DEFICIENT DOPAMINE METABOLISM IN THE CELIAC GANGLION OF SPONTANEOUSLY HYPERTENSIVE RATS

BENNO E. LÜTOLD, FAROUK KAROUM,* AND NORTON H. NEFF

SUMMARY It has been established by others that dopamine (DA), in the small intensely fluorescent (SIF) cells of a sympathetic ganglion, is released on to sympathetic neurons as a result of preganglionic cholinergic stimulation. DA is known to suppress transmission in sympathetic ganglia. The concentration of 3,4-dihydroxyphenylacetic acid (DOPAC) within the ganglion is a relative measure of DA release and metabolism. We assayed DA, norepinephrine (NE), and DOPAC in the celiac ganglia of spontaneously hypertensive rats (SHR), during the development of hypertension, and in control Wistar-Kyoto rats (WKR). Blood pressure begins to rise in SHR after 4 to 6 weeks of age and becomes stable after about 10 weeks. In WKR, DOPAC concentrations are highest in the ganglion at 4 weeks and decline by about 45% at 6 weeks to levels at which they remain for 20 weeks. In contrast, DOPAC concentrations in SHR are lowest in the ganglion at 4 weeks, increase in parallel to blood pressure, and at 20 weeks are comparable to the concentration in WKR. Sectioning the greater splanchnic nerves does not induce major changes in the content of NE and DA in the celiac ganglion but strikingly reduces DOPAC concentrations in both SHR and WKR, supporting the notion that the conversion of DA to DOPAC is modulated by preganglionic cholinergic neuronal activity. Sectioning the nerves in SHR delays the development of hypertension but does not significantly alter blood pressure in WKR. Our studies suggest the hypothesis that young SHR are not able to modulate the sensitivity of sympathetic neurons to incoming cholinergic activity to the celiac ganglion because of a deficiency of SIF cell DA metabolism, and the consequences of this may play a critical role in the development of hypertension. Circ Res 44: 467–471, 1979

PREGANGLIONIC cholinergic fibers synapse on postganglionic sympathetic neurons and on ganglionic interneurons, the small intensely fluorescent (SIF) cells (Williams, 1967). The SIF cells, in turn, synapse on the postganglionic sympathetic neurons (Williams et al., 1976; Chiba et al., 1977). In most mammals, the neurotransmitter of the interneurons apparently is dopamine (DA) (Bjorklund et al., 1968, 1970). Catecholamines have long been known to suppress synaptic transmission in sympathetic ganglia (Marrazzi, 1939; Willems, 1973; Costa et al., 1961). Suppression of transmission by DA appears to be associated with a hyperpolarization of postganglionic sympathetic neurons, the slow inhibitory postsynaptic potential (S-IPSP) (Libet and Tosaka, 1970). DA was also found to facilitate a slow muscarinic depolarizing response, the slow excitatory postsynaptic potential (S-EPSP) (Libet and Tosaka, 1970). Thus, DA released from sympathetic ganglia SIF cells might modulate transmission of central signals to peripheral adrenergic end organs by suppressing ganglionic transmission.

Celiac ganglia of young spontaneously hypertensive rats (SHR), before the onset of hypertension,
show elevated activity of choline acetyltransferase, and tyrosine hydroxylase (Nakamura and Nakamura, 1977). Moreover, the activity of the splanchnic nerve, which is the major cholinergic input to the ganglia, is greater than normal in SHR (Judy et al., 1976; Okamoto et al., 1967). Our objective was to compare catecholamine metabolism in the celiac ganglion of SHR and Wistar-Kyoto rats (WKR) to determine whether abnormal metabolism in young rats could contribute to the development of hypertension. Catecholine metabolism was assessed from the concentration of DA, norepinephrine (NE), and 3,4-dihydroxyphenylacetic acid (DOPAC) before and after the greater splanchnic nerves were sectioned. Catecholamines and metabolites were analyzed by mass fragmentography (Karoum et al., 1977).

Methods

Three-week-old SHR and WKR were obtained from the National Institutes of Health colony and allowed to mature in our animals facility. Celiac ganglia were removed after decapitation and immediately frozen. The greater splanchnic nerves were sectioned just below the diaphragm to decentralize the celiac ganglia. In one series of experiments, the adrenal glands were bilaterally demedullated by making a small incision in the gland and scooping out the darker central core with a small spatula. Surgery was performed under anesthesia with ketamine hydrochloride, 25 mg/kg, iv, with supplemental injections as required. The details of the time course of decentralization, measurement of blood pressure, and biochemical determinations are given in the Results.

