Responsiveness of Noradrenergic Neurons in Rat Experimental Hypertension

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We investigated possible abnormalities of cholinergic-noradrenergic neurotransmission in superior cervical ganglia in vitro in spontaneously hypertensive, Dahl salt-sensitive and deoxycorticosterone-salt-hypertensive rats by measuring the de novo synthesis of catecholamines from their labeled precursor tritiated tyrosine in response to cholinergic stimulation. Labeled tyrosine was incorporated into dopamine and its main neuronal metabolite dihydroxyphenylacetic acid as well as into norepinephrine. Dihydroxyphenylacetic acid and norepinephrine, but not dopamine, generation was linear with time under basal and stimulated conditions. However, norepinephrine incorporation remained similar before and after cholinergic stimulation of ganglionic neurons. Only young, prehypertensive spontaneously hypertensive rats showed altered responses when compared with their controls. Although endogenous dihydroxyphenylacetic acid content and baseline tyrosine incorporation into dihydroxyphenylacetic acid were lower in 4-week-old spontaneously hypertensive rats than in age-matched Wistar-Kyoto rats, cholinergic stimulation increased labeled dopamine and dihydroxyphenylacetic acid generation significantly more in juvenile spontaneously hypertensive rats. Such a hyperresponsiveness was not observed in either young Dahl rats or in any of the other models when they became hypertensive. These results probably reflect a genuine hyperreactivity of postganglionic noradrenergic neurons to acetylcholine or their increased catecholamine-synthesizing ability after centrally evoked enhanced sympathetic outflow known to occur during the early development of hypertension in spontaneously hypertensive rats. (Hypertension 1989;13:712-715)

Increased central sympathetic outflow has been implicated in the development and maintenance of human and experimental hypertension. There is also evidence that spontaneously hypertensive rats (SHR) exhibit several abnormalities in peripheral sympathetic activity. However, there has been less focus on possible alterations in neurotransmission at the level of the sympathetic ganglia, the intermediate station between the central and peripheral nervous system. Changes in ganglionic neurotransmission have been indicated by several observations. For example, the sympathetic ganglia of SHR contain different concentrations of some catecholamines (CAs) and peptides, or these ganglia express receptors differently for peptides, such as atrial natriuretic factor, when compared with Wistar-Kyoto (WKY) rats. Principal ganglionic neurons of SHR also exhibit an abnormally high uptake of amino acids with increased protein synthesis.

The purpose of this study was to evaluate the responsiveness of noradrenergic neuron perikarya in two genetic models of hypertension, SHR and Dahl rats, and in an acquired model, deoxycorticosterone acetate (DOCA)-salt-hypertensive rats. We set up an in vitro system to measure CA synthesis responses to cholinergic stimulation in rat superior cervical ganglia.

Materials and Methods

Male SHR and WKY rats (Taconic Farms Inc., Germantown, New York), Dahl salt-resistant and salt-sensitive rats (Brookhaven National Laboratory, Upton, New York), and Sprague-Dawley rats (Charles River Canada Inc., St. Constant, Quebec, Canada) were used in this study. They were kept under standard conditions with rat chow and water ad libitum. The Dahl groups were fed an 8% NaCl diet (Ralston Purina, Indiana City, Indiana) for 5 weeks. DOCA-salt hypertension was produced in uninephrectomized Sprague-Dawley rats by administering a 50-mg DOCA pellet i.p. and providing a 1% saline drinking solution ad libitum for 3 weeks.
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FIGURE 1. Time course of [3H]tyrosine incorporation into [3H]dopamine, [3H]dihydroxyphenylacetic acid (DOPAC), and [3H]norepinephrine stimulated with 0.1 mM carbachol in superior cervical ganglia of Sprague-Dawley rats (weight 200–225 g) in vitro. Each point represents an average of four determinations.

Systolic blood pressure was measured by the tail-cuff method. The weight and blood pressure values were given elsewhere.10 Superior cervical ganglia were removed after decapitation and processed as described previously.14 To randomly placed ganglia (1 ganglion/well), 10^6 cpm L-3,5-[3H]tyrosine (specific activity 50 Ci/mmol) (Amersham International PLC, Amersham, United Kingdom) was added, together with carbachol (Sigma Chemical Co., St. Louis, Missouri), a nonselective agonist of cholinergic receptors, at a final concentration of 0.1 mM, which causes maximal stimulation of tyrosine hydroxylase, the rate-limiting enzyme in CA synthesis. Incubation was stopped after 1 hour by adding 3.0N HClO₄, and the ganglia were homogenized in an ultrasound homogenizer. The homogenates were processed to prepare for high-performance liquid chromatography separation, as described elsewhere.15 High-performance liquid chromatography fractions corresponding to peaks of endogenous dopamine (DA), dihydroxyphenylacetic acid (DOPAC), and norepinephrine (NE) were collected and counted for tritium content. The data were evaluated by two variable linear regression correlations and by the unpaired Student’s t test.

