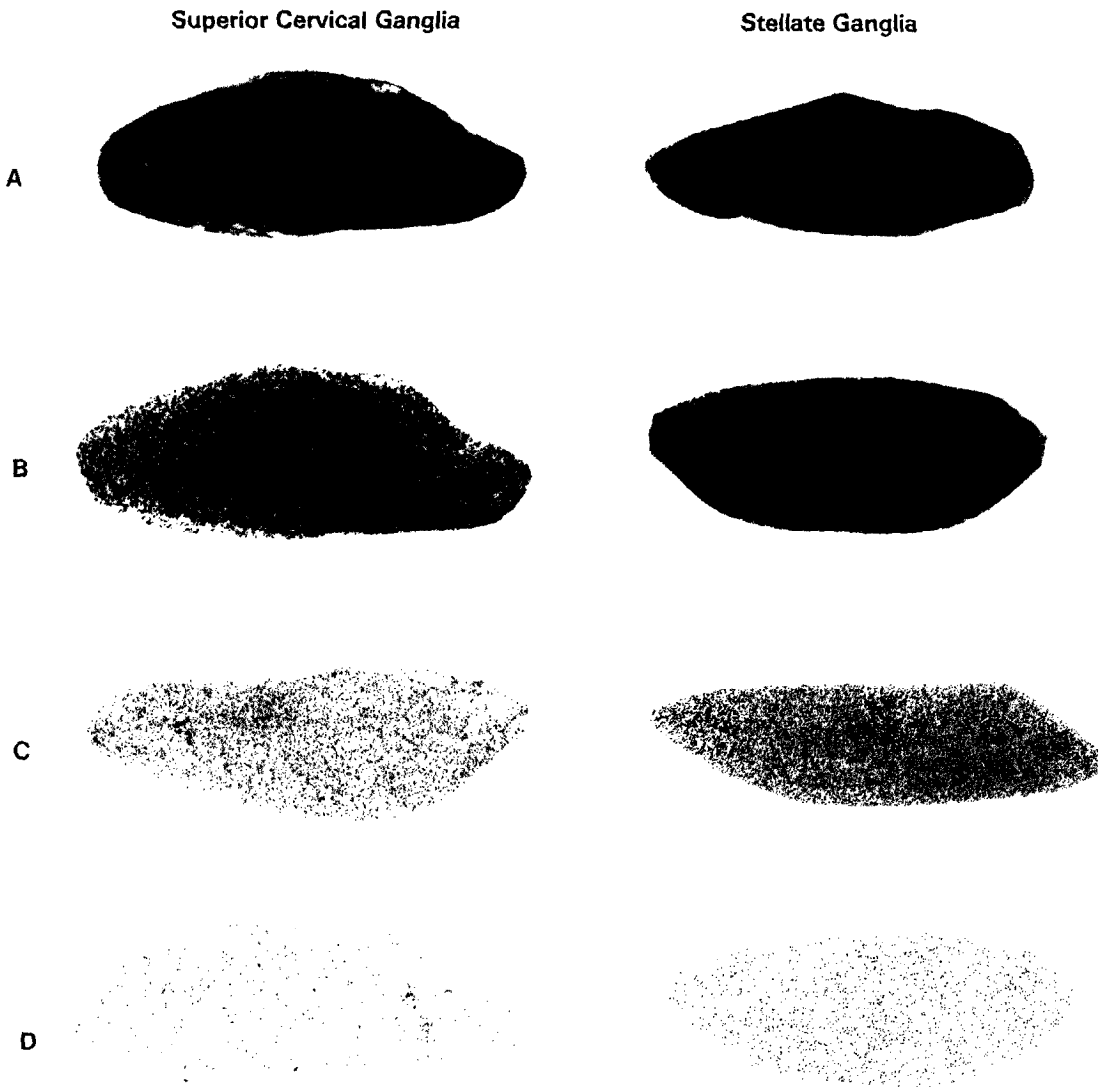

Figure 1. Autoradiographic images of ^{125}I-ANP(99-126) binding to the superior cervical and stellate ganglia from 4-week-old SHR and WKY rats. ANP binding sites were labeled with 0.5 nM ^{125}I-ANP with (nonspecific binding) or without (total binding) of 1.0 uM unlabeled ANP. Sections were exposed to ^{3}H-Ultrofilm for 3 days. A: sections from superior cervical and stellate ganglia stained with Hematoxylin and Eosin; B: total binding in WKY rat; C: total binding in SHR; D: section adjacent to B showing nonspecific binding. Pictures were magnified 8x.

(Fig. 2). The maximum binding capacity in superior cervical and stellate ganglia of adult WKY rats was higher than that of young WKY rats ($p < 0.05$ for both ganglia), while the binding affinity in sympathetic ganglia of adult WKY rats was not significantly different from that of young WKY rats (Table 1).

