Atrial Natriuretic Factor in Specific Brain Areas of Spontaneously Hypertensive Rats

UDO BAHNER, HELMUT GEIGER, MIKLÓS PALKOVITS, AND AUGUST HEIDLAND

SUMMARY Atrial natriuretic peptides (atrial natriuretic factor, ANF) are present in a great number of brain areas inside and outside of the blood-brain barrier. The pattern of distribution implies the involvement of ANF in different physiological functions, such as blood pressure regulation, electrolyte and fluid homeostasis, and modulation of the neuroendocrine system. To further investigate a possible involvement of central ANF in spontaneous hypertension, we measured levels of ANF in 18 selected, microdissected brain areas of prehypertensive (4-week-old) and hypertensive (12-week-old) spontaneously hypertensive rats (SHR) and their normotensive control, Wistar-Kyoto rats (WKY), by radioimmunoassay. ANF was significantly decreased in seven brain areas in SHR at both ages investigated; the most pronounced decreases were found in the subfornical organ, in the perifornical and periventricular hypothalamic nuclei, and in the medial preoptic nucleus. In addition, in young SHR ANF was significantly decreased in the organum vasculosum laminae terminalis and increased in the median eminence. After the development of hypertension, a significant decrease of ANF could be detected in four more brain areas (bed nucleus of the stria terminalis, paraventricular and arcuate nuclei, dorsal raphe nucleus) of SHR, as compared with normotensive controls, and the increase in the median eminence was no longer detectable. These results suggest a role for ANF in genetic hypertension and the specific importance of certain brain regions. (Hypertension 12: 519–524, 1988)

KEY WORDS • atrial natriuretic factor • brain • hypertension • spontaneously hypertensive rats

Atrial natriuretic peptides (atrial natriuretic factor, ANF) were first described in the mammalian cardiac atrium, where they are released into the general circulation to take part in the regulation of fluid homeostasis and blood pressure by acting on the kidneys, the adrenals, and the blood vessels. In addition to the peripheral actions of ANF, certain effects of circulating ANF may be centrally mediated, since binding sites for ANF have been localized in specific brain areas that lack a blood-brain barrier. Furthermore, several lines of evidence indicate the existence of brain-born ANF. Immunohistochemical studies have demonstrated ANF-like material in neuronal perikarya, fibers, and terminals throughout the central nervous system, as well as a wide distribution of their receptors in brain areas. Messenger RNA coding for ANF peptides has been isolated from the hypothalamus of rats, and its sequence is identical to that found in the atria of the heart. However, brain ANF is stored and released as the 24 and 25 amino acid form, in contrast to plasma, where the 28 amino acid form predominates, and to the atria, where the larger prohormone is the stored form.

There is increasing evidence that ANF may be involved in the central regulation of blood pressure. For example, in certain conditions, ANF has hypotensive effects in normal rats and antihypertensive actions in experimentally hypertensive rats (SHR). ANF is also thought to be involved in the pathomechanism of spontaneous hypertension in the rat. Altered metabolism, release, and function of ANF has been found in the SHR. Moreover, the number of ANF binding sites was decreased in the subfornical organ and other brain areas of SHR, as compared with that in normotensive controls.

In the present study, ANF levels in 18 selected and microdissected brain areas of young and adult SHR and age-matched normotensive Wistar-Kyoto rats (WKY) were determined by radioimmunoassay. The brain areas represent all those cell groups of the central nervous system that are directly or indi-
Materials and Methods

Animals

Four and 12-week-old male SHR and WKY (24 per group) of the same strain were housed at 23 °C with illumination from 0600 to 1800 and given Altromin standard food (Lage, FRG) and tap water ad libitum. Arterial blood pressure was measured in the conscious animals during their development using the tail-cuff plethysmographic technique.

Tissue Dissection and Extraction

The animals were decapitated between 0800 and 1000, and brains were quickly removed and frozen on dry ice. Serial coronal sections of 300-μm thickness were cut in a cryostat at −10 °C. Brain regions were removed by the micropunch technique. Identical brain samples of four animals were pooled (i.e., six assay sets per experimental group were formed). The samples were collected in 1.5-ml conical Eppendorf tubes containing 60 μl of 0.1 N HCl and put in a boiling water bath for 10 minutes. After the addition of 100 μl of radioimmunoassay buffer (0.1% bovine + 0.1% Triton X-100, pH 7.4), the samples were homogenized and 20 μl aliquots of the homogenates were removed for protein determination according to the method of Lowry et al. The remaining sample was centrifuged at 2000 g for 15 minutes at 4 °C, and 100 μl of the supernatant fluid was used for ANF determination by radioimmunoassay.