Ganglia were prepared and assayed mass-fragmentographically as previously described (Karoum et al., 1977). In brief, a ganglion was homogenized with 0.3 ml 1 N HCl, centrifuged at 20,000 g for 10 minutes, and the clear supernatant fluid was transferred and stored at −14 °C until analyzed. A 0.1-ml portion of the supernatant fluid was analyzed for NE and DA and another 0.1-ml portion for DOPAC. Deuterated isomers of NE (NE, α-d1, α-d2) DA (DA, α-d1, α-d2), and DOPAC (DOPAC, d6) were added to the appropriate samples and the whole aqueous mixture was evaporated to dryness under N2. NE and DA were converted to their pentafluoropropionate and DOPAC to its methylester/pentafluoropropionate derivatives.

For combined gas chromatography-mass spectrometry, a model 4000 Finnigan gas chromatograph-quadrupole mass spectrometer was used. The ions selected for mass fragmentography were [atomic mass unit (AMU) of the authentic compound, AMU of deuterated isomer]: NE (590,592), DA (428,431), DOPAC (387,392). The lower limit of detection of the method employed corresponded to about 0.1 pmol/ganglion.

We assayed protein by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

Blood pressure was measured with a tail sphygmomanometer (model PE-300 E and M Inst. G. Houston, Texas).

Student's t-test was used to evaluate the significance of the differences between groups of rats.

Results

Relationship between Blood Pressure and Catecholamine Metabolism in the Celiac Ganglion of Intact WKR and SHR

At 4 weeks of age the blood pressures of WKR and SHR were similar (filled symbols, Fig. 1). After 6 weeks of age the blood pressure of SHR was significantly greater than that of WKR. By about 10 weeks the blood pressure of SHR reached a plateau and remained relatively stable until 20 weeks.

The concentration of DA in the celiac ganglion was essentially the same when age-matched WKR and SHR were compared (Table 1). In contrast, NE concentrations were higher in SHR than in WKR at 4 weeks, before the blood pressure rose. NE concentrations were still higher at 6 and at 10 weeks, but were similar at 20 weeks of age.

A major difference in the DOPAC concentration in the two groups (filled symbols, Fig. 2) was found at the age of 4 weeks. This corresponds to the time at which blood pressure appeared normal (filled symbols, Fig. 1). There was more than twice as much DOPAC present in the celiac ganglion of WKR than in the SHR. For WKR, DOPAC concentrations decreased from about 450 to 250 pmol/
**TABLE 1** Catecholamine Concentrations in Innervated and Decentralized Celiac Ganglia of WKR or SHR during Development

<table>
<thead>
<tr>
<th>Age (wks)</th>
<th>Rate per group</th>
<th>NE (pmol/mg protein)</th>
<th>DA (pmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>WKR</td>
<td>SHR</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>2574 ± 206</td>
<td>3075 ± 161*</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>1308 ± 115</td>
<td>1766 ± 128†</td>
</tr>
<tr>
<td>(6)</td>
<td>5</td>
<td>(1233 ± 96)</td>
<td>(1381 ± 101)</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>885 ± 78</td>
<td>1170 ± 132‡</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>1011 ± 99</td>
<td>1211 ± 92</td>
</tr>
<tr>
<td>(22)</td>
<td>5</td>
<td>(824 ± 49)</td>
<td>(776 ± 63)</td>
</tr>
</tbody>
</table>

Rats were decapitated and the ganglion removed and assayed by mass fragmentography. Values are presented as the mean ± SEM. Parentheses indicate that the celiac ganglion was decentralized 14 days before the rats were killed for analysis. Student’s t-test was used to compare values for age-matched SHR and WKR.

* P < 0.001; † P < 0.01; ‡ P < 0.05.

The onset of development of hypertension in SHR (triangles, Fig. 1).

DA concentrations in the celiac ganglia in SHR and WKR were not significantly different when the ganglia were decentralized at 4 weeks and rats studied at 6 weeks or when decentralized at 20 weeks and studied at 22 weeks (Table 1). In contrast, the concentration of NE was significantly higher in unoperated SHR than in WKR at 6 weeks. This difference in NE concentration was eliminated by decentralization. At 20 weeks of age there were no differences in the concentration of NE in the ganglia of WKR and SHR. Decentralization at 20 weeks of age resulted in lower concentrations of NE in the ganglia of both groups of rats when measured at 22 weeks of age. In spite of this, however, the concentration of NE in the two groups remained similar.

Decentralization of the celiac ganglion resulted in a significant reduction in DOPAC concentrations at all ages in both groups of rats (unfilled symbols, Fig. 2). However, there was still a significantly higher concentration of DOPAC in the ganglia of decentralized SHR when compared with those of decentralized WKR of all ages.