Conversely, the number of specific ANP binding sites in both superior cervical and stellate ganglia of young and adult SHR was very low (< 15

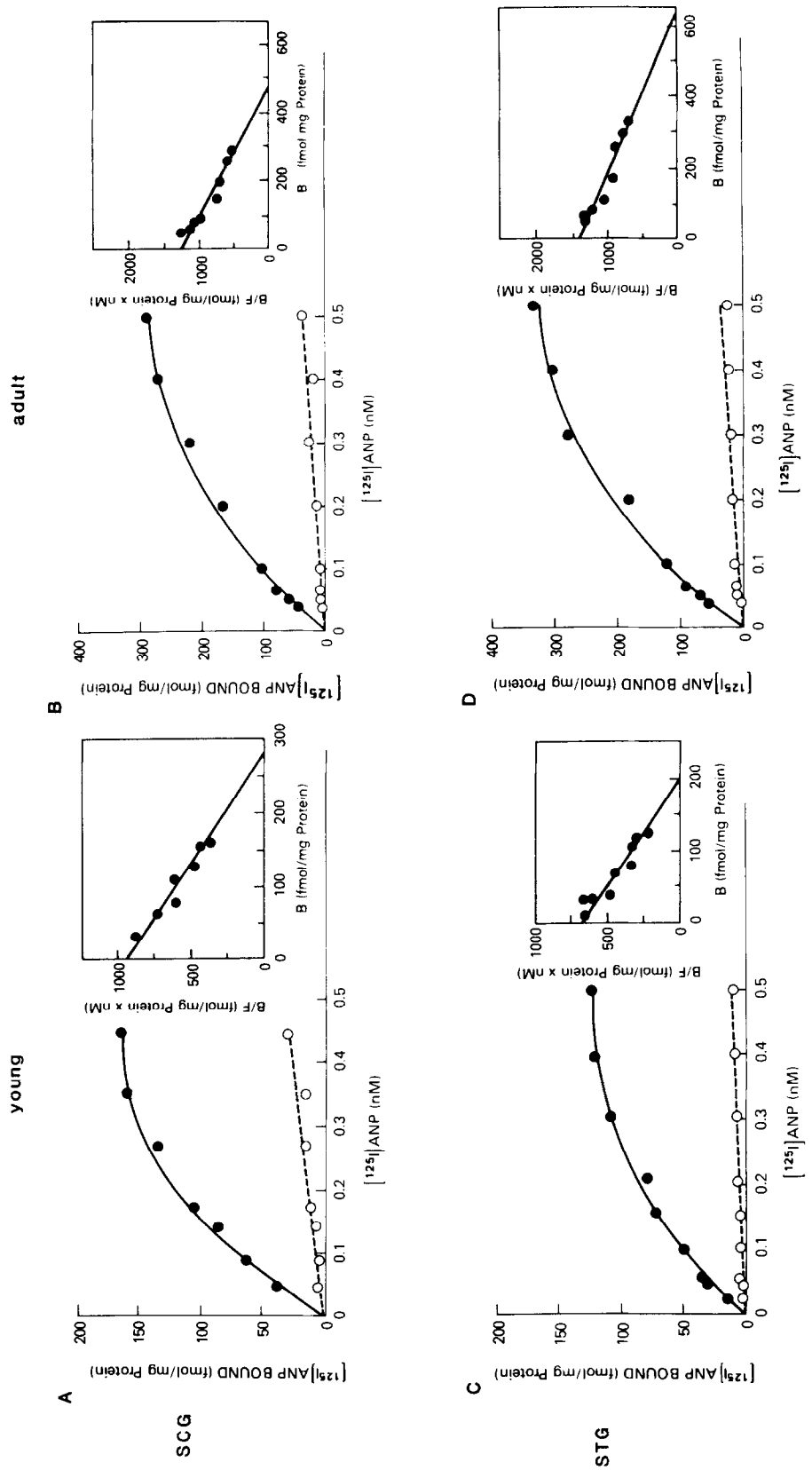
young
adult
SCG
STG
A
B
C
D
[125I]ANP BOUND (fmol/mg Protein)
[125I]ANP (nM)
B/F (fmol/mg Protein x nM)
B (fmol/mg Protein)

Table 1. ^{125}I-ANP Binding Sites in Rat Sympathetic Ganglia

Age	Ganglia	Binding Capacity Bmax (fmol/mg protein)	Dissociation Constant Kd (nM)
4-wk	Superior cervical	388 ± 30	0.33 ± 0.03
	Stellate	152 ± 14	0.32 ± 0.04
14-wk	Superior cervical	507 ± 43*	0.38 ± 0.03
	Stellate	604 ± 38*	0.41 ± 0.04

Data represent mean ± S.E.M. from 5 WKY rats assayed individually by duplicates. * $p<0.05$ vs young animals.

fmol/mg protein) and could not be quantified reliably under our experimental conditions (Fig. 1C).

The basal cyclic GMP content was similar in the stellate ganglia from adult SHR and WKY rats (0.68 ± 0.10 and 0.75 ± 0.06 pmoles cyclic GMP/ganglion in SHR and WKY rats, respectively). In the stellate ganglia of adult SHR and WKY rats, addition of ANP stimulated cyclic GMP formation in a concentration-dependent manner (Fig. 3). Notably, there was no significant difference in the magnitude of response between SHR and WKY rats at any concentration of ANP.