Measurement of ANF

A commercial radioimmunoassay kit was used (No. 8798, Peninsula Laboratories, Belmont, CA, USA). The antiserum fully recognizes the following peptides: human ANF-(99-126), rat ANF-(99-126), and rat ANF-(102-126). The cross-reactivity with rat ANF-(101-126) is 90%; with rat ANF-(103-126), 50%; with rat ANF-(103-125), 27%; with rat ANF-(102-125), 10%; and with rat ANF-(103-123), 3%. The ANF antiserum does not cross-react with somatostatin, oxytocin, and arginine8-vasopressin. The sensitivity of the radioimmunoassay (i.e., the amount of peptide that displaced 50% of the label) was 23 pg/tube. The intra-assay and interassay coefficients of variation were 10 and 14%, respectively.

FIGURE 1. Coronal sections of the rat brain (drawings and coordinates according to Palkovits9). Rostrocaudal coordinates: A) 300 μm rostral to the bregma level; B) bregma (0) level; C) 900 μm, D) 1800 μm, E) 2700 μm, F) 3300 μm, G) 9600 μm, and H) 13,200 μm caudal to the bregma. Microdissected brain areas: 1) organum vasculosum laminae terminalis; 2) bed nucleus of the stria terminalis; 3) periventricular (median) preoptic nucleus; 4) medial preoptic nucleus; 5) subfornical organ; 6) cerebral (frontal) cortex; 7) periventricular hypothalamic nucleus; 8) supraoptic nucleus; 9) medial amygdaloid nucleus; 10) paraventricular nucleus; 11) arcuate nucleus; 12) median eminence; 13) perifornical nucleus; 14) medial forebrain bundle (MFB; lateral hypothalamus); 15) locus ceruleus; 16) tegmentum pontis (dorsal and dorsolateral tegmental nuclei); 17) nucleus tractus solitarii. (The dorsal raphe nucleus is not shown.) A = anterior commissure; F = fornix; IC = internal capsule; OC = optic chiasma; OT = optic tract; S = superior cerebellar peduncle; T = tractus solitarius; IV = fourth ventricle.
Statistical Analysis
For statistical evaluation of the data, the Student’s t test was applied. A p value below 0.05 was considered statistically significant. Values are given as means ± SEM.

Results

Systolic Blood Pressure
At 4 weeks of age there was no difference in mean systolic blood pressure between SHR and WKY (95 ± 4 vs 93 ± 3 mm Hg). At 12 weeks of age, blood pressure was significantly elevated in SHR (170 ± 6 vs 101 ± 3 mm Hg; p < 0.001, n = 10).

ANF Concentration
In normotensive animals, high concentrations of ANF were measured in certain preoptic (medial, periventricular) and hypothalamic (arcuate, paraventricular) nuclei as well as in the median eminence. Average concentrations were found in all other regions measured except the cerebral cortex, where the concentration was relatively low (Table 1). These data are in good agreement with those of Zamir et al. 32 ANF concentration was on average 1.5-fold higher in the brain areas of adult, as compared with young, animals. In SHR, lower ANF concentrations were measured in nearly all brain nuclei; the only exception was the significant ANF increase noted in the median eminence of 4-week-old SHR. In animals of both ages, ANF was significantly decreased in the subfornical organ, the periforimal and periventricular hypothalamic nuclei, the medial preoptic nucleus, the tegmentum pontis, the locus ceruleus, and the cerebral cortex of SHR, as compared with WKY. The decrease of ANF in the organum vasculosum laminae terminalis was statistically significant only in young SHR, although it was also apparent in adult SHR. Furthermore, in adult SHR a significant decrease in the ANF concentration was found in the bed nucleus of the stria terminalis, in the paraventricular and arcuate nuclei, as well as in the dorsal raphe nucleus, whereas the increase in ANF in the median eminence, as found in young SHR, was no longer detectable. Surprisingly, no changes were found in the periventricular (median) preoptic nucleus or in the nucleus of tractus solitarii (see Table 1).

Discussion
The factors responsible for the elevation of arterial blood pressure in SHR have not yet been identified, but ANF may contribute to this form of hypertension. Indeed, a variety of differences in the ANF system have been found between SHR and WKY. Although there are some opposite findings, most authors have reported unchanged cardiac and plasma ANF levels in young (prehypertensive) SHR, but significantly decreased ANF concentrations in atria and ventricles with a simultaneously increased plasma ANF level in adult (hypertensive) SHR. 14, 17–27 Furthermore, differences in the renal and blood pressure responses to ANF have been detected. 16, 17, 27 In certain brain areas the number of ANF binding sites was decreased in SHR compared with WKY.

