Early changes in noradrenaline content of some brain nuclei in spontaneously hypertensive rats

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Summary

1. The noradrenaline content of individual brain nuclei was measured by using high-pressure liquid chromatography coupled with electrochemical detection.

2. This study was performed on spontaneously hypertensive rats during the development of hypertension (4 and 12 weeks) and also on deoxycorticosterone–salt hypertensive rats after 1 and 4 weeks of treatment.

3. In young spontaneously hypertensive rats a significant decrease in noradrenaline content was observed in some medullary and hypothalamic nuclei which are involved in cardiovascular regulation. No change was observed after deoxycorticosterone–salt treatment.

4. It is proposed that a change in adrenergic activity, restricted to brain cardiovascular centres, could represent a mechanism triggering spontaneous hypertension.

Key words: cardiovascular centres, catecholamines, electrochemical detection, high-pressure liquid chromatography, spontaneous hypertension.

Introduction

The early onset of hypertension in spontaneously hypertensive rats of the Okamoto strain (SH rats) may be central in origin (Haeusler, Finch & Thoenen, 1972; Loewy, McKellar, Swensson & Panneton, 1980). Such a trigger function of the central nervous system may be related to modifications of central adrenergic activity in the cardiovascular centres of young SH rats. Changes in aminergic biosynthetic enzymes have indeed been reported in specific brain areas during the development of hypertension (Saavedra, Grobecker & Axelrod, 1978). However, the determination of the catecholamine content of these nuclei with radio-enzymatic methods has led to controversial results.

The aim of the study was to reinvestigate catecholamine content of brain nuclei in SH rats and deoxycorticosterone (DOCA)–salt-treated rats at various periods, by using high-pressure liquid chromatography coupled with electrochemical detection.

Methods

Four and 12 week old male SH rats were compared with their Wistar–Kyoto (WK rats) controls. Experimental hypertension was induced in male Wistar rats by uninephrectomy at 6 weeks of age followed by administration of DOCA (5 mg week⁻¹ kg⁻¹ subcutaneously) and 1% NaCl to drink. After 1 and 4 weeks of treatment rats were compared with control uninephrectomized male Wistar rats given tap water to drink.

Rats were killed by decapitation. The brain was quickly removed and frozen in liquid nitrogen. Serial sections of 500 μm were cut in a cryostat at −6°C. Specific areas were punched out with a needle with an internal diameter of 1.0 mm. Forebrain and hindbrain areas were located according to the atlases of König & Klippel (1963) and Palkovits & Jacobowitz (1974) respectively. The mean weight of one punch was 0.38 mg and protein content determined with the method of Lowry, Rosebrough, Farr & Randall (1951) was 32 μg. Catecholamine content was assayed on a single punch for the nucleus commissuralis and on two contralateral punches.
for other areas. Punches were homogenized and sonicated in 50 μl of perchloric acid (0.4 mol/l) containing ascorbic acid (10-5 mol/l), then centrifuged at 15 000 g. The supernatants were stored at -20°C for up to 3 weeks before assay. Separation of catecholamines was performed through a column (250 mm length and 2 mm interior diameter) packed with microparticulate octadecyl bound silica (Micropak CH-10 Varian). The elution solution was a sodium phosphate buffer (0.1 mol/l; pH 6.7) containing sodium octyl sulphate (1.43 mmol/l). The electrochemical detection described by Keller, Oke, Mefford & Adams (1976) was used, with a carbon paste working electrode and a LC-4 electronic controller (Bioanalytical Systems). The potential of the working electrode against the Ag/AgCl reference electrode was set at +0.8 V. Samples of 5–20 μl were injected. The response of the cell was compared with standard curves obtained with reference solutions and was linear from 0.05 pmol to 5 nmol.

Results

Noradrenaline content of 12 specific hindbrain and forebrain areas of SH and WK rats (ages 4 and 12 weeks) are represented in Fig. 1. Dopamine was mainly concentrated in hypothalamic areas and was not modified in SH rats, compared with WK rats. Adrenaline was detected only occasionally in hypothalamic nuclei with the present method.

No change in noradrenaline content was observed in DOCA–salt-treated rats.

Discussion

A decrease in noradrenaline content of the main cardiovascular centres was observed in young SH rats compared with WK rats. These nuclei included nucleus commissuralis, area A2 and the lower portion of the rostral nucleus tractus solitarii in the medulla and nucleus anterior hypothalami, nucleus paraventricularis, medio-basal hypothalaminus (ventral and dorsal) in the diencephalon. These changes may reflect the same alteration since the medullary and hypothalamic nuclei are connected (Ricardo & Koh, 1978; Palkovits, Mezey & Zaborszky, 1979). These modifications disappeared with age and were no longer detectable in 12-week old rats. Analysis of the same nuclei in 1 and 4 week DOCA–salt-treated rats failed to show any change in noradrenaline content, indicating that the abnormality observed in young SH rats was not secondary to the rising blood pressure and thus could reflect a primary change in central adrenergic activity. Our results differ from those of Saavedra et al. (1978) and of Wijnen,

![Graph](image-url)
Spierenburg, De Kloet, De Jong & Versteeg (1980) as these authors did not observe any difference in noradrenaline content of medullary centres of SH rats compared with WK rats. However, Saavedra et al. (1978) did find a reduction in the noradrenaline content of anterior and medio-basal hypothalamic nuclei in 4 week old SH rats, a result which is compatible with our data. However, these authors reported that this change persisted in 14 week old SH rats. Wijnen et al. (1980) failed to observe changes in 3 and 7 week old SH rats, but observed a reduction in noradrenergic content of nucleus anterior hypothalami in 10 week old SH rats.

A change in the endogenous concentration of noradrenaline suggests an abnormal adrenergic transmission, but cannot be used alone as an index of the rate of nerve activity (Chalmers, 1975). The activity of synthesizing enzymes now under investigation in our laboratory could give more insight into the adrenergic activity of the brain nuclei. Saavedra et al. (1978) have reported a decrease in dopamine-β-hydroxylase activity in the hypothalamic nuclei where noradrenaline was diminished, indicating a selective deficiency of some central noradrenaline neurons in SH rats.

In conclusion, a decrease in noradrenaline content of certain brain nuclei corresponding mainly to cardiovascular centres was observed in young SH rats. This central abnormality may be causally related to genetic hypertension as no such change was secondary to DOCA–salt hypertension.

References