Choice of control groups in the appraisal of sympathetic nervous activity in essential hypertension

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Summary

1. Plasma noradrenaline concentrations were similar in normotensive and hypertensive outpatients, but were significantly lower in laboratory control subjects.

2. Standing plasma noradrenaline concentrations were similar in all three groups.

3. Urinary vanillyl mandelic acid, catecholamines and metanephrines were also similar in the normotensive and hypertensive groups.

4. Laboratory controls, possibly because of familiarity with the techniques of sphygmomanometry and blood sampling, may attain a 'basal' resting level of sympathetic nervous discharge more readily and rapidly than subjects who are unfamiliar with such procedures.

5. After orthostatic stimulation by standing for 2 min, the activity of the sympathetic nervous system, as determined by pulse rate and plasma noradrenaline concentrations, was similar in the three groups, despite the lower starting values in the laboratory staff.

6. The absence of differences in plasma noradrenaline or urinary catecholamine and metabolite concentrations does not support the hypothesis of excessive sympathetic nervous activity in essential hypertension.

Key words: catecholamine excretion, metabolite excretion, hypertensive outpatients, noradrenaline,

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Introduction

‘Normal ranges’ for a given variable, particularly in the early phases of evaluation, are often calculated by collecting measurements from a readily available source, such as healthy laboratory personnel or volunteer subjects, who may be medical or paramedical staff. When the range is defined, it is then used as a reference when the measurement is performed in abnormal or pathological states. This arrangement does not usually cause problems, but, if the index being studied is readily influenced by stress, anxiety, fear, physical activity etc., the application of such a ‘normal range’ may be inappropriate. This is true of plasma noradrenaline, which is influenced by physical or psychological stress.

There has been much investigation into the role of the sympathetic nervous system in hypertension, and, until 1970, the only consistently reliable measurements were of urinary catecholamines and their metabolites (de Champlain, 1977). Over the last decade, specific, sensitive radioenzymatic assay techniques have been developed and applied to the measurement of catecholamines in plasma. Plasma noradrenaline is now regarded as the single most useful index of sympathetic neuronal activity (Lake, Ziegler & Kopin, 1976). Whereas urinary catecholamine measurements provide an integrated index of sympathetic nervous activity, plasma noradrenaline measurements can reflect short-term variation in sympathetic discharge. Plasma noradrenaline
estimation has superseded the urinary measure-
ments in many studies aimed at investigating the con-
tribution of the sympathetic nervous system to the
determination and regulation of blood pressure. How-
ever, the concentrations of plasma noradrenaline, because of its intimate association with sympa-
thetic efferent activity, can be affected by anxiety and physical activity.

The present study describes the measurement of blood pressure, pulse rate and plasma noradrenaline in supine and standing subjects, and examines the differences that are obtained when laboratory personnel, normotensive outpatients and hypertensive patients are compared. It serves to illustrate the importance of selecting appropriate controls for the determination of the 'normal range' and how incorrect choice of controls may produce misleading results.

**Subjects and methods**

Three groups of subjects were studied.

Group 1. Twelve healthy male laboratory staff, ten of whom were medically qualified, one was a postgraduate pharmacology student and one was a third-year preclinical medical student.

Group 2. Untreated normotensive outpatients (resting supine blood pressure less than 150/100 mmHg) attending a hospital outpatient department for the first time for assessment of symptoms such as headache, tiredness and weakness, dizziness, chest discomfort and abdominal pain. No abnormal pathology was detected at consultation or during routine investigation in this group of patients (n = 16).

Group 3. Hypertensive outpatients (resting supine blood pressure greater than 150/100 mmHg) who were attending the clinic for the first time for assessment of their blood pressure, and who were not receiving treatment. Subsequent investigation did not reveal an underlying secondary cause for the hypertension (n = 31).

Having been seen in the outpatient clinic, the patients were asked to return on a separate occasion for a blood pressure check and for blood sampling. They were also requested to provide a 24 h urine collection. All the measurements were made between 09.00 and 12.00 hours, the subjects having fasted from midnight on the previous evening. An indwelling needle, filled with heparin/saline, was inserted into the antecubital vein of the resting, supine patient, and a blood pressure cuff was placed on the contralateral arm. During the following 10 min period the blood pressure was checked at intervals, and the readings were generally stable (consecutive recordings within 5 mmHg) after 7–8 min. At 10 min the pulse was counted for 1 min, and duplicate blood pressure readings were taken. Blood was withdrawn into a chilled heparin-treated tube for measurement of supine plasma noradrenaline concentration. The patient then stood up for 2 min and duplicate blood pressure recordings were made at the end of this period. The pulse rate was counted during the period 2–3 min, and a blood sample was withdrawn after 2½–3 min standing. All blood pressure recordings were made with the automatic ultrasound sphygmomanometer (Arteriosonde 1217, Roche). None of the subjects was taking any medication.