Table 1. Concentrations of ANF in Selected Brain Regions of 4- and 12-Week-Old SHR and Normotensive Control WKY

<table>
<thead>
<tr>
<th>Brain area</th>
<th>WKY (pg/mg protein)</th>
<th>SHR (pg/mg protein)</th>
</tr>
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<tbody>
<tr>
<td>Cerebral cortex</td>
<td>8.5±1.1</td>
<td>5.6±0.7*</td>
</tr>
<tr>
<td>Organum vasculosum laminae terminalis</td>
<td>20.0±1.7</td>
<td>14.6±0.8*</td>
</tr>
<tr>
<td>Subfornical organ</td>
<td>28.8±3.7</td>
<td>15.2±1.2†</td>
</tr>
<tr>
<td>Periventricular preoptic nucleus</td>
<td>131.6±10.9</td>
<td>118.9±9.6</td>
</tr>
<tr>
<td>Medial preoptic nucleus</td>
<td>139.4±5.7</td>
<td>107.3±3.8†</td>
</tr>
<tr>
<td>Bed nucleus of the stria terminalis</td>
<td>63.9±5.8</td>
<td>53.5±4.2</td>
</tr>
<tr>
<td>Periventricular hypothalamic nucleus</td>
<td>133.9±8.7</td>
<td>98.5±9.4†</td>
</tr>
<tr>
<td>Supraoptic nucleus</td>
<td>12.0±1.3</td>
<td>15.8±1.5</td>
</tr>
<tr>
<td>Paraventricular nucleus</td>
<td>100.0±2.2</td>
<td>76.6±14.4</td>
</tr>
<tr>
<td>Arcuate nucleus</td>
<td>144.7±13.9</td>
<td>158.7±11.7</td>
</tr>
<tr>
<td>Median eminence</td>
<td>126.2±12.3</td>
<td>179.4±13.3*</td>
</tr>
<tr>
<td>Periforimal nucleus</td>
<td>105.6±5.5</td>
<td>83.5±4.2†</td>
</tr>
<tr>
<td>Medial forebrain bundle</td>
<td>52.2±4.0</td>
<td>58.8±4.6</td>
</tr>
<tr>
<td>Medial amygdaloid nucleus</td>
<td>26.2±5.6</td>
<td>19.8±1.2</td>
</tr>
<tr>
<td>Dorsal raphe nucleus</td>
<td>106.8±7.9</td>
<td>111.0±10.2</td>
</tr>
<tr>
<td>Tegmentum pontis</td>
<td>50.2±5.1</td>
<td>35.7±1.0†</td>
</tr>
<tr>
<td>Locus ceruleus</td>
<td>44.8±4.5</td>
<td>31.0±4.3*</td>
</tr>
<tr>
<td>Nucleus tractus solitarii</td>
<td>21.2±1.4</td>
<td>22.7±1.5</td>
</tr>
</tbody>
</table>

Values are means±SEM of six/group.
*p < 0.05, †p < 0.01, ‡p < 0.001, compared with respective values for WKY.
before and after the development of hypertension.\textsuperscript{3, 11} The marked decrease in the steady state levels of ANF in selected brain areas of young and adult SHR, as found in the present study, indicates that central ANF may be involved in the development as well as in the maintenance of genetic hypertension in rats. This hypothesis is strengthened by the finding that the pattern of alterations in the central ANF system is quite different in renal and doxycorticosterone acetate-salt hypertension (unpublished observations, 1988).

It has been suggested that ANF and angiotensin II may act as physiological antagonists in the brain, as in the periphery,\textsuperscript{3, 11} and that the actions of ANF within the brain are well coordinated with its peripheral actions to regulate blood pressure and electrolyte and fluid homeostasis.\textsuperscript{13} This idea is supported by recent findings that centrally administered ANF inhibits angiotensin II-induced water drinking.\textsuperscript{33, 34} Salt appetite,\textsuperscript{35} vasopressin secretion,\textsuperscript{36, 37} and corticotropic (ACTH) secretion\textsuperscript{46}; antagonizes the centrally, but not intravenously, angiotensin II–induced increase in blood pressure\textsuperscript{39}; and potentiates the hypotensive action of the angiotensin blocker saralasin in SHR.\textsuperscript{40}

