Ganglionic Immunoreactive Atrial Natriuretic Factor in Rat Experimental Hypertension

OTTO KUCHEL, WALDEMAR DEBINSKI, NGUYEN T. BUU, MARC CANTIN, AND JACQUES GENEST

SUMMARY Because previous data have suggested a dependence of ganglionic atrial natriuretic factor (ANF) content on preganglionic cholinergic input, we investigated the possibility that the increased neural activity observed in spontaneously hypertensive rats (SHR) may be reflected by ganglionic immunoreactive ANF levels. Four-week-old normotensive SHR had celiac ganglionic immunoreactive ANF values comparable to those of Wistar-Kyoto rats (WKY). When they became hypertensive, however, at 12 weeks of age, the SHR manifested higher immunoreactive ANF levels in celiac ganglia than the WKY group (25.3 ± 2.6 vs 14.5 ± 1.7 pg/ganglion; p<0.01), but there were no differences in levels in the superior cervical and nodose ganglia. The values in celiac ganglia were quadrupled on the average in hypertensive Dahl salt-sensitive rats under the influence of an 8% salt intake for 5 weeks, but no differences was noted in any of these ganglia between this group and their salt-resistant partners. The celiac and superior cervical ganglionic immunoreactive ANF content in normotensive Sprague-Dawley rats was higher with high salt than with normal salt intake. Hypertensive rats treated with deoxycorticosterone acetate (DOCA)-salt and sham-treated controls showed immunoreactive ANF concentrations in celiac ganglia similar to those detected in Dahl rats but, again, no differences were found between groups. Thus, hypertensive SHR, compared to WKY, have higher celiac ganglionic immunoreactive ANF levels, unlike Dahl salt-sensitive and DOCA-salt animals relative to their respective controls. This increase is unique to SHR (although all three models have elevated plasma immunoreactive ANF when they are hypertensive) and to the celiac ganglia. Hypertensive SHR, celiac ganglia (Th8-L2 roots) carry the most evidently enhanced neural splanchnic traffic, which may be reflected by increased immunoreactive ANF concentrations.

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KEY WORDS • atrial natriuretic factor • ganglia • spontaneously hypertensive rats • Dahl rats • salt

THE recent finding of immunoreactive atrial natriuretic factor (irANF) activity in neural structures outside the brain, such as the parasympathetic and sympathetic ganglia, and baroreceptor area of the aortic arch, suggests a previously unsuspected neuromodulating role of this peptide along these neural pathways. The sympathetic ganglia occupy a special position in the efferent blood pressure (BP)-regulating pathway, contrary to the parasympathetic nodose ganglia (NG), which are in the afferent baroreceptor pathway. A functional link between ganglionic ANF and cholinergic neurotransmission can be suspected, since surgical decentralization of the sympathetic ganglia produces a parallel decrease of ganglionic ANF and acetylcholine. Glycogenic ANF thus appears to be independent of circulating ANF and, hypothetically, as a result of axonal transport of ANF from spinal centers, may reflect the state of preganglionic cholinergic neurotransmission through the ganglia.

It has been shown that the elevated BP in spontaneously hypertensive rats (SHR) parallels a generalized increase in neural activity in the preganglionic (splanchnic and cervical sympathetic) and postganglionic (splanchnic and renal) nerves. A comparison of irANF content in several ganglia of normotensive and hypertensive SHR with that in Wistar-Kyoto rats.
(WKY) therefore appeared to be warranted. The same measurements in Dahl salt-sensitive (S) rats, in which neural abnormalities of a permissive nature seem to be induced by a high salt intake, allowed us, with controls for salt consumption in Sprague-Dawley rats, to evaluate what changes, if any, in ganglionic ANF are unique to SHR. Finally, rats with deoxycorticosterone acetate (DOCA)–salt hypertension served as controls for a possible nonspecific effect of hypertension on ganglionic ANF. All three hypertension models previously found to have elevated plasma irANF, which also increases in response to high salt intake, permitted us to ascertain whether changes in ganglionic irANF follow those observed in plasma.