**Discussion**

A multiplicity of etiological factors probably is responsible for the development of hypertension in SHR. One of the possible sites for pathogenesis is the celiac ganglion (Nakamura and Nakamura, 1977; Judy et al., 1976). The ganglion is a site for modulating transmission of central information to the periphery and not just a simple link between pre- and postganglionic neurons. Within the ganglion, DA released from SIF cells apparently is responsible for an initial S-IPSP followed by a S-EPSP of the principal neurons to a direct muscarinic action of acetylcholine (Libet and Owman, 1974; Libet, 1977). The first response to DA is associated with suppression of ganglionic transmission, whereas the second response to DA may be associated with enhanced ganglionic transmission, although this remains to be demonstrated. Release
of DA from the SIF cells following preganglionic cholinergic nerve activity, therefore, could modulate effectively postganglionic sympathetic activity (Della Bella and Benelli, 1977) to the kidneys and visceral arteries and thus affect blood pressure.

We found that the celiac ganglion from young SHR contained more NE than the ganglion from WKR. The concentration decreased to about normal levels after decentralization of the ganglion. At 20 weeks of age there were no differences in NE concentrations between SHR and WKR, but there was a decline in NE concentration in both groups after decentralization. Presumably, preganglionic cholinergic neuronal activity was responsible for the elevated concentration of NE in the young SHR. SHR and WKR had similar concentrations of DA in their ganglia before and after decentralization at all ages, suggesting that SIF cells were present and capable of forming and storing DA. The relatively constant concentration of DA with maturation vs. the declining levels of NE suggests that SIF cells and sympathetic neurons develop independently.

Our most striking observation was the great difference in the concentration of DOPAC found in the ganglion from SHR and WKR at 4 weeks of age, before the blood pressure increased in the SHR. DOPAC concentrations are a direct indicator of DA formation in ganglion (Karoum et al., 1977). In support of this notion, decentralization of the ganglion at any age studied produced a significant decrease in DOPAC in both SHR and WKR. Apparently, DA is being formed at about half the rate in SHR as compared with WKR at 4 weeks of age. At 10 weeks the formation rate is higher in SHR and by 20 weeks the rates are similar.

Decentralization of the celiac ganglion at 4 weeks of age reduced blood pressure in both groups of rats when measured at 5 weeks of age. By 6 weeks of age the pressure appeared normal and remained essentially normal until 8 weeks of age. After 8 weeks of age the blood pressure of operated SHR began to increase and by 12 weeks was similar to the pressure in intact SHR. However, decentralization of the celiac ganglion at 10 or 20 weeks (data not shown) did not change the course of development of blood pressure in either group of rats. It should be noted that sectioning the splanchnic nerves also removes the central influence from the adrenal medulla, a major source of circulating catecholamines which could be partially responsible for the delayed hypertension in the SHR. Adrenal demedulation alone, however, did not appreciably delay the development of hypertension in SHR.

Our studies suggest a new hypothetical model which might, in part, explain the development of hypertension in SHR. At about 5 weeks of age the sympathetic system reaches maturity (De-Champlain et al., 1970). In normal rats, SIF cells apparently release and metabolize DA within the celiac ganglion at a rapid rate even before 4 weeks of age. The rapid metabolic rate in young rats probably plays a major role in rendering NE neurons that are developing less sensitive to cholinergic nerve activity and thus prevents excessive sympathetic input to the vessels of the kidneys and the viscera. In contrast to those in WKR, cholinergic neurons to the ganglion are more active than normal in SHR, yet the SIF cell-dopaminergic system is not functioning at a sufficiently accelerated rate in the young rats to dampen the responsiveness of the sympathetic neurons to the incoming traffic. The consequence is that more catecholamine is released at sympathetic nerve endings, causing vasoconstriction (Molinoff and Axelrod, 1971; Grober et al., 1975; Nagatsu et al., 1976). Perhaps young SHR can compensate for the vasoconstriction, but older rats cannot. The disease process may not be reversible after 10 weeks when DA metabolism is enhanced in SHR. In support of this notion, we found that the development of hypertension in SHR was not altered by decentralizing the celiac ganglion at 10 or 20 weeks. Decentralizing the celiac ganglion of SHR at 4 weeks of age terminates the central input to the sympathetic nerves and therefore the blood pressure does not exceed normal values. At about 8 weeks of age, following decentralization, the ganglion may be reinnervated to initiate the pathological process.

The low DOPAC concentration in young SHR might be explained by the possibility that the SIF cells in SHR may be less responsive to cholinergic input for several reasons. They may not be innervated normally by cholinergic neurons or they may lack acetylcholine receptors and therefore cannot respond to released acetylcholine. With age, SIF cells may become more responsive to acetylcholine, but it may be too late to reverse the disease process. Clearly, the hypertension that develops in SHR cannot be explained solely as a problem of DA metabolism in the celiac ganglion. Our results and those of others showing a deficiency of catecholamine biosynthetic enzymes in brain of young SHR but not in adult SHR suggest that the lack of responsive negative feedback neuronal loop systems in brain as well as ganglion may be one of the causes of hypertension (Grober et al., 1975; Nagatsu et al., 1976; 1977). The celiac ganglion of SHR may be a useful, simple model for evaluating these problems.

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B E Lütold, F Karoum and N H Neff

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