Results
[3H]Tyrosine was incorporated into tritiated DA, DOPAC, and NE during incubation with superior cervical ganglia. As seen in Figure 1, carbachol-stimulated CA synthesis was linear when plotted against time for DOPAC and NE for at least 2 hours of incubation but was hyperbolic for DA. The DOPAC and NE curves achieved a highly significant correlation under stimulated conditions. Basal [3H]tyrosine incorporation into CA presented a similar pattern, and the DOPAC and NE curves had correlation coefficients r=0.995, p<0.005 and r=0.994, p<0.01, respectively. NE incorporation remained similar before and after cholinergic stimulation of ganglionic neurons. Basal [3H]DOPAC synthesis was lower in 4-week-old SHR than in WKY rats (167±16 vs. 213±15 cpm, mean±SEM, n=8, p<0.05), which was not observed for [3H]DA and [3H]NE (158±14 vs. 148±14 cpm and 692±68 vs. 560±43 cpm, respectively). However, the superior cervical ganglia of young SHR (Figure 2) manifested significantly greater [3H]DA and [3H]DOPAC synthesis after cholinergic stimulation than those of WKY rats. Carbachol did not raise [3H]NE in both groups of rats. No difference was observed in Dahl rats in which the increment of [3H]DA and [3H]DOPAC generation was 184±29% and 217±27% of baseline values (126±8 vs. 102±7 cpm) in the salt-resistant groups and 158±17% and 207±15% (138±15 and 129±10 cpm) in the salt-sensitive groups, respectively. Basal and carbachol-stimulated CA synthesis in hypertensive 12-week-old SHR, severely hypertensive Dahl rats, and DOCA-salt rats was similar to that in their respective controls (Table 1). Measurement of endogenous CA content revealed no difference in DA and NE between prehypertensive SHR and their controls, but DOPAC content was lower in SHR than in WKY rats (1.34±0.10 ng/ganglion vs. 2.39±0.21 ng/ganglion, p<0.05).

Discussion
The present study demonstrates differences in ganglionic CA synthesis in prehypertensive SHR compared with WKY rats under both normal and stimulated conditions. Cholinergic stimulation with carbachol was equally effective in older hypertensive SHR and their age-matched WKY controls, in Dahl rats, whether prehypertensive or hypertensive, and in the DOCA-salt model. Juvenile SHR had lower concentrations of endogenous DOPAC in superior cervical ganglia; this occurrence confirmed a finding previously reported in celiac ganglia. It was suggested that this decrease in DOPAC is
related to a diminished metabolism of DA, having an exceptionally high turnover in ganglia compared with other neuronal tissues. Our study did not address this question, and the cause of this phenomenon remains unclear. \[^{3}H\]DOPAC generation from \[^{3}H\]tyrosine was also lower in SHR superior cervical ganglia, but cholinergic activation evoked a greater increment of newly synthesized \[^{3}H\]DOPAC and \[^{3}H\]DOPA than in WKY rats. This is the first demonstration that the perikarya of postganglionic noradrenergic neurons may respond in an exaggerated manner to cholinergic stimulation in young SHR, as measured by biochemical variables of such stimulation. This response may be attributed to increased tyrosine hydroxylase activity, induced by stimulation of both nicotinic and muscarinic receptors along with its phosphorylation. Thus, acute stimulation of this rate-limiting enzyme may result in a different degree of CA synthesis activation in SHR and WKY rats. Alternatively, differences in affinity and density of cholinergic receptors in noradrenergic neurons may be involved since neuronal receptor abnormalities were frequently demonstrated in SHR. It is also plausible that membrane permeability for cations, especially for Ca\(^{2+}\), which plays an important role in tyrosine hydroxylase activation, is impaired in neurons of SHR. SHR with established hypertension have already been shown to have abnormal Ca\(^{2+}\) conductance. Interestingly, principal ganglionic neurons were found to have higher amino acid uptake and protein synthesis in young SHR up to at least 30 days of age. Some of these amino acids can be used for CA synthesis. Therefore, the increased responsiveness of neurons of SHR may also arise as a consequence of higher substrate availability caused by a more avid uptake of tyrosine, an amino acid. Further work is required to explore which of the above-mentioned possibilities is involved in the hyperreactivity of postganglionic neurons in juvenile SHR.

Our study confirms a general pattern of exaggerated sympathetic outflow documented in several reports on prehypertensive SHR. Young SHR have a higher level of circulating CA,


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