Materials and Methods

Male SHR and WKY rats (Charles River Canada Inc., St.-Constant, Quebec), were housed in groups of five to six animals per cage. They ate ordinary rat chow and water ad libitum and had a 12-hour dark-light cycle until the age of 12 weeks. Dahl rats of the salt-resistant (R) and S strains (Brookhaven National Laboratory, Upton, NY, USA), delivered to our animal facilities a few days before weaning, were kept ad libitum on ordinary chow and water for 10 days. After this acclimatization period, the rats were fed a diet containing 8% NaCl (Ralston Purina, Indiana City, IN, USA) for 5 weeks. In addition, water was provided ad libitum. Rats of Sprague-Dawley origin of similar age were fed either ordinary chow and water, or an 8% NaCl diet for 5 weeks. DOCA-salt hypertension was produced in uninephrectomized Sprague-Dawley rats by administering a 50-mg DOCA pellet and providing 1% saline solution for drinking ad libitum for 3 weeks. The control group was sham-treated with the same salt regimen for 3 weeks.

Systolic BP was measured by the tail-cuff method in the SHR and Dahl rat groups at 4 weeks of age and before the end of the experiment: at age 12 weeks in SHR, at 10 weeks in Dahl rats, and at the height of hypertension in the DOCA-salt–treated group. Body weight was determined in all cases.

Preparation of Samples

The celiac ganglia (CG), superior cervical ganglia (SCG), and NG were removed after decapitation. They were homogenized individually or two per tube in an ultrasound homogenizer in 0.1 M acetic acid containing endogenous protease inhibitors, as described elsewhere. The homogenates were centrifuged at 10,000 × g for 20 minutes, and the supernates were further diluted in acetic acid. They were processed in Sep-Pak C18 cartridges (Waters Associates, Milford, MA, USA) according to an established procedure. The eluates were evaporated in a Speed-Vac concentrator and dissolved in buffer (as recommended by Peninsula Laboratories Inc., Belmont, CA, USA) just before radioimmunoassay.

Radioimmunoassay

ANF iodination was performed according to the lactoperoxidase technique described by Murthy et al. When the reaction was completed, the reaction mixture was diluted in 4% acetic acid and purified in a Sep-Pak cartridge. The eluate was kept frozen and appropriately diluted in radioimmunoassay buffer. The assay was performed in polystyrene tubes. The antibody (rabbit anti-a-natriuretic peptide serum) and synthetic rat ANF (99–126) employed for preparation of the standard were purchased from Peninsula Laboratories. The standard curve ranged from 0.75 to 390 pg/tube, and 100 µl of the standard or sample dissolved in radioimmunoassay buffer were mixed with 100 µl of rabbit antiserum before overnight incubation at 4°C. After 24 hours, 100 µl of 125I-ANF was added to the tubes (6000–10,000 cpm) and kept for another day at 4°C. For the separation of free and bound labeled ANF, 100 µl of goat antirabbit immunoglobulin G serum (Peninsula Laboratories) and 100 µl of normal rabbit serum, pretitered accordingly, were added before incubation for 2 hours at ambient temperature. At the end of the incubation period, 1.0 ml of 6% polyethylene glycol was vortexed with the samples, and the set of tubes was centrifuged simultaneously at 3000 g for 20 minutes at 4°C. The supernatants were then discarded and the pellets counted in a gamma counter.

The detection limit of this assay was 3 pg/tube. The binding of labeled ANF varied from 20 to 35%, with very low nonspecific binding. The IC50 was 20 pg/tube, and the interassay and intra-assay variations were 8.6% and 16.9%, respectively. Ganglionic protein content was determined by the method of Lowry et al. The data were statistically analyzed by Student’s t test.

