The urinary excretion of catecholamines and their derivatives in primary hypertension in man

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(Received 28 May 1976; accepted 22 October 1976)

Summary
1. The 24 h urinary excretion of adrenaline, noradrenaline, normetadrenaline, metadrenaline and vanilloylmandelic acid has been compared in 17 male normotensive subjects and 25 age-matched male hypertensive patients studied under similar in-patient conditions.

2. 24 h urinary metadrenaline was significantly lower in the hypertensive patients. With this exception, no significant differences were found between the two groups when the total 24 h excretion of free catecholamines and their metabolites was analysed.

3. Diurnal variation in free catecholamine excretion was found in both normotensive and hypertensive subjects. There was no corresponding variation in metabolite excretion.

4. No correlation could be established between systolic or diastolic blood pressure and the amounts of the catecholamines or their metabolites in the urine of either group.

5. The results are considered in the light of recent work demonstrating high plasma catecholamine concentrations in hypertension. They lend no support to the concept that excessive circulating catecholamines are responsible for the elevated blood pressure in essential hypertension.

Key words: catecholamines, hypertension, vanilloylmandelic acid.

Abbreviations: MA, metadrenaline; NMA, normetadrenaline.

Introduction
It is a matter of continuing debate whether or not patients with primary (or essential) hypertension can be shown to have enhanced activity of the sympathetic nervous system when compared with normal subjects. Sympathetic activity in hypertension has been assessed by physiological tests (Fröhlich, Tarazi, Ulrych, Dustan & Page, 1967), by the measurement of urinary catecholamines and their derivatives (Nestel & Esler, 1970), by the assay of plasma noradrenaline and dopamine β-hydroxylase (Geffen, Rush, Louis & Doyle, 1973) or by the turnover of radioactively labelled noradrenaline (Gitlow, Mendlowitz, Wilk, Wilk, Wolf & Naftchi, 1964). The work of De Quattro & Sjoerdsmwma (1968) seemed to point conclusively to normal sympathetic activity in primary hypertension, but the topic has recently assumed importance again with the work of De Quattro & Chan (1972) and Louis, Doyle & Anavekar (1973), who found elevated noradrenaline in urine, or plasma, in either a selected group of hypertensive subjects or in labile hypertension.

If elevated plasma concentrations of catecholamines exist in some or all patients with hypertension and the normal methods and routes of plasma clearance are not disturbed, this should be reflected in an enhanced urinary excretion of unchanged noradrenaline and adrenaline or their derivatives. One of the obstacles to accepting some of the earlier work on this topic as...
conclusive has been the relatively small number of subjects studied and the lack of controls matched for age, sex and activity. For this reason we have re-examined the urinary excretion of noradrenaline, adrenaline and their principal metabolites, normetadrenaline, metadrenaline and vanilloylmandelic acid, and their circadian variation in a group of hypertensive subjects and a matched group of normotensive control subjects.

Methods

Subjects

All subjects studied were male in-patients in the same hospital. A group of 25 were untreated hypertensives (blood pressure 166.0 ± 24.3 mmHg/106.8 ± 13.7 mmHg: mean values ± SD) admitted for investigation and treatment. Preliminary investigations carried out before admission had demonstrated no abnormalities in renal function (plasma urea, creatinine clearance, intravenous nephropyelography) or in plasma electrolytes. Femoral pulses were normal in all subjects and there was no clinical suggestion of Cushing's syndrome in any. The control group of 17 were normotensive subjects admitted for investigation of conditions unrelated to the cardiovascular system and receiving no medication (blood pressure 125.4 ± 11.0 mmHg/77.6 ± 6.0 mmHg: mean values ± SD). The mean ages of the groups were 45.4 ± 9.7 years and 43.3 ± 11.1 years respectively. Urine collections (collected in acid: 5 ml of HCl, 6 mol/l, final pH <3) over three successive periods of 8 h were made between 14.00 hours on the second and 14.00 hours on the third in-patient days. The subjects were all ambulant in the hospital ward and were receiving standard hospital diet. None received drugs of any description before the collections were completed. The hypertensive group could be further subdivided, on the basis of the subsequent fall or stability of blood pressure while untreated in hospital, into a 'labile' group of nine and a 'fixed' group of 16.

