The sympathetic nervous system and the renin–angiotensin system in borderline hypertension

Department of Pharmacology and University Hospital, University of Limburg Medical School, Maastricht, The Netherlands

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Summary

1. Plasma catecholamine levels as well as plasma renin activity and plasma renin concentration were compared in normotensive volunteers and in subjects with borderline hypertension. All subjects were studied at rest and during bicycle ergometry.

2. The two groups of volunteer subjects did not differ in the plasma concentrations of noradrenaline and adrenaline, both at rest and during physical activity. The same was true for plasma renin activity and plasma renin concentration. Furthermore, urinary excretion of noradrenaline, adrenaline and 4-hydroxy-3-methoxymandelic acid was similar in both groups.

3. The results do not support the assumption that there is increased sympathetic activity in subjects with borderline hypertension.

Key words: borderline hypertension, catecholamines, ergometry, renin.

Abbreviation: HMMA, 4-hydroxy-3-methoxymandelic acid.

Introduction

The role of the sympathetic nervous system in the pathogenesis of essential hypertension is still controversial. Upon stimulation of sympathetic nerves, noradrenaline is released from the nerve endings. The majority is taken up again into the neurons but a small fraction reaches the systemic circulation. Thus plasma noradrenaline concentration can be used as an index of sympathetic nervous activity. In man, most if not all adrenaline in plasma stems from the adrenal medulla. Plasma adrenaline levels thus give an impression of the activity of this organ.

In patients with essential hypertension, increased plasma catecholamine levels have been found at rest (Engelman, Portnoy & Sjoerdsm, 1970; De Quattro & Chan, 1972; Louis, Doyle, Anavekar, Johnston, Geffen & Rush, 1974) and during physical activity (Chodakowska, Nazar, Wocial, Jarecki & Skórka, 1975; Planz, Gierlichs, Hawlina, Planz, Stephany & Rahn, 1976). Other investigators did not find differences in plasma catecholamine concentrations between patients with essential hypertension and normotensive control subjects at rest (Pedersen & Christensen, 1975; Lake, Ziegler, Coleman & Kopin, 1977; Taylor, Pool, Lake, Ziegler, Rosen, Rollins & Mitchell, 1978). The discrepancies might be due to differences in the composition of the groups of subjects studied. Thus it could be that elevated plasma catecholamine concentrations exist only in an early phase of hypertension. In order to test this hypothesis, a study was designed to compare plasma catecholamine levels in subjects with borderline hypertension and in normotensive controls.

It seemed possible that differences in plasma catecholamine concentrations might become more evident during physical exercise, that is in a situation with increased sympathetic activity. Furthermore, the sympathetic nervous system is known to be a mediator of renin secretion by the kidney (Davis & Freeman, 1976). Thus there could be altered plasma renin activities concomitant with changes of plasma catecholamine
levels. Therefore renin estimations, as well as measurements of both catecholamines and renin, at rest and during physical activity were performed.

Some of these results were presented at the Seventh International Congress of Pharmacology in Paris (1978).

Subjects studied and methods

The study was performed on 50 male non-hospitalized subjects whose age ranged from 18 to 30 years. Twenty-five of the subjects were normotensive: blood pressures after 2 min standing \( \leq 125/85 \) mmHg at two measurements performed with an interval of 1 week. The other 25 subjects were borderline hypertensive: blood pressure \( \geq 140/90 \) to \( <160/100 \) mmHg at two measurements with 1 week’s interval. In order to recruit volunteers for the study, all 18–30 year old men attending an outpatient clinic of the Maastricht University Hospital were asked to participate, provided that no diagnosis other than mild hypertension had been made. Furthermore, three general practitioners were requested to recruit volunteers in the same way. Finally, those ready to participate in the study were asked to invite friends of theirs to volunteer. Diseases other than borderline hypertension were excluded by taking a history and performing a physical examination. In the borderline hypertensive subjects, secondary hypertension was ruled out by a physical examination and performing a physical examination. In the borderline hypertensive subjects, secondary hypertension was ruled out by clinical examination, by determination of potassium and creatinine in serum, urine analysis, measurement of urinary excretion of 4-hydroxy-3-methoxymandelic acid (HMMA) and by intravenous pyelography. None of the subjects with borderline hypertension had retinal changes. None of them ever had received antihypertensive therapy. Informed consent was obtained from all subjects participating in the study. The investigation was approved by the research committee of the University of Limburg School of Medicine.