The plasma noradrenaline concentrations were estimated with a sensitive, specific radioenzymatic assay (Henry, Starman, Johnson & Williams, 1975), the inter-assay and intra-assay coefficients of variation for our laboratory being 12% and 8% respectively. In the outpatient subjects, urinary noradrenaline was measured by the fluorimetric method of von Euler & Lishajko (1961). Urinary metanephrines were measured spectrophotometrically by Pisano's (1960) method, and urinary vanillyl mandelic acid by another spectrophotometric technique (Pisano, Crout & Abraham, 1962). Creatinine was measured in urine by a spectrophotometric method utilizing the Jaffé reaction (Owen, Iggo, Scandrett & Stewart, 1954). Blood pressure, pulse rate and biochemical results were compared by Student's t-test for unpaired data, and by linear regression analysis.

**Results**

Table 1 summarizes the supine and standing blood pressures, pulse rates and plasma noradrenaline values in the three groups of subjects. The results of the urinary catecholamine and metabolite excretion for the normotensive and hypertensive outpatient groups are also shown, as are the mean ages, and the results of the statistical comparisons.

The laboratory staff had a low resting supine blood pressure and pulse rate, and these are significantly lower (P < 0.001) than those of the normotensive outpatients. The standing systolic pressure is also lower in the volunteer subjects than in the normotensive subjects, but there are no significant differences between the standing diastolic pressures and pulse rates (P > 0.5 and >0.1 respectively). The mean supine plasma noradrenaline concentration in the laboratory staff is lower than in
Controls in assessment of sympathetic activity

The urinary results from the two outpatient groups are also shown. N.S., Not significant. Results shown as means ± SEM.

<table>
<thead>
<tr>
<th>Laboratory staff</th>
<th>Normotensive outpatients</th>
<th>Hypertensive outpatients</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Age (years)</td>
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<tr>
<td></td>
<td></td>
<td>31 ± 2</td>
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<tr>
<td><strong>Supine</strong></td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
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<td>104.7 ± 4.3</td>
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<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td></td>
<td>66.1 ± 2.1</td>
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<tr>
<td>Pulse rate (beats/min)</td>
<td></td>
<td>66 ± 3</td>
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<tr>
<td>Plasma noradrenaline (nmol/l)</td>
<td></td>
<td>1.42 ± 0.12</td>
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<tr>
<td><strong>Standing</strong></td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
<td></td>
<td>112.1 ± 2.5</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td></td>
<td>84.3 ± 3.1</td>
</tr>
<tr>
<td>Pulse rate (beats/min)</td>
<td></td>
<td>88 ± 3</td>
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<tr>
<td>Plasma noradrenaline (nmol/l)</td>
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<td>3.19 ± 0.47</td>
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<td><strong>Urinary studies</strong></td>
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<tr>
<td>Vanillyl mandelic acid (µmol/24 h)</td>
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<tr>
<td>Catecholamines (nmol/nmol of creatinine)</td>
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<td>Metanephrines (nmol/nmol of creatinine)</td>
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</table>

the normotensive patients (mean ± SEM: 1.42 ± 0.12 and 2.60 ± 0.41 nmol/l, P < 0.05), but the standing concentrations are not significantly different (3.19 ± 0.12 and 3.78 ± 0.53 nmol/l, P > 0.5). The mean ages of the two groups are also significantly different (31 ± 2 and 41 ± 3 years, P < 0.01).

Comparison of the outpatient groups shows that the hypertensive patients have supine and standing blood pressure values that are all significantly higher (P < 0.001) than those of the normotensive subjects. The plasma noradrenaline concentrations for the two groups are similar [supine values: 2.60 ± 0.41 and 2.42 ± 0.24 nmol/l (P > 0.7) for the normotensives and hypertensives respectively; standing values: 3.78 ± 0.53 and 3.78 ± 0.35 nmol/l respectively (P > 0.9)]. There is no significant difference (P > 0.05) in the mean ages of these groups (41 ± 3 and 47 ± 2 years respectively). The plasma noradrenaline results are represented in scattergram form in Fig. 1 and Fig. 2.

No relationship was found in any group between blood pressure and plasma noradrenaline concentration, neither was there any correlation between the change in blood pressure and the change in plasma noradrenaline concentrations on standing. Urinary concentrations of vanillyl mandelic acid, noradrenaline and metanephrines show no significant differences when the results of the two outpatient groups are compared (P > 0.2, >0.05 and >0.6 respectively).

Fig. 1. Scattergram of supine plasma noradrenaline concentrations in laboratory staff, normotensive outpatients and hypertensive outpatients. The mean values ± SEM are also shown.

