Role of the sympathetic nervous system in spontaneous hypertension: changes in central adrenoceptors and plasma catecholamine levels

ANNA PALERMO, CARLO COSTANTINI, GABRIELE MARA AND ARNALDO LIBRETTI
Clinica Medica, L. Sacco Hospital, University of Milan, Milan, Italy

Summary
1. There is evidence that dysfunction of central adrenergic neurons and the peripheral sympathetic nervous system are implicated in the development and maintenance of spontaneous hypertension.

2. Accordingly, adrenoceptors of brain areas related to cardiovascular control (hypothalamus and lower brain stem) and plasma catecholamine levels were measured in male spontaneously hypertensive rats and in their normotensive Wistar-Kyoto controls at 11 weeks of age.

3. Assays of $\beta_1$, $\alpha_1$, and $\alpha_2$ receptor binding were performed by using respectively $[^3H]$dihydroalprenolol, $[^3H]$clonidine and $[^3H]$labelled WB4101 as ligand. Plasma catecholamines were assessed by high performance liquid chromatography with electrochemical detection.

4. No difference was observed on $\beta$-receptor binding sites in the two animal groups. On the other hand, $\alpha_2$ receptors were increased by 29% in the lower brain stem and $\alpha_2$ receptors showed an increase of 46% in the hypothalamus of the hypertensive rats. By Scatchard plot analysis, these changes were attributable to variations in the number rather than in the affinity of binding site.

5. Plasma noradrenaline levels were more elevated in the hypertensive rats, and no difference was observed in plasma adrenaline concentrations.

6. The increase in hypothalamic $\alpha_1$ receptors and the probably compensatory increase in lower brain stem $\alpha_2$ receptors suggest enhanced stimulation of the vasomotor centre. Hyperactivity of the peripheral sympathetic nervous system in spontaneous hypertension could therefore be secondary to a dysfunction of central adrenergic neurons projecting to the vasomotor centre of the brain stem.

Key words: central adrenoceptors, plasma catecholamines, spontaneous hypertension.

Introduction
Many experimental studies support the view that central adrenergic neurones are implicated in the development and maintenance of genetic hypertension [1–4]. Moreover, hyperactivity of peripheral sympathetic nervous system has been proposed in spontaneously hypertensive rats (SHR), because of the observed increases in catecholamine plasma levels and catecholamine turnover [5–8].

The present report was designed firstly to study central neuronal activity in spontaneously hypertensive rats through the measurement of the adrenoceptor binding in the brain areas related to cardiovascular control (hypothalamus and lower brain stem), and secondly to assess peripheral sympathetic nervous activity through the evaluation of the plasma catecholamine levels.

Methods
The study was carried out in eight male SHR (Charles River, France) and in eight normotensive Wistar–Kyoto (WKY) controls at 11 weeks of age. Animals were killed by inhalation of ethyl ether. Brains were removed, and hypothalamus and lower brain stem dissected and stored at $-80^\circ$C until analysis.
Assays for receptor binding were performed by incubating at 25°C the brain area homogenates with the following ligands: [3H]dihydroalprenolol, μmol/l (DHA, sp. activity 45Ci/mmol, N.E.N.), for the β receptors, [3H]-labelled WB4101, 0.375 mmol/l (sp. activity 24·5 Ci/mmol, N.E.N.) for the αi receptors and [3H]clonidine, 6 mmol/l (sp. activity 20 Ci/mmol, R.C.A.) for the α2 receptors. Binding assays were terminated by rapid filtration under vacuum and the filters counted by liquid scintillation spectrometry.

Specific ligand binding was defined as the difference between total and non-specific binding obtained by concurrent incubation with (-)-propranolol (1 μmol/l) as displacing agent for the β receptors and noradrenaline (100 μmol/l) for the αi, and α2 receptors [9].

Blood samples (3 ml) for plasma catecholamine assay were collected from the inferior vena cava in heparinized cold tubes and centrifuged at 6500 rev./min at 4°C for 20 min. Plasma catecholamines were measured in duplicate by high performance liquid chromatography with electrochemical detection [10].

**Results**

No difference in β receptors was observed between SHR and WKY in both the brain areas, since [3H]DHA binding was respectively 1·16 ± 0·39 vs 1·18 ± 0·14 pmol/g of tissue in hypothalamus and 1·15 ± 0·50 vs 1·01 ± 0·34 pmol/g of tissue in lower brain stem. The hypothalamus [3H]-labelled WB4101 binding was significantly (P < 0·05) increased, by 46% in SHR compared with the controls (1·5 ± 0·28 vs 1·03 ± 0·22 pmol/g of tissue). The αi-receptors of lower brain stem did not show any difference (1·07 ± 0·081 vs 1·06 ± 0·04 pmol/g of tissue).