In the subfornical organ of the brain, a much lower number of ANF receptors was found in SHR than in normotensive controls,\textsuperscript{11} whereas the number of angiotensin II binding sites was higher in SHR, as compared with WKY.\textsuperscript{3} The subfornical organ contains one of the highest concentrations of ANF receptors\textsuperscript{24} as well as angiotensin II receptors\textsuperscript{41} in the brain and has long been implicated in the central control of body fluid homeostasis and blood pressure.\textsuperscript{42, 43} It has been demonstrated to be one of the main target sites for circulating angiotensin II\textsuperscript{44, 45} and could mediate the central actions of circulating ANF. Nerve terminals, but not cell bodies, immunostained for ANF have been reported in the subfornical organ. In the present study, markedly reduced ANF levels (52 and 45%, respectively, of control) were measured in this brain area of young and adult SHR, as compared with normotensive rats. The decreased ANF concentration may be the result of a diminished uptake of circulating ANF or a decreased ANF input from connected brain areas (or both). Conversely, angiotensin II–like immunopositive nerve cells are concentrated in this organ,\textsuperscript{46} and dense neuronal inputs from the subfornical organ are confined to several preoptic and hypothalamic nuclei.\textsuperscript{47, 48} Many of these are rich in ANF-containing cell bodies and terminals,\textsuperscript{7-9} and their ANF levels are diminished substantially in young and adult SHR, as compared with normotensive rats (see Table 1).

Of special interest are projections from the subfornical organ to cells in the paraventricular nucleus (probably ANF-containing cells, which are present there in high number,\textsuperscript{7-9}) which in turn sends axons to preganglionic cells in the spinal cord and the lower brainstem.\textsuperscript{48, 49} Furthermore, ANF-containing cells in the paraventricular nucleus are the major, if not the only, source of ANF-containing nerve fibers and terminals in the median eminence, which constitutes the final common pathway for signals from the brain to the pituitary gland.\textsuperscript{50} A decreased ANF level is also remarkable in the perifornical nucleus (cells in the lateral hypothalamus around the fornix) where many ANF-containing cells are present.\textsuperscript{9}

About one third of the total perifornical cell population consists of large cells sensitive to changes in body fluid homeostasis.\textsuperscript{51} Neurons in this area project to preganglionic baroreceptor efferents in the intermediolateral cell column of the spinal cord.\textsuperscript{48, 49}

The subfornical organ is also closely connected with the region of the anteroven tral third ventricle,\textsuperscript{52} which includes the organum vasculosum laminae terminalis, the periventricular (median) preoptic nucleus and a part of the medial preoptic nucleus. This area contains the largest accumulation of ANF-containing cells in the brain.\textsuperscript{8, 9} Large numbers of angiotensin II–containing nerve terminals,\textsuperscript{46} and angiotensin II receptors.\textsuperscript{23} It has been described as a critical brain area for the development and maintenance of experimental hypertensions and for the regulation of fluid and electrolyte homeostasis.\textsuperscript{52, 53} However, lesioning of this region blocks only the development of various types of angiotensin II–vasopressin–mediated renal hypertension (Goldblatt,\textsuperscript{54, 55} Dahl,\textsuperscript{56} or doxycorticosterone acetate–salt\textsuperscript{55} hypertension), but destruction of this region does not affect the blood pressure of SHR.\textsuperscript{57} Thus, the region of the anteroven tral third ventricle may not be directly involved in the pathomechanism of this form of genetic hypertension, and the alterations of ANF in this area may be secondary. Since the ANF content of the periventricular nuclei was reduced after adrenalectomy and since intracerebroventricular administration of ANF can inhibit angiotensin II–induced ACTH release,\textsuperscript{46} the decrease in ANF in these nuclei of SHR may be related to the altered ACTH secretion in SHR.\textsuperscript{58}

The nucleus tractus solitarii in the medulla oblongata is the primary center of baroreception. Lesioning\textsuperscript{59} or transecting peripheral inputs\textsuperscript{60} of this nucleus results in a fulminating hypertension. This type of hypertension is produced by an increase in sympathoadrenal activity and an increase in vasopressin release. Blocking both systems, but not one of them, prevents the hypertension caused by lesioning of the nucleus tractus solitarii.\textsuperscript{61} Although ANF is present in cell bodies and nerve terminals in the nucleus tractus solitarii,\textsuperscript{7-9} unchanged ANF levels in SHR are inconsistent with a fundamental neurotransmitter role of this peptide in relay mechanisms at the level of the primary baroreceptor center. However, altered ANF concentrations in connected brain areas (locus ceruleus, tegmentum pontis) imply at least an indirect influence of central ANF on the baroreceptor reflex arc.
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