Discussion

Engelman, Portnoy & Sjoerdsma (1970) stated that their measurement in 32 'normal, resting subjects has defined a normal range of values both for total catecholamine content . . . of plasma . . . and for norepinephrine and epinephrine'. Their value of 1·36 nmol/l for total catecholamines for their 'normals' is significantly lower (P < 0.001) than the value of 2·62 nmol/l for 18 resting
patients. However, no information is given as to the selection of the 'normal' controls or of the age groups.

The hypertensive patients studied by De Quattro & Chan (1972) had a mean catecholamine concentration higher \( P < 0.05 \) than that of the 'control' group; the latter comprised two groups, volunteers and hospitalized normotensive patients (these groups having similar mean catecholamine concentrations). Details of age-matching are lacking, but the authors are reserved in their conclusion that 'excessive plasma catecholamines in some patients may reflect increased sympathetic tone, and be a pathogenic factor in their hypertension'.

Louis, Doyle & Anavekar (1975) reported a mean plasma noradrenaline value of 1.18 nmol/l in 'normotensive volunteers' and 2.36 nmol/l in essential hypertension. No description of the method of selection of volunteers was given, however, and no details of age-matching.

Lake et al. (1976) quoted values of 1.71 ± 0.12 and 1.83 ± 0.18 nmol/l for the mean plasma noradrenaline concentrations in 'resting, supine, normal subjects' and 'hypertensive subjects' respectively. Details are not included as to how many laboratory staff are incorporated in the 'normal subjects', but a later communication by this group (Lake, Ziegler, Coleman & Kopin, 1977) described similar plasma noradrenaline concentrations in normotensive and hypertensive subjects when the results are age-adjusted.

de Champlain (1977) and de Champlain & Cousineau (1977) reported a significantly higher plasma catecholamine concentration in hypertensive subjects, compared with normotensive subjects, this being independent of age and sex.

In the study by Sever, Birch, Osikowska & Tunbridge (1977), the normotensive and hypertensive subjects were age-matched, and no difference was found between the supine plasma noradrenaline concentrations of the two groups (2.36 ± 0.12 and 2.42 ± 0.12 nmol/l respectively). The majority of the normotensive controls in their study were from a civil service health screen, but hospital or laboratory personnel comprised 20%, and were particularly prominent in the younger age group. Although no difference was demonstrated between the mean plasma noradrenaline concentrations in the normotensive and hypertensive subjects, the relationship between noradrenaline and age differed in these two groups. The present study, like the results of Sever et al. (1977), shows that mean supine plasma noradrenaline concentrations are the same in normotensive and hypertensive outpatients of similar age.

The supine plasma noradrenaline concentration in the laboratory staff is significantly lower than in the other two groups. The blood pressure values are also lower in this group, but this cannot be proposed as a reason for the difference in plasma noradrenaline concentrations, because a similar difference in blood pressure is seen between the normotensive and hypertensive groups. However, there are at least two other important differences between the laboratory staff and the normotensive outpatients: the mean age and the pulse rate.

Age-related rises in plasma noradrenaline concentrations have been described (Lake et al., 1976, 1977; Sever et al., 1977; Franco-Morselli, Elghozi, Joly, DiGiulio & Meyer, 1977; Jones, Hamilton & Reid, 1978), and the argument could be proposed that the lower noradrenaline concentration in the laboratory personnel was due to a difference in age. However, the age-related rise is modest, and the application of an age-correction factor (from the regression equations described by Lake et al., 1977, and Sever, 1978) still results in significantly different mean plasma noradrenaline concentrations in these two groups.

The mean pulse rate in the laboratory volunteer group is significantly lower than that of the normotensive outpatient group. This, together with a
were familiar with venepuncture and sphygmomanometry and thus might attain a more basal sympathetic discharge much sooner than their outpatient counterparts. The much smaller scatter of noradrenaline results in the supine position (Fig. 1), which is reflected in the smaller variance, also lends support to this argument.

Comparison of the blood pressures, pulse rates and plasma noradrenaline concentrations in the laboratory staff and the hypertensive patients shows similar results to those obtained with the laboratory subjects and the normotensive patients. In the absence of a normotensive group, it might be tempting to say that the elevated pulse and blood pressure in the hypertensive patients could be the result of increased sympathetic nervous activity. However, inclusion of the normotensive group (who show pulse rates and plasma noradrenaline concentrations similar to those of the hypertensive patients) detracts from this argument, and a reasonable conclusion from the comparison of the three groups is that both outpatient groups have not attained the same ‘basal’ level of sympathetic discharge as the volunteers (as implicated by the large variance of the noradrenaline values in Fig. 1). This difference in sympathetic nervous activity cannot, however, be proposed as a cause of raised blood pressures in the hypertensive patients. The groups were allowed 10 min in the supine position, and consideration must be given to the fact that a longer resting period might allow the patients to assume a more basal sympathetic discharge, to approximate to that of the laboratory staff. Whether this would have occurred to a similar degree in both normotensive and hypertensive patients is conjectural.