After entering the study, all subjects collected a 24 h urine sample for measurement of the excretion of sodium, creatinine, noradrenaline, adrenaline and HMMA. The next day, maximum work load during bicycle ergometry was determined. The subjects started with a work load of 124 W. The work load was increased stepwise by 31 W every 4 min until the maximum work load was reached. This was defined as the work load where either heart rate reached a maximal value or where the subjects became exhausted. The electrocardiogram was continuously monitored during physical exercise.

Not earlier than 5 days after the determination of maximum work capacity, the subjects came back to the laboratory. They rested for 30 min in the supine position after a needle had been placed into a forearm vein. Thereafter, heart rate and blood pressure were measured and a blood sample was drawn for the estimation of the packed cell volume, as well as the measurement of noradrenaline concentration, adrenaline concentration, renin activity and renin concentration in plasma. Then the subjects cycled in the sitting position, subsequently at 50% and at 75% of their maximum work capacity. Each work load lasted 5 min. In the last 2 min of each ergometry period, heart rate and blood pressure were measured. Furthermore, at the end of the work period with 75% of maximum capacity a blood sample was drawn to determine the same parameters as during supine rest.

All ergometric studies were done between 15.00 and 17.00 hours. For the individual subject, room temperature at the days of bicycle ergometry did not differ by more than 1°C. The volunteers did not smoke during the hour preceding the measurements as this might have increased plasma catecholamine levels (Cryer, Haymond, Santiago & Shah, 1976).

Blood pressure was always measured by the same observer (J.W.H.), using a mercury manometer. The point of muffling of the Korotkoff sounds was read as diastolic value. Heart rate was determined by electrocardiography.

Plasma catecholamine concentrations were measured as has been described (Rahn, Gierlichs, Planz, Planz, Schols & Stephany, 1978), except for the initial extraction of the metanephrines formed by enzymatic conversion. For this extraction ether and tetraphenylborate were used as suggested by Da Prada & Zurcher (1976). Furthermore, the metanephrines were separated by using thin-layer chromatography (Da Prada & Zürcher, 1976) in place of paper chromatography (Rahn et al., 1978). Within-assay coefficient of variation was 7% for both noradrenaline and adrenaline. Inter-assay coefficient of variation was 10% for both amines.

Urine was collected (24 h) in bottles containing 10 ml of HCl (6 mol/l). Catecholamine excretion in urine was determined by means of a double-isotope technique. For this, \( 10^7 \) d.p.m. of \(^{[3]H}\)noradrenaline (specific radioactivity 15 Ci/mmol; New England Nuclear, Boston, U.S.A.) as well as \( 10^7 \) d.p.m. of \(^{[3]H}\)adrenaline (specific radioactivity 15 Ci/mmol; New England Nuclear) were added as internal standards to 20 ml of urine. The urine samples were then subjected to ion-exchange chromatography as described.
Sympathetic nervous system in hypertension by Rahn (1973). After addition of reduced glutathione to obtain a 6·5 mmol/l solution, the eluate was adjusted to pH 8·2 and a portion (1 ml) was used for radiometric estimation as described for plasma, with the only exception that S-adenosyl[methyl-14]C]methionine (specific radioactivity 56 mCi/mol; New England Nuclear) was added as methyl donor. Within-assay coefficient of variation was 5% and inter-assay coefficient of variation was 8% for both noradrenaline and adrenaline. The [3H]noradrenaline and \([3H]\)adrenaline were purified by ion-exchange chromatography before use.

HMMA in urine was estimated by means of a spectrophotometric method (Pisano, Crout & Abraham, 1962).

Plasma renin activity was determined by incubating the plasma for 3 h at 37°C (pH 5·5). The angiotensin I generated was isolated by ion-exchange chromatography (Drayer & Benraad, 1975; Lijnen, Amery & Fagard, 1976) and measured by radioimmunoassay. Plasma renin concentration was estimated after adding plasma from nephrectomized sheep (Skinner, 1967). All measurements were done in duplicate. Within-assay coefficient of variation was 9%; inter-assay coefficient of variation was 12% for both plasma renin activity and plasma renin concentration.