Results

The data summarized in Table 1 show that the SHR and two of the other hypertensive models had expected elevations of BP. The CG protein content increased with age, but no difference was evident between the normotensive and hypertensive partners in any of these three hypertensive paradigms at any age. The irANF concentrations in the CG, expressed in picograms per milligram protein, and the individual values, expressed in picograms per ganglion (Figure 1), revealed no difference between normotensive SHR and WKY. The hypertensive SHR, however, had an increase of irANF, which was significant under both forms of expression (see Table 1). The more evident difference of the values expressed as picograms per ganglion may have been due to the fact that the rise in irANF was partly underestimated but nevertheless significantly higher in SHR than in WKY, because of the age-related augmentation of CG protein content and weight of the animals. Thus irANF in SHR keeps pace with increased ganglionic protein, unlike in WKY, in which the values, corrected for protein, decrease. Dahl rats had elevated irANF levels in the CG between 4 and 10 weeks of age, with a threefold increase in the R strain and a fourfold increase in the S animals, but there were no differences between groups (see Table 1). Rats with DOCA-salt hypertension, studied at
Table 1. Biological and Immunoreactive ANF Data on Several Models of Hypertension in Rats

<table>
<thead>
<tr>
<th>Age of rats</th>
<th>No. of rats</th>
<th>Weight (g)</th>
<th>BP (mm Hg)</th>
<th>Protein content of CG (pg/ganglion)</th>
<th>irANF content of CG (pg/mg protein, pg/ganglion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>WKY</td>
<td>20</td>
<td>71±5</td>
<td>94±3</td>
<td>271±11</td>
<td>69.8±7.9, 11.6±1.1</td>
</tr>
<tr>
<td>SHR</td>
<td>19</td>
<td>68±2</td>
<td>110±2*</td>
<td>272±11</td>
<td>63.7±5.0, 13.1±1.6</td>
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<tr>
<td>12 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>17</td>
<td>284±4.4</td>
<td>110±3</td>
<td>337±19</td>
<td>44.7±6.1, 14.5±1.7</td>
</tr>
<tr>
<td>SHR</td>
<td>19</td>
<td>249±3</td>
<td>165±5*</td>
<td>395±26</td>
<td>62.3±5.2*, 25.3±2.6*</td>
</tr>
<tr>
<td>4 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR</td>
<td>7</td>
<td>97±2</td>
<td>76±2</td>
<td>278±11</td>
<td>43.2±4.0, 12.0±1.5</td>
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<tr>
<td>DS</td>
<td>9</td>
<td>112±3</td>
<td>85±5</td>
<td>267±17</td>
<td>42.4±4.5, 10.8±0.9</td>
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<tr>
<td>10 weeks</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR</td>
<td>9</td>
<td>242±7</td>
<td>115±5</td>
<td>—</td>
<td>36.2±6.6, 36.2±8.9</td>
</tr>
<tr>
<td>DS</td>
<td>10</td>
<td>227±11</td>
<td>210±8*</td>
<td>—</td>
<td>41.5±8.9</td>
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<tr>
<td>12 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham-salt</td>
<td>9</td>
<td>252±6</td>
<td>100±3.5</td>
<td>402±31</td>
<td>80±10, 31.4±4.4</td>
</tr>
<tr>
<td>DOCA-salt</td>
<td>9</td>
<td>300±6</td>
<td>183±6*</td>
<td>511±39</td>
<td>74±12, 36.4±5.1</td>
</tr>
</tbody>
</table>

Values are means ± SEM. CG = celiac ganglia; irANF = immunoreactive ANF; DR = Dahl salt-resistant rats; DS = Dahl salt-sensitive rats.

*Different from controls of the same age (p<0.05).

At the end of the experiment, had increased ganglionic irANF values similar to those of Dahl rats but, again no differences were apparent between the normotensive and hypertensive groups.

The irANF content of the SCG did not present any difference between hypertensive SHR and WKY (20.3 ± 2.8 and 21.9 ± 3.7 pg/ganglion, respectively) and between Dahl S and R rats (33.3 ± 10.8 and 37.4 ± 8.8 pg/ganglion, respectively). The irANF content of the NG did not show any difference between SHR and WKY (2.6 ± 0.5 and 4.5 ± 1.3 pg/ganglion, respectively) or between Dahl S and R rats (2.1 ± 0.3 and 1.8 ± 0.4 pg/ganglion, respectively), even after correction per milligram protein.

