Plasma concentrations of catecholamines in two strains of spontaneously hypertensive rats at different ages

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Summary
1. Blood pressure was measured and plasma levels of noradrenaline and adrenaline were determined radioenzymatically under basal conditions and after 10% blood volume reduction in blood drawn through catheters previously implanted in young and adult rats of two different genetically hypertensive strains: the Kyoto strain (SHR) and the Milan strain (MHS), and in their respective controls: Wistar-Kyoto strain (WKY) and Milan normotensive strain (MNS).
2. Under basal conditions no differences were observed between plasma noradrenaline and adrenaline levels in SHR and MHS rats and in the controls, at any age. Haemorrhage produced a greater fall in the blood pressure (P < 0.01) of young and adult hypertensive strains (SHR-MHS) than in WKY and MNS rats, and a greater rise in plasma adrenaline (P < 0.01).
3. These results suggest that: (a) there may be differences in involvement of the sympathetic nervous system in the pathogenesis of hypertension in SHR and MHS rats but not such as to cause differences in plasma catecholamine levels in either young or adult rats; (b) haemorrhage activates the sympathetic-adrenal systems more in SHR and MHS rats, than in controls, and the greater percentage fall in blood pressure is probably due to a difference in reflex venoconstriction.

Key words: catecholamines, haemorrhagic stress, spontaneously hypertensive rats.

Abbreviation: MBP, mean blood pressure.

Introduction
Several investigators have measured plasma concentrations of noradrenaline and serum dopamine-β-hydroxylase in Kyoto spontaneously hypertensive rats (SHR) on the assumption that they might serve as biochemical indices of sympathetic nervous system activity [1, 2]. In the published results there are some discrepancies: when blood for catecholamine assay was obtained by decapitation, plasma noradrenaline is always higher in young SHR than in their normotensive controls (WKY), whereas in adult rats these differences disappear [3–6]. However, when blood was drawn from chronically cannulated rats, one group of investigators [7] still found higher levels of noradrenaline in young SHR than in WKY rats, and another group found no differences in the resting condition, but only after stress [8].

Our work had two purposes: (1) to measure plasma levels of noradrenaline and adrenaline under basal conditions and after haemorrhage, from chronically cannulated young and adult SHR rats; (2) to compare SHR and the Milan hypertensive strain (MHS) [9] under the same experimental conditions to see if different patterns of plasma catecholamines lend support to the different pathogenetic mechanisms proposed for the hypertension in these two kinds of genetically hypertensive rats [10–14].

Methods

Animals.

Male MHS and the control normotensive rats (MNS), isolated from the same Wistar stock colony, were obtained from our animal colony. SHR and WKY controls were obtained from a
commercial supplier (Charles River, France) and after arrival they were housed in cages for 3–4 days before use. Animals had free access to food and water until the morning of the experiment. We have studied the MHS and MNS rats at three ages: 25 days, 31 days and 60 days. The SHR and WKY rats were studied at 25 and 60 days of age.

Experimental procedure

The day before the experiment, chronic catheters (PE 50 + Silastic) were implanted in right femoral arteries of rats 31 and 60 days of age under halothane anaesthesia. In 25 day old rats, cannulation was performed the same day, 5 h before the experiment, to avoid an excessive weight loss from surgical stress in these small animals (60 g body weight). The catheters were filled with 0.9%-sodium chloride solution (saline) containing 500 units of heparin/ml and were kept open by flushing with 0.2-0.3 ml of the heparinized saline once a day. After surgery the rats were replaced in their cages without any restrictions, with the tubing extending out of the cage.

Before the experiment, mean blood pressure (MBP) and heart rate were measured without disturbing the rats with an arterial pressure transducer (Hewlett Packard 1280 B) connected to a recorder (Hewlett Packard model 770 2B). When the animals were completely quiet a first sample of 0.3-0.4 ml of blood was drawn for basal measurement of plasma catecholamines. After 10 min, blood volume was reduced by removing 10% of the total blood volume (previously determined with the Evans blue method; [15]) in 15–20 s with an heparinized syringe and, after 1 min, another sample of 0.3–0.4 ml of blood was collected for basal measurement of plasma catecholamines. After 10 min, blood volume was reduced by removing 10% of the total blood volume (previously determined with the Evans blue method; [15]) in 15–20 s with an heparinized syringe and, after 1 min, another sample of 0.3–0.4 ml of blood was collected for catecholamine assay. After each sampling, MBP and heart rate were measured. Thereafter, the blood in the syringe was rein infused into the animal and MBP and heart rate were again measured.

For the 25 day old rats we have considered the first basal removal of 0.4 ml of blood to be the haemorrhagic withdrawal, because 0.4 ml is 10% of the blood volume in these young rats. The blood samples were collected into heparinized tubes and the plasma was stored at $-80^\circ$C until assayed.