All other reported parameters measured in blood and plasma were estimated at the routine laboratory of Maastricht University Hospital.

Results are expressed as mean values ± SEM. Statistical significance was tested with Student's t-test for unpaired data.

**Results**

There were no differences between the borderline hypertensive group and the normotensive controls in age and height as well as in the serum concentrations of sodium, potassium, creatinine and uric acid ($P > 0·1$). Furthermore, both groups did not differ in the urinary excretion of sodium, creatinine, HMMA, noradrenaline and adrenaline ($P > 0·1$). However, the borderline hypertensive group as compared with the normotensive group had a higher body weight ($78·2 ± 2·0$ vs $70·1 ± 1·2$ kg, $P < 0·005$), higher serum cholesterol levels ($5·31 ± 0·24$ vs $4·41 ± 0·18$ mmol/l, $P < 0·005$) and higher serum triglyceride concentrations ($1·74 ± 0·22$ vs $1·19 ± 0·09$ mmol/l, $P < 0·05$). Maximum work capacity averaged to $210 ± 7$ W in the normotensive group as compared with $185 ± 5$ W in the borderline hypertensive group. The difference is statistically significant ($P < 0·005$).

Heart rate was higher in the borderline hypertensive group than in the normotensive group after 30 min supine rest, after 2 min standing as well as during ergometer work corresponding to 50 and 75% of maximum work capacity (Table 1). Only at maximum work capacity had both the groups of subjects studied identical heart rates. Systolic and diastolic blood pressure was higher in the borderline hypertensive than in the normotensive group at rest, after standing and at all levels of exercise (Table 1).

Plasma concentrations of noradrenaline and adrenaline increased substantially in both groups of subjects during exercise corresponding to 75% of maximum work capacity, as compared with the data obtained at supine rest (Table 2). Neither at rest nor during ergometry at 75% of maximum work capacity did normotensive and borderline hypertensive subjects differ in the plasma levels of noradrenaline and adrenaline (Table 2). Fifteen

**Table 1. Systolic blood pressure, diastolic blood pressure and heart rate in (a) the normotensive controls and (b) the borderline hypertensive subjects**

<table>
<thead>
<tr>
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<th>Supine (30 min)</th>
<th>Standing (2 min)</th>
<th>Exercise 50% maximum work capacity</th>
<th>Exercise 75% maximum work capacity</th>
<th>Maximum work capacity</th>
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<td></td>
<td>(a)</td>
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<tr>
<td>Heart rate (beats/min)</td>
<td>$63 ± 2$</td>
<td>$73 ± 2$</td>
<td>$77 ± 2$</td>
<td>$88 ± 3$</td>
<td>$120 ± 3$</td>
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<tr>
<td>$P$</td>
<td>&lt;0·001</td>
<td>&lt;0·01</td>
<td>&lt;0·05</td>
<td>&lt;0·05</td>
<td>&gt;0·1</td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>$119 ± 1$</td>
<td>$143 ± 1$</td>
<td>$120 ± 1$</td>
<td>$148 ± 1$</td>
<td>$157 ± 3$</td>
</tr>
<tr>
<td>$P$</td>
<td>&lt;0·001</td>
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<td>&lt;0·05</td>
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<td>Diastolic blood pressure (mmHg)</td>
<td>$74 ± 1$</td>
<td>$91 ± 1$</td>
<td>$81 ± 1$</td>
<td>$95 ± 1$</td>
<td>$72 ± 2$</td>
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<tr>
<td>$P$</td>
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subjects with borderline hypertension had the same maximum work capacity as eight normotensive subjects, namely 200 W. At 75% of their maximum work capacity, that is 150 W, plasma noradrenaline concentration was 6·93 ± 0·95 nmol/l in the borderline hypertensive and 6·16 ± 0·95 nmol/l in the normotensive subjects. At the same level of exercise, plasma adrenaline level was 0·93 ± 0·16 nmol/l in the former and 0·87 ± 0·05 nmol/l in the latter. The differences between the borderline hypertensive and the normotensive groups are not statistically significant (P > 0·1).