Analytical

Adrenaline and noradrenaline were measured by the modification of the fluorimetric trihydroxyindole conversion technique described in an earlier paper (Townshend & Smith, 1973). Metadrenaline and normetadrenaline were extracted as described by Bigelow & Weil-Malherbe (1968), 20 ml of acetic acid (0.5 mol/l) being used instead of 10 ml of formic acid (1.0 mol/l) for the elution from Amberlite. Eluates were stored at −20°C until analysed. The O-methylated derivatives were measured with an automated fluorimetric method similar to that used for noradrenaline and adrenaline, but incorporating the addition of zinc ions to catalyse oxidation at pH 5.5 by potassium ferricyanide (Brunjes, Wybenga & Johns, 1964). The resulting lutine derivatives were made strongly alkaline with sodium hydroxide (2.5 mol/l) to produce the fluorescent trihydroxyindoles. Ascorbic acid stabilized the fluorescence of both metadrenaline and normetadrenaline derivatives, whereas mercaptoethanol (0.143 mol/l) stabilized the fluorescence of the normetadrenaline derivative only. It proved impossible to use a series of extracted standards as described in our free catecholamine procedure and internal standards were necessary to allow for variation in the quenching effects produced by extracts from different urine samples. From each eluate, a series of five solutions in 3 ml of acetic acid (0.5 mol/l) was prepared. These contained 0.1 µg of MA, 0.05 µg of MA, 0.1 µg of NMA, 0.2 µg of NMA or no standard added to a constant volume of eluate (usually 0.5–1.0 ml). These five samples were cycled four times through the Auto-Analyzer and flow cell of the fluorimeter (Hitachi 203). Ascorbic acid was used as stabilizer in the first cycle, and replaced by water in the second. The excitation wavelength was 405 nm and emission wavelength 500 nm in these two cycles. During the third and fourth cycles with water and mercaptoethanol respectively, excitation wavelength was 400 nm and emission wavelength 485 nm (uncorrected wavelengths). Fluorimeter readings for both MA and NMA were linear over the range of concentrations used. Analysis of nine mixtures of solutions of MA and NMA in acetic acid (0.5 mol/l) gave recoveries averaging 95% (84–105%) for MA and 112% (108–116%) for NMA. The coefficients of variation of the determinations of MA and NMA in urine (19 extractions and estimations on the same sample) were ±22.6% for MA and ±13.8% for NMA. Mean amounts (±SD) of methylated catecholamines found in the 19 10 ml samples of a single
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Results
No significant differences were found between the hypertensive group and normal group in the 24 h excretion of noradrenaline, adrenaline, NMA or vanillylmandelic acid. The difference between the amounts of urinary MA was significant at the 5% level. Differences between mean systolic and diastolic blood pressures were highly significant. (Analysis of variance $P<0.01$: Table 1.)

Circadian variation in the urinary excretion of free catecholamines was identical in both groups of subjects; the levels were lowest during the period of sleep. There was no corresponding night-time fall in the excretion of the metabolites metadrenaline, normetadrenaline and vanillylmandelic acid (Fig. 1) (Klepping, Goudonnet, Didier & Escousse, 1971; Wisser & Knoll, 1975). However, the hypertensive subjects showed a mean excretion of NMA in the

| Table 1. Urinary excretion in 24 h of adrenaline, noradrenaline, metadrenaline, normetadrenaline and vanillylmandelic acid in male patients with high and normal blood pressure

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Normotensive</th>
<th>Hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>Age (years)</td>
<td>$43.3 \pm 11.1$</td>
<td>$45.4 \pm 9.7$</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic (mmHg)</td>
<td>$125.4 \pm 11.0$</td>
<td>$166.0 \pm 24.3^{* *}$</td>
</tr>
<tr>
<td>Diastolic (mmHg)</td>
<td>$77.6 \pm 6.0$</td>
<td>$106.8 \pm 13.7^{* *}$</td>
</tr>
<tr>
<td>Adrenaline (nmol/24 h)</td>
<td>$66.0 \pm 26.7$</td>
<td>$77.5 \pm 41.5$</td>
</tr>
<tr>
<td>Noradrenaline (nmol/24 h)</td>
<td>$215.1 \pm 85.1$</td>
<td>$195.0 \pm 112.3$</td>
</tr>
<tr>
<td>MA (nmol/24 h)</td>
<td>$975.2 \pm 245.4$</td>
<td>$779.9 \pm 259.6^{*}$</td>
</tr>
<tr>
<td>NMA (nmol/24 h)</td>
<td>$2236 \pm 978.2$</td>
<td>$2044 \pm 806.8$</td>
</tr>
<tr>
<td>Vanillylmandelic acid ($\mu$mol/24 h)</td>
<td>$15.9 \pm 3.8$</td>
<td>$15.5 \pm 4.2$</td>
</tr>
</tbody>
</table>

| Table 2. Proportions of 24 h urinary free catecholamines (expressed as unity) to total O-methylated derivatives and vanillylmandelic acid in normal subjects, hypertensive subjects with primary hypertension and subjects with phaeochromocytoma

