I) Abnormality in Catecholamine Metabolism in the Brain of Spontaneously Hypertensive Rats (SHR) - Analysis by In Vivo Voltammetry

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Summary

The abnormality in catecholamine metabolism in the brain of SHR has been examined by use of in vivo voltammetry technique. In vivo release of dopamine and serotonin in the striatum under acute stress was more prominent in SHR at 4 weeks of age than in normotensive Wistar-Kyoto rats (WKY). Acute activation after stress of in vitro tyrosine hydroxylase activity was observed only in SHR, but tryptophan hydroxylase activity did not change significantly. It is concluded that central monoaminergic neurons are more susceptible to stress in SHR than those in WKY.

Introduction

We have examined the activities of catecholamine synthesizing enzymes (tyrosine hydroxylase and dopamine β-hydroxylase) relevant to catecholaminergic functions in the adrenal medulla, peripheral sympathetically innervated tissues, blood, and brain in SHR (1) and in stroke-prone SHR (SHRSR) (2). The activity of the peripheral sympathetic nerves is increased only at young ages before the onset of hypertension and may trigger off the onset of hypertension (3-8). In contrast, the abnormality in central catecholaminergic neurons in SHR remains to be further elucidated. Several reports support the hypoactivity of noradrenergic neurons (4,6,9,10) and hyperactivity of adrenergic neurons (10) only at young ages of SHR, which may cause the hyperactivity of the peripheral sympathetic neurons. However, some controvertial results have also be presented (11,12).

We have studied the abnormality in catecholaminergic neurons in the brain of SHR under conscious and freely-moving conditions in monitoring the released catecholamines by newly developed in vivo voltammetry technique. In this technique, dopamine and serotonin released as neurotransmitters can be identified as their metabolites such as 3,4-dihydroxyphenylacetic acid (DOPAC) from dopamine and 5-hydroxyindoleacetic acid (5-HIAA) from serotonin. Since dopamine release can be measured most easily by in vivo voltammetry and recently acquires considerable interest in hypertension researches, we have measured release of dopamine and serotonin by stress in the striatum of SHR and WKY at 4 weeks of age.

Materials and Methods

All experiments were carried out on male SHR and WKY, weighing 70 to 75 g at 4 weeks of age. In vivo voltammetry was carried out by the method of Ikeda et al. (13). A three-electrode system was employed for differential pulse voltammetry. A carbon fiber electrode (7 μm in diameter) was inserted into the caudate nucleus as a working electrode, and reference (Ag/AgCl wire, 250 μm in diameter) and auxiliary (silver wire, 200 μm in diameter) electrodes were placed on the dura surface of the frontal cortex area. Two days after electrode implantation recordings were made at intervals of 15 min for 60 min before and for 105 min after stress (forced swimming in water at 15°C for 5 min).

Recording parameters of differential pulse voltammetry were as follows: potential range, -200 mV to +400 mV vs reference electrode; pulse amplitude, 25 mV; scan rate, 50 mV/sec; pulse frequency, 10 Hz; and pulse duration, 50 msec. Results were expressed as percentage of the mean.
control amplitude of the voltammograms calculated by averaging the initial fire values before stress. In vitro tyrosine hydroxylase (14) and tryptophan hydroxylase (15) activities were also measured at 5 min after 5 min swimming stress.

Results

Differential pulse voltammograms recorded from the caudate nucleus of WKY and SHR under normal conditions showed three peaks; peak 1 at -50 mV corresponding to ascorbic acid, peak 2 at +120 mV corresponding to DOPAC derived from dopamine, and peak 3 at +270 mV corresponding to 5-HIAA derived from serotonin. No appreciable differences in voltammograms were observed between SHR and WKY, indicating that DOPAC and 5-HIAA levels in the caudate nucleus of the WKY and SHR are equally detectable.

Swimming stress in water at 15°C for 5 min resulted in rapid increase in peak 2 (DOPAC) and peak 3 (5-HIAA) in WKY and SHR. The maximum amplitude of peak 2 (DOPAC) of SHR and WKY was 167 ± 15 % and 120 ± 7 %, respectively. The maximum amplitude of peak 3 (5-HIAA) of SHR and WKY was 345 ± 40 % and 236 ± 16 %, respectively. Throughout all the experiments the amplitudes of peaks 2 and 3 of SHR were always larger than those of WKY at any recordings made after stress.

Tyrosine hydroxylase activity measured in vitro in the striatum homogenate obtained 5 min after stress was significantly higher in SHR than in WKY (WKY, 223 ± 7.1; SHR, 271 ± 10.5 pmol/min/mg protein in the presence of 0.2 mM 6-methyltetrahydropterin, n=5), but tryptophan hydroxylase activity did not change significantly.

Discussion

Elevation of the amplitude of peaks 2 (DOPAC) and 3 (5-HIAA) after stress by in vivo voltammetry can be considered as the results of increased release of dopamine and serotonin. However, elevated amplitude of peaks 2 and 3 to stress was higher in SHR as compared with that in WKY, suggesting that central dopaminergic and serotonergic neurons of SHR may respond more sensitively to short-term swimming stress. The increased response of peripheral noradrenergic sympathetic nerves to stress was reported previously (12, 15, 16). This study also showed increased neural response to stress in the brain of SHR for the first time. In vitro tyrosine hydroxylase activity was also acutely increased only in SHR after stress, whereas in vitro typtophan hydroxylase activity did not change significantly after stress even in SHR. This suggests that tyrosine hydroxylase is more rapidly activated accompanied with release of dopamine by stress than tryptophan hydroxylase.

In conclusion, it is indicated that the central dopaminergic and serotonergic neurons in SHR may have higher response to stress than those in WKY. The changes in central catecholaminergic neurons can be further followed during the onset of hypertension by the in vivo voltammetry.

References