The α2 receptors of SHR were unchanged with respect to WKY in hypothalamus (1·98 ± 0·36 vs 1·98 ± 0·28 pmol/g of tissue) whereas they were significantly (P < 0·05) raised by 29% in lower brain stem (1·03 ± 0·11 vs 0·74 ± 0·12 pmol/g of tissue).

In order to determine whether the increased binding observed in SHR was due to an increased number of the binding sites or to a change in their affinity, samples of hypothalamus and lower brain stem were incubated with increasing concentrations respectively of [3H]-labelled WB4101 (from 0·125 to 2 nmol/l) and of [3H]clonidine (from 1 to 8 nmol/l).

By Scatchard plot analysis no significant difference between Kd values of SHR and WKY controls was observed; the contrast, a significant rise in the Bmax values (i.e. the x intercept on a Scatchard plot) occurred in SHR (Fig. 1).

Thus the changes in SHR of [3H]-labelled WB4101 and [3H]clonidine binding were attributable to an increase in the number of αi and α2 adrenoceptors rather than in their binding affinity.

Plasma noradrenaline levels were significantly (P < 0·01) more elevated in SHR (7·13 ± 0·41 vs 4·56 ± 0·88 ng/ml); no difference was observed in plasma adrenaline concentrations (3·40 ± 0·4 vs 2·54 ± 0·91 ng/ml).
Discussion

Previous studies have shown that brain areas involved in cardiovascular regulation are characterized in SHR by changes in enzymatic activity, histological structure and catecholamine content [2, 4, 11-13]. Our findings have demonstrated that variations in the number of hypothalamus \(\alpha_{1}\) and lower brain stem \(\alpha_{2}\) receptors are also present.

The increased number of \(\alpha_{2}\) receptors found in the hypothalamus of SHR could provide an explanation for neuronal hyperactivity observed in the posterior hypothalamic area in many experimental studies [14-20].

It is well known that an excitatory noradrenergic pathway arises from this area to the lower brain stem vasomotor centre and it has been reported that pressor responses elicited by electrical stimulation of the posterior hypothalamus [14, 15], or vasodepressor effects produced by cutting the neural connections with the lower brain stem [16], are more pronounced in SHR than in normotensive WKY rats. Moreover, SHR appear to display exaggerated pressor responses to stressful stimuli, also during the early prehypertensive phase, thus suggesting that normal environmental stimuli interact with an inherent hyper-reactivity of the 'defence area' located in posterior hypothalamus [17, 18]. It is likely therefore that more frequent and exaggerated neurogenic pressor responses, from increased sympathetic vasomotor discharge from posterior hypothalamus, can contribute to the development of the so-called 'spontaneous hypertension'. The increase in \(\alpha_{1}\) receptors in the hypothalamus might also be a compensatory change to counteract a transient decrease in the activity of inhibitory noradrenergic neurons. Many experimental studies [2, 13] indicate that the activity of catecholaminergic neurons projecting to the anterior hypothalamus is lowered during the early development of genetic hypertension, with a consequent decrease of the depressor influence of the anterior hypothalamus on the lower brain stem vasomotor centre. In the present study low levels of endogenous noradrenaline in this area may have caused an increase in the \(\alpha_{2}\) adrenoceptors through the 'up-regulation' mechanism. Moreover, our findings may explain the augmented vasodepressor responsiveness observed in SHR after centrally administered phenylephrine [19].

Since the anterior hypothalamus receives direct input from the nucleus tractus solitarius, which contains the primary synapses of the baroreceptor fibres, the decreased noradrenergic activity in this area could be related to a reduced baroreceptor inhibitory activity during the onset of spontaneous hypertension [20]. This interpretation can be supported by the hypotensive effect of clonidine, which probably acts on an inhibitory \(\alpha_{2}\)adrenergic system in the nucleus tractus solitarius, with the consequent stimulation of baroreceptor reflexes [21].

Therefore the increase in \(\alpha_{2}\) receptors shown in the lower brain stem of SHR might represent a compensatory increase in central postsynaptic adrenoceptors of inhibitory neurons. On the other hand, our results might be also interpreted as a compensatory increase in presynaptic adrenoceptors of facilitatory neurons, related to the augmented stimulation of the vasomotor centre by the noradrenergic pathway arising from the posterior hypothalamus.

In conclusion, the changes in adrenoceptor numbers observed in hypothalamus and lower brain stem of SHR suggest an enhanced stimulation of the vasomotor centre. Therefore our results suggest the hypothesis that the increased activity of the peripheral sympathetic nervous system, shown by the rise in plasma noradrenaline levels, is probably secondary to a central influence.

References