Plasma catecholamine assay

Adrenaline and noradrenaline were measured simultaneously in 50 $\mu$l of plasma by the radioenzymatic method of Da Prada & Zürcher [16].

Analysis of data

Student's $t$-test for paired data was employed to compare the cardiovascular and biochemical parameters before and after haemorrhage in each group. Student's $t$-test for unpaired data was employed to compare MBP, heart rate and basal plasma levels of catecholamines between groups.

Results

MPB values and plasma levels of noradrenaline and adrenaline are shown in Table 1. MBP was already significantly higher in SHR than in WKY rats at 25 days of age, and in MHS than in MNS rats at 31 days of age. After haemorrhagic shock, a significant fall in MBP in hypertensive and normotensive rats of both strains appeared as soon as the arterial catheter was connected to the pressure transducer (at 20 s from the start of the haemorrhage), and the MBP remained almost unchanged until the next removal of blood for catecholamine assay. This fall in MBP was greater in the hypertensive rats than in their respective controls.

Heart rate tended to rise slightly in all four strains after haemorrhage with the difference significant in young WKY and adult MHS and MNS rats (WKY $= 460 \pm 16$ vs $380 \pm 9$ beats/min, $P < 0.005$; MHS $415 \pm 16$ vs $364 \pm 9$, $P < 0.01$; MNS $480 \pm 17$ vs $431 \pm 10$, $P < 0.05$).

After haemorrhage, plasma levels of adrenaline rose significantly in SHR and MHS rats of all ages, whereas in the WKY and MNS rats it rose to a lesser degree, but significantly in adult WKY. Plasma levels of noradrenaline rose in all rats, but the increase was significant only in adult MHS and MNS and in young SHR. After reinjection of blood, the MBP returned to basal values in all the rats.

Discussion

Our main findings were: (1) there were no differences between basal plasma levels of catecholamines in SHR and MHS rats and their controls at any age considered; (2) haemorrhage produced a fall in MBP in all four strains, at each age, more pronounced in MHS and SHR, and a greater rise in plasma A than in controls.

To avoid all stress, blood samples were drawn from conscious and undisturbed rats, completely
TABLE 1. Mean blood pressure, plasma noradrenaline and adrenaline in Milan hypertensive (MHS), Milan normotensive (MNS), spontaneously hypertensive (SHR) and Wistar-Kyoto normotensive (WKY) rats at different ages

A, Basal state; B, after haemorrhage; C, after re-injection of blood. Values are means ± SEM. Numbers in parentheses are the numbers of rats. Significant differences between hypertensive and control strains (†) and those between basal state and after haemorrhage (*): †† P < 0.05; ††† P < 0.01; †††† P < 0.001.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Milan strain</th>
<th>Kyoto strain</th>
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<tr>
<td></td>
<td>MHS (6)</td>
<td>MNS (6)</td>
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<td>MHS (8)</td>
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<td>MHS (13)</td>
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<td>Mean blood pressure (mmHg)</td>
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<td>B</td>
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<td>C</td>
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<td>Noradrenaline (pmol/ml)</td>
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unaware of the procedure. Plasma adrenaline and noradrenaline levels under resting conditions were much lower than those after decapitation, reported by some authors [3–6], but are in good agreement with basal values found with the chronic cannulation technique [7, 17].

Our first results suggest that any differences there may be in involvement of the sympathetic nervous system in the pathogenesis of hypertension in SHR–MHS strains are not such as to cause differences in plasma catecholamine levels, either in the resting condition or after haemorrhagic shock. Of course, this does not rule out that there might be other differences in sympathetic activity between the two strains.

After haemorrhage we observed a general activation of the sympatho–adrenergic system in all groups of rats [18], but there was a greater increase of plasma adrenaline in the hypertensive strains than in their controls, and a greater fall in MBP. The blood pressure fall is probably not related to basal blood pressure values, but is a peculiarity of the hypertensive strains, because in adult WKY rats with the same basal pressures as adult MHS rats, MBP falls 6% against the 24% in MHS rats.

Our data do not explain the greater MBP fall in hypertensive strains after haemorrhage. However, they may be taken as evidence of abnormal cardiovascular responses to haemorrhage in 25 day old MHS and SHR. As this abnormality could be observed immediately (a few seconds) after haemorrhage, it is most likely that it is due to a defect in sympathetically mediated reflex venoconstriction [19]. Whether this defect is nervous or venous is open to discussion. However, the data for plasma catecholamines do not indicate defective activation of the sympathetic nervous system in the hypertensive rats. Therefore abnormal contractility of the venous walls in hypertensive rats seems to be the most likely explanation for our findings.

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References