The control experiment with 8% salt-feeding of rats of Sprague-Dawley origin for 5 weeks demonstrated that rats on high salt intake had significantly higher SCG and CG irANF concentrations than those on ordinary chow diet corresponding to the ones previously reported2 (Figure 2). That this rise in irANF was due to high salt consumption and not to aging was established by an additional study on WKY of comparable age and weight that ate a normal diet. These animals showed no change in irANF content in the CG.

Discussion

The data indicate that at age 4 weeks, when the groups are normotensive (except for a borderline higher BP in SHR), irANF levels in the CG are comparable in SHR and WKY as well as in Dahl S and R animals. Upon becoming hypertensive at 12 weeks, the SHR, compared to WKY, have elevated irANF values in the CG but not in the SCG or NG. The irANF concentration in superior cervical and celiac ganglia in normotensive Sprague-Dawley rats is higher after high salt than normal salt intake. An elevation also is observed in Dahl rats but without significant differences between the S and R groups. The DOCA- and sham-treated, salt-fed animals also have increased ganglionic irANF, but, again, there is no difference between

Figure 1. Celiac ganglionic immunoreactive ANF in WKY and SHR at 4 and 12 weeks of age.

Figure 2. Effect of feeding 8% NaCl to Sprague-Dawley rats for 5 weeks on immunoreactive ANF levels in the superior cervical and celiac ganglia. The data are compared to celiac ganglionic levels of immunoreactive ANF in WKY of a similar age and weight kept on normal salt intake.
DOCA-treated hypertensive and sham-treated control rats, indicating that the augmented CG level of irANF in hypertensive SHR is an abnormality that is unique to this model and to the CG. It does not occur in Dahl hypertensive rats and, as the DOCA-salt paradigm suggests, it is not a nonspecific response to elevated BP. An increased plasma irANF concentration was noted earlier in hypertensive Dahl S and DOCA-salt rats. The present observation that the CG irANF levels were indistinguishable in these two models from those of their normotensive partners and the decreased ganglionic level after ganglionic decentralization speaks against any relationship between circulating and ganglionic irANF.

The generalized rise in ganglionic irANF in normotensive rats fed a high salt diet and the more selectively increased CG content in hypertensive SHR seem to correlate best with enhanced sympathetic outflow; this may relate to changes in norepinephrine release, metabolism or its action after high salt intake, and the appearance of heightened neural activity in SHR when they become hypertensive. Although coincidence of the two phenomena does not link their causality, some data support such a possibility.

The most recent description of ANF receptors in sympathetic ganglia and of the decrease of ANF binding sites in SHR is compatible with a possible biological role of increased ganglionic irANF. The increased neural activity affecting hypertensive SHR, probably due to a central resetting of sympathetic centers, is predominant in the splanchnic region, particularly in the renal nerves, and thus in the region of the CG corresponding to the TH8-L2 roots. It is not clear why only the CG and not the SCG have elevated irANF levels in hypertensive SHR, although neural activity is also augmented in the cervical sympathetic nerves. In view of the possible spinal origin of ganglionic irANF, an explanation may be that the highest spinal content is found in the lumbar-sacroccral spinal cord. Alternatively, ganglionic irANF being dependent on preganglionic axonal transport may reflect better the neural influx into those ganglia having long preganglionic axons (such as CG) than others with short preganglionic axons (such as SCG).

The present study is only a first step in the search for a possible role of neural ANF in some abnormalities of neural activity in SHR and, more generally, its possible participation in adjustments to high salt intake. We recently found (Debinski et al., unpublished data, 1987) increased irANF content in the spinal cord of SHR and confirmed the finding of increased hypothalamic irANF in SHR. This may indicate that the increased CG irANF content rather reflects in SHR its changes in the spinal cord and possibly also brainstem centers, which are most important for BP regulation.

Neural ANF apparently behaves differently from "hormonal" plasmatic ANF in several models of hypertension. Thus ANF seems to be one of the recently recognized neuromodulatory peptides possibly involved in neurotransmission. As the preliminary observation of increased ganglionic irANF in response to high salt intake suggests, such involvement may be of homeostatic relevance as a factor modulating the adjustment of sympathetic tone to changes in salt balance. This role of ANF may be complementary to its many interactions with the autonomic nervous system.

References

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