When the erect posture is assumed, the scatter of the standing plasma noradrenaline values is much greater, and displays a pattern similar to that of the normotensive outpatients (Fig. 2). The mean standing plasma noradrenaline values (3·19 ± 0·47 nmol/l in the laboratory staff, 3·78 ± 0·53 nmol/l in the normotensive outpatients, and 3·78 ± 0·35 nmol/l in the hypertensive outpatients) are not significantly different, suggesting that for a given stimulus (postural change for 2½ min in this case), the response of the sympathetic nervous system is independent of its basal discharge. Hence, the percentage increase in plasma noradrenaline concentration is much greater in the laboratory staff (120%) as compared with the normotensive outpatients (38%) and the hypertensive outpatients (42%). The standing diastolic blood pressures and pulse rates in the laboratory personnel and normotensive subjects were not statistically significantly different (P > 0·5 and >0·1 respectively) and these again suggest that the response of the sympathetic nervous system to standing for this time is independent of its basal activity.

de Champlain & Cousineau (1977), in a study of 24 normotensive and 44 hypertensive patients, suggest that in some hypertensive patients there is an ‘hyperadrenergic’ state, and that sympathetic function is abnormal in such patients, the dysfunction playing an important role in the maintenance of their hypertension. In the present study, the range of plasma noradrenaline was similar in hypertensive and normotensive subjects, so that a ‘hyperadrenergic’ group could be identified only if the hypertensive patients are compared with the laboratory staff. This comparison does not appear

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**Table 2. Comparison of plasma noradrenaline concentrations and urinary catecholamines and metanephrines in outpatients with normal or borderline elevated vanillyl mandelic acid**

<table>
<thead>
<tr>
<th>Urinary metanephrines</th>
<th>Normal vanillyl mandelic acid (&lt;35 μmol/24 h)</th>
<th>Raised vanillyl mandelic acid (&gt;35 μmol/24 h)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of urine samples analysed</td>
<td>22</td>
<td>12</td>
<td>N.S. (P &gt; 0·7)</td>
</tr>
<tr>
<td>Urinary catecholamines (nmol/mmol of creatinine)</td>
<td>29 ± 5</td>
<td>32 ± 7</td>
<td>N.S. (P &gt; 0·5)</td>
</tr>
<tr>
<td>Urinary metanephrines (nmol/mmol of creatinine)</td>
<td>370 ± 44</td>
<td>330 ± 40</td>
<td>N.S. (P &gt; 0·2)</td>
</tr>
<tr>
<td>Supine plasma noradrenaline (nmol/l)</td>
<td>2·18 ± 0·30</td>
<td>2·83 ± 0·41</td>
<td>N.S. (P &gt; 0·5)</td>
</tr>
<tr>
<td>Standing plasma noradrenaline (nmol/l)</td>
<td>3·42 ± 0·47</td>
<td>3·84 ± 0·53</td>
<td>N.S. (P &gt; 0·5)</td>
</tr>
</tbody>
</table>
justified. In addition, no support for the concept of a separate 'hyperadrenergic' group was obtained from study of urinary catecholamine excretion.

When patients are assessed in a hypertension clinic, they are often screened for the rare, but potentially curable, phaeochromocytoma, as a secondary cause of the hypertension. Such a screen usually involves measurement of vanillyl mandelic acid quantitatively in a 24 h urine collection. Even when the quantitative spectrophotometric method of Pisano et al. (1962) is used, modest elevation of vanillyl mandelic acid excretion may be noted in patients who, on further investigation, are shown not to have a phaeochromocytoma. In the study described, there were 12 subjects with vanillyl mandelic acid concentrations at, or above, the upper limit of the normal range (<35 μmol/24 h) from the 34 urine samples analysed from the combined outpatient group. Comparison of the values for urinary metanephrines, urinary catecholamines and supine and standing plasma noradrenaline concentrations in these patients with raised vanillyl mandelic acid with those of the patients with normal vanillyl mandelic acid values showed no significant differences between the values (Table 2). Hence, analysis of the results by this alternative approach again does not support an 'hyperadrenergic' concept.

The study thus emphasizes that the appropriate choice of controls is essential in the definition of a 'normal range', especially when the variables investigated are under the influence of the central nervous system and sympathetic activity. If this is not considered, inappropriate conclusions may be drawn. Further, as a result of critical group selection, there is no good evidence to support the hypothesis of excessive sympathetic nervous activity as reflected by plasma noradrenaline and urinary catecholamine measurements in patients with essential hypertension.

Acknowledgments

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References