<table>
<thead>
<tr>
<th>Patients</th>
<th>Urinary adrenaline and noradrenaline</th>
<th>Total O-methyl derivatives</th>
<th>Vanillylmandelic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive ($n = 17$)</td>
<td>$1$</td>
<td>$12.4$</td>
<td>$64.9$</td>
</tr>
<tr>
<td>Labile hypertensive ($n = 9$)</td>
<td>$1$</td>
<td>$11.2$</td>
<td>$60.8$</td>
</tr>
<tr>
<td>Stable hypertensive ($n = 16$)</td>
<td>$1$</td>
<td>$11.2$</td>
<td>$67.4$</td>
</tr>
<tr>
<td>Phaeochromocytoma I (urinary noradrenaline 17.2 $\mu$mol/24 h)</td>
<td>$1$</td>
<td>$4.2$</td>
<td>$7.1$</td>
</tr>
<tr>
<td>Phaeochromocytoma II (urinary noradrenaline 2.2 $\mu$mol/24 h)</td>
<td>$1$</td>
<td>$14.5$</td>
<td>$18.7$</td>
</tr>
</tbody>
</table>
from our 25 hypertensive subjects were divided into two groups, 16 'fixed' and nine 'labile', and compared. No significant differences were found between 24 h or 8 h excretion data for any of the compounds measured. Systolic and diastolic blood pressure did not correlate with urinary free catecholamines for the hypertensive group as a whole.

The proportions of 24 h urinary total free catecholamines/total O-methylated derivatives/vanilloylmandelic acid were the same in normotensive, 'labile' and 'fixed' hypertensive subjects and contrasted markedly with these proportions in two patients with phaeochromocytomas, whose elevated blood pressure was cured by removal of their tumours and in whom the proportions thereafter approximated to those found in our control and hypertensive subjects (Table 2).

Discussion

Three pressor substances can be identified, which may be present in excess in some forms of human hypertension: noradrenaline in phaeochromocytoma, sodium and water in, for example, renal failure or mineralocorticoid excess, and angiotensin II in some forms of renovascular disease. However, these pressor mechanisms can only be related aetiologically to the elevation of blood pressure in a minority of hypertensive patients. Many workers have examined the possibility of increased sympathetic activity in a larger number of patients than the very few whose raised blood pressure is sustained by catecholamines secreted by a phaeochromocytoma, but our results are at variance with several recent papers which revive the hypothesis that small increases in catecholamine output may be responsible for early labile or even established hypertension.

We have measured only the urinary excretion of free catecholamines, O-methylated derivatives and vanilloylmandelic acid and have not been able to examine at the same time plasma noradrenaline or urinary dopamine or phenylglycols and to this extent our survey of chemical indices of sympathetic function in our two groups is incomplete. However, if plasma noradrenaline is elevated in hypertension, an increase in urinary excretion of either free catecholamines or their principal metabolites would be expected. We have been unable to

Fig. 1. Mean values (± sd) for urinary excretion per 8 h of adrenaline, noradrenaline, metadrenaline, normetadrenaline and vanilloylmandelic acid (VMA) in groups of (a) 17 normotensive and (b) 25 hypertensive subjects.

'morning' period (06.00–14.00 hours) which was significantly lower than the 'afternoon' period (14.00–22.00 hours) (P < 0.001: paired t-test), a difference that was not apparent in the normotensive group and for which we can find no explanation.

As it has been suggested that elevated catecholamine concentrations may be found particularly in labile hypertensive subjects, the data
demonstrate such an increase and our findings are supported by similar recent data reported by Berglund, Tibblin & Aurell (1975).

Is it then possible to reconcile our results with those of Louis et al. (1973), who found in their subjects a linear correlation between recumbent diastolic blood pressure and plasma noradrenaline? If both sets of observations are true, a defect in renal clearance of catecholamines may exist in hypertensive patients which might be causally or consecutively related to the blood pressure elevation. There are several precedents for an abnormality in renal function consequent on hypertension, notably in relation to sodium and water excretion and uric acid elimination.

However, the suggestion that the elevation in plasma noradrenaline reported by Louis et al. (1973) is sufficient to account for and is causally related to the measured hypertension demands further examination. The administration of α-receptor-blocking drugs such as phentolamine or phenoxybenzamine will nearly always reduce blood pressure in phaeochromocytoma, as might be expected if noradrenaline excess is causally related to the hypertension, but commonly has little effect in essential hypertension. Secondly, peripheral noradrenaline plasma concentration may be very high (120 nmol/l in one of our recent patients) in phaeochromocytoma without elevation of the blood pressure to values comparable with those commonly seen in benign essential hypertension. Thirdly, when the tyrosine hydroxylase inhibitor α-methylparatyrosine was given to hypertensive patients in order to inhibit the synthesis of catecholamines, blood pressure was not substantially reduced although catecholamine output was reduced by approximately 50% (Sjoerdsma, 1967).

For all these reasons it seems difficult to sustain the argument that an elevation in plasma noradrenaline is causal in many patients with hypertension. It might be appropriate to examine further the hypothesis that in some patients raised plasma catecholamines may be a consequence rather than a cause of this condition.

Acknowledgments
We thank the Endowment Research Fund of the former United Sheffield Hospitals for financial support for R.F.B. and M.M.T.

References