Plasma renin activity and plasma renin concentration were almost identical in the two groups both at supine rest and during exercise at 75% of maximum work capacity (Table 2). When plasma renin activity measured during supine rest was plotted against daily urinary sodium excretion, three borderline hypertensive subjects were above (group A) and three were below (group B) the range of values obtained in the normotensive group. The same was true when in place of resting plasma renin activity values the data measured during ergometry at 75% of maximum work capacity were used. In group A, plasma noradrenaline concentration was 3·32 ± 1·30 nmol/l at rest and 7·22 ± 2·25 nmol/l at 75% of maximum work capacity. The corresponding values of group B were 2·78 ± 0·89 and 6·97 ± 0·71 nmol/l. Plasma adrenaline concentration in group A was 0·46 ± 0·05 nmol/l at rest and 0·79 ± 0·16 nmol/l at 75% of maximum work capacity. The data in group B were 0·61 ± 0·38 and 0·82 ± 0·33 nmol/l respectively. Thus plasma catecholamine levels in the borderline hypertensive subjects with high (group A) and low (group B) plasma renin activity were comparable with the values measured in the normotensive group (Table 2).

Discussion

In the present study there was no difference between borderline hypertensive and normotensive subjects in the plasma levels of noradrenaline and adrenaline as well as in plasma renin activity and plasma renin concentration at rest and during physical activity. Furthermore, the two groups of subjects did not differ in the urinary excretion of noradrenaline, adrenaline and HMMA. Similar results were obtained by Weidmann, Keusch, Flanner, Ziegler & Reubi (1979). Esler, Julius, Zweifler, Randall, Harburg, Gardiner & De Quattro (1977) studied a group of patients with mild hypertension whose blood pressures ranged from 150/90 and 175/105 mmHg, these being not much higher than the values in our study. These authors found elevated plasma noradrenaline concentrations in subjects with mild hypertension and high plasma renin activity, whereas plasma noradrenaline levels in hypertensive subjects with normal plasma renin activity were identical with the concentrations measured in normotensive subjects. Thus, as far as normal renin hypertensive subjects are concerned, the data of Esler et al. (1977) are comparable with those of the present study. In contrast to Esler et al. (1977), plasma noradrenaline levels in the few high renin borderline hypertensive subjects studied by us were comparable with the values in normotensive subjects. However, the number of borderline hypertensive subjects with high plasma renin activity in our study is too small to allow any conclusions.

Miura, Kobayashi, Tomioka, Adachi & Yoshinaga (1978) described increased noradrenaline concentrations in plasma from patients with labile hypertension. It could be that in stressful situations such subjects are particularly prone to react with an enhanced release of catecholamines into the circulation. The same might be true for the volunteers studied by Cousineau, de Champlain & Lapointe (1978).

In the study by Robertson, Shand, Hollifield, Nies, Fröhlich & Oates (1979), there were no differences in plasma noradrenaline levels at
supine rest between normotensive and borderline hypertensive subjects. The latter were defined as having blood pressure readings greater than 150/95 mmHg separated by a reading of less than 140/90 mmHg. However, plasma noradrenaline concentrations in borderline hypertensive subjects were higher than in normotensive subjects during physical activity and during sodium deprivation. The conclusions of Robertson et al. (1979) are based on a rather small number of subjects (seven normotensive and eight to nine borderline hypertensive). There is substantial overlap of the plasma noradrenaline levels in the two groups. Inclusion of a few more subjects could make the differences to be no longer statistically significant. Furthermore, these authors used exercise on a treadmill without taking into account that there might be differences between the two groups of subjects studied in the maximum tolerated work load. Thus differences in conditioning could have caused the different plasma levels of noradrenaline. Finally, sodium deprivation caused a somewhat more pronounced fall of mean blood pressure in borderline hypertensive than in normotensive subjects. Although the differences were not statistically significant with the small number of subjects studied, they might have been sufficient to cause a greater degree of sympathetic stimulation in the borderline hypertensive group.