DISCUSSION

Our results are the first demonstration of the presence of specific ANP binding sites in the rat sympathetic ganglia. These findings and the ability of ANP to increase cyclic GMP content in stellate ganglia, as described in other target organs (4-7), suggest the existence of physiologically active ANP receptors in rat sympathetic ganglia.

Recently, ANP-like immunoreactivity was found in sympathetic and parasympathetic rat ganglia (12-14). Previous studies have reported that ANP-like immunoreactivity localizes in small intensely fluorescent (SIF) cells or in preganglionic terminals, but does not localize in principal ganglion cells (14). Our results, however, suggest that the majority of the ANP binding sites are associated with principal ganglion cells. ANP receptors in sympathetic ganglia may be stimulated by either circulating ANP or intrinsic ANP, which are possibly synthesized in SIF cells or released from preganglionic terminals.

Figure 2. ^{125}I-ANP binding in sympathetic ganglia of WKY rats.
A and C: Saturation curves and Scatchard plots of ^{125}I-ANP binding to the superior cervical ganglia (SCG) of young (A) and adult (C) WKY rats.
B and E: Saturation curves and Scatchard plots of ^{125}I-ANP binding to the stellate ganglia (STG) of young (B) and adult (E) WKY rats.
Solid lines = specific binding; dashed lines = nonspecific binding.
Scatchard plots were analyzed with the LIGAND program (24). Each point represents the mean of duplicates from one typical animal from each group.

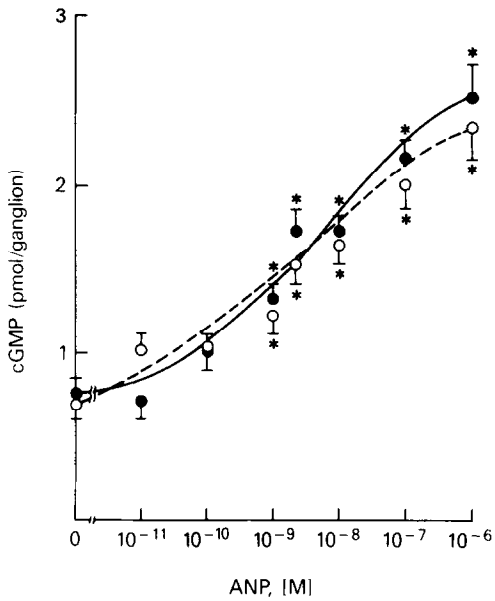

Figure 3. Effect of ANP on the cyclic GMP content in stellate ganglia of adult SHR (open symbols) and WKY rats (closed symbols). Each point represents mean ± S.E.M. of 4-6 individual ganglia measured in duplicate.
* $p<0.05$ vs basal levels.

In young and adult SHR, both superior cervical and stellate ganglia had few ANP binding sites. Earlier reports have shown that compared with age-matched WKY rats, SHR had a very low number of ANP receptors in the subfornical organ and choroid plexus (19), but did not have low numbers of receptors in the adrenal zona glomerulosa (25). Alterations of ANP metabolism have also been reported in SHR. Adult SHR with established hypertension show increased plasma level of ANP (20-22). However, our data from young SHR indicate that low ANP receptor binding in ganglia is already present before the development of hypertension. Thus, in our study of SHR, we could not determine whether lower ANP binding sites in ganglia were of genetic origin or were related to a down-regulation process.

The EC_{50} for ANP-stimulated cyclic GMP formation in sympathetic ganglia (approx. 3.0 nM) was higher than the K_d for ^{125}I-ANP in stellate ganglia (Table 1). A comparable discrepancy between ANP receptor affinity and second messenger response has also been reported in other systems (8,9).

ANP-stimulated cyclic GMP formation in stellate ganglia from SHR did not differ from that obtained from age-matched WKY rats, which indicates a discrepancy between the number of ANP binding sites and the ANP-mediated biochemical response in SHR. Recently, the existence of multiple ANP receptors was demonstrated in different target organs and cultured cell systems (4-9,26). However, since these ANP receptors have an identical

affinity for the radiolabeled ANP, the presence of two sites could not be detected in binding isotherms. Furthermore, only a small portion of the total number of ANP binding sites have been linked to the activation of guanylate cyclase (4). A similar situation may explain the discrepancy between receptor number and guanylate cyclase activation in sympathetic ganglia of SHR. It could be postulated that the decreased number of ANP binding sites in SHR reflects a specific alteration of those receptors that are uncoupled to the activation of guanylate cyclase. We are currently performing experiments to test this hypothesis.

ANP binding sites increased with age in stellate ganglia of WKY rats. The possible significance of this developmental change is not yet known.

Our results indicate that either intrinsic or circulating ANP may affect the regulation of sympathetic tone through high affinity ANP receptors located in sympathetic ganglia. Since it was found that ANP decreases the carbachol-stimulated catecholamine synthesis in superior cervical ganglia (16) the lower number of ANP receptors in sympathetic ganglia of SHR could be related to sympathetic hyperactivity in this model (17,18) and to the pathophysiology of genetic hypertension.

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