The plasma catecholamine concentrations measured in normotensive subjects at supine rest in the present investigation are comparable with levels reported by other authors using radiometric techniques (Pedersen & Christensen, 1975; Lake et al., 1977; Sever, Birch, Osiowska & Turnbridge, 1977; Taylor et al., 1978). Lower noradrenaline concentrations in plasma from normotensive subjects were found by Engelman & Portnoy (1970), De Quattro & Chan (1972), Louis et al. (1974) and Esler et al. (1977). The differences may be due to the fact that these authors used hospital and laboratory personnel as normotensive subjects (Engelman & Portnoy, 1970; De Quattro & Chan, 1972) or studied hospitalized subjects after a prolonged period of rest (Louis et al., 1974; Esler et al., 1977). Jones, Hamilton & Reid (1979) have shown that in studies of plasma catecholamines in hypertensive subjects the choice of appropriate controls is most important. Plasma catecholamine levels measured during physical exercise in the present study are comparable with data obtained under similar conditions (Rahn et al., 1978; Christensen, Galbo, Hansen, Hesse, Richter & Trap Jensen, 1979).

Our plasma renin activity and plasma renin concentration values measured at rest and during physical activity in normotensive subjects are similar to data reported for subjects with a comparable salt intake (Kotchen, Hartley, Mougey, Jones & Mason, 1971; Brunner, Laragh, Baer, Newton, Goodwin, Krakoff, Bard & Bühler, 1972; Costill, Branam, Fink & Nelson, 1976; Kosunen & Pakarinen, 1976). Like Werning, Fischer, Kaip, Stiel, Triebstein & Vetter (1972) and Robertson et al. (1979), we were unable to find differences in plasma renin activity between normotensive and borderline hypertensive subjects at supine rest. Furthermore, in our study both groups did not differ in resting plasma renin concentration values as well as in plasma renin activity and plasma renin concentration data estimated during physical activity. There are conflicting results in the literature for plasma renin activity in borderline hypertensive subjects during orthostasis. Werning et al. (1972) reported higher values in borderline hypertensive than in normotensive subjects during active orthostasis, whereas Robertson et al. (1979) found no difference between the two groups of subjects. An explanation for the discrepancies might be that Werning et al. (1972) did not take into account differences in sodium balance whereas Robertson et al. (1979) studied the volunteers under metabolic ward conditions with a constant sodium intake. Robertson et al. (1979) found lower plasma renin activity in normotensive than in borderline hypertensive subjects during sodium deprivation. However, as has been mentioned earlier, this may be due to the fact that low sodium intake caused a more pronounced fall of mean arterial pressure in the latter group.

The lack of a difference in plasma catecholamine levels between normotensive and borderline hypertensive subjects in the present study does not support the assumption of enhanced sympathetic activity in borderline hypertension. However, one has to consider that only a portion of borderline hypertensive subjects will reach definitively hypertensive blood pressures, that is blood pressures of 160/100 mmHg or above, in the course of years. It could be that plasma catecholamines rise only in these subjects, either as a cause or as a consequence of increasing blood pressure.

It seems surprising that, in spite of comparable plasma catecholamine concentrations, normotensive and borderline hypertensive subjects differed in heart rate at rest and during physical exercise, except for the level of maximum work load. Increased heart rates at rest and during
activity have been described in a substantial proportion of subjects with borderline hypertension, as has been reviewed by Julius & Schork (1971).

Higher heart rates in borderline hypertensive than in normotensive subjects together with similar plasma catecholamine levels in both groups could be explained by assuming that there is enhanced sympathetic stimulation of the heart in the former which is poorly reflected in peripheral venous catecholamine concentrations. This possibility cannot be excluded at present. However, there are haemodynamic data obtained after autonomic blockade which suggest that enhanced sympathetic activity is at least not the only cause of the increased heart rate found in borderline hypertensive subjects (Sannerstedt, Julius & Conway, 1970; Julius, Pascual & London, 1971). A second explanation of the differences in heart rate in spite of similar plasma catecholamine levels would be a higher sensitivity of cardiac β-adrenoreceptors in borderline hypertensive subjects. This assumption, too, is very unlikely since the positive chronotropic effect of isoprenaline was similar in normotensive and borderline hypertensive subjects (Safar, London, Weiss & Milliez, 1975). A third explanation of the higher heart rates measured in borderline hypertensive subjects could be a decreased parasympathetic tone in these subjects. This assumption gets support from the study by Julius et al. (1971) and could explain the disappearance of differences in heart rate during physical exercise at a high work load. As Robinson, Epstein, Beiser & Braunwald (1966) have shown, both the sympathetic and the parasympathetic nervous system control resting heart rate, whereas the sympathetic system predominates during strenuous exercise.

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Sympathetic nervous system in hypertension


