Variations in individual organ release of noradrenaline measured by an improved radioenzymatic technique; limitations of peripheral venous measurements in the assessment of sympathetic nervous activity

M. J. BROWN, D. A. JENNER, D. J. ALLISON AND C. T. DOLLERY
Departments of Clinical Pharmacology and Radiology, Royal Postgraduate Medical School, London

(Received 28 November 1980/6 March 1981; accepted 11 May 1981)

Summary

1. The validity of plasma noradrenaline as an index of sympathetic nervous activity was assessed by estimating variation in individual organ contribution to circulating concentrations.

2. Arteriovenous (A–V) differences in noradrenaline and adrenaline concentration were measured across several organs in nine patients with mild essential hypertension, in five with renal artery stenosis and 15 phaeochromocytoma patients.

3. In patients with phaeochromocytomas the percentage extraction of noradrenaline and adrenaline (estimated from the A–V differences) was similar across all organs, suggesting that adrenaline extraction could be used as a marker for noradrenaline extraction.

4. In the non-tumour patients the A–V difference for noradrenaline was less than that for adrenaline across most organs studied, reflecting the net result of noradrenaline release and extraction. The estimated contribution of various organs to the noradrenaline concentrations in their venous effluent was: heart 21%; kidney 47%; legs 68%.

5. This pattern of A–V difference proved a positive diagnostic feature for non-tumour patients since it was not found even in the patients with small phaeochromocytomas, whose peripheral venous noradrenaline concentration alone did not distinguish them.

6. The venous–arterial difference across the adrenal glands of non-tumour patients was more than 10-fold greater for adrenaline than that for noradrenaline. Since the mean arterial concentration of noradrenaline was more than fivefold higher than that of adrenaline, the normal adrenal contribution to circulating noradrenaline is likely to be less than 2%.

7. In the patients with renal artery stenosis renal venous concentrations of noradrenaline (from the ischaemic kidney) were higher than arterial values, but mean arterial values were no higher than in the essential hypertensive patients.

8. Local variations in sympathetic activity may occur without altering the plasma noradrenaline concentration measured in peripheral plasma.

Key words: adrenal medulla, heart, kidney, noradrenaline, sympathetic activity.

Introduction

The measurement of plasma noradrenaline concentration remains the most convenient tool for estimating short-term changes in sympathetic nervous activity in man [1, 2]. Several unproven assumptions are, however, involved in the use of such an index. One of these is that an increased peripheral vein concentration of noradrenaline reflects a general increase in noradrenaline release throughout the body or, conversely, that such a general increase will necessarily cause a rise in peripheral venous noradrenaline concentration.
further assumption is that the escape of noradrenaline from synaptic clefts into the bloodstream is uniform in different organs. An opportunity to test these assumptions was provided in the present study by the measurement of the arteriovenous (A–V) difference of noradrenaline across several organs in patients undergoing catheter studies in their clinical investigation. Since these differences are the net result of extraction and release, the use of adrenaline differences as a marker for noradrenaline extraction was investigated. The A–V difference for adrenaline across all organs except the adrenal medulla reflects extraction alone, since only the adrenal (outside the brain) can synthesize adrenaline [3]. Since adrenaline is cleared by the same routes as noradrenaline [4], its extraction by a tissue is likely to be similar to that of noradrenaline. So the adrenaline difference could be used to predict that of noradrenaline if it was extracted but not released; the discrepancy between the predicted and observed A–V difference for noradrenaline would then be due to noradrenaline release by the tissue. The validity of this model was tested in patients with large phaeochromocytomas, in whom virtually all circulating catecholamines were derived from one site, with little contribution from other organs.

Subjects and methods

Studies were performed in patients being investigated for suspected phaeochromocytomas (24), renal artery stenosis (five) or coronary artery disease (four). None of the first or third group had received any medication for at least 2 weeks before investigations. All of the second group were receiving hydralazine, a β-adrenoceptor antagonist (propranolol or atenolol) and a diuretic. Studies were performed 1 h after premedication with pethidine (50 mg) and promazine (50 mg); general anaesthesia was not used. In the patients with elevated plasma catecholamine concentrations selective venous sampling was performed to determine whether there was a single (tumour) source for the elevated secretion and, if so, to localize it [5]. In 13 patients a tumour was identified (and subsequently removed) in one or both adrenals, and in two others an extra-adrenal phaeochromocytoma was found. No tumour was found in the other nine patients (of this group) and introduction of the pentolinium suppression test [6] subsequently enabled the diagnosis of phaeochromocytoma to be excluded; these patients all had mild hypertension [blood pressure (BP) 140/95–160/110] and their vanillylmandelic acid excretion and plasma noradrenaline concentrations were elevated on at least two occasions up to two-fold above normal; urinalysis, creatinine clearance, ECG, chest X-ray and intravenous pyelogram were all normal.

In all the suspected phaeochromocytoma patients blood samples (5 ml) were obtained from the adrenal, renal, hepatic, iliac (internal and external), antecubital and brachial veins, from several levels in the inferior and superior vena cava, and from the right atrium. A catheter was inserted by the Seldinger technique under local anaesthesia into the right femoral vein and introduced into most of these sites, under fluoroscopic control; its position was ascertained by the hand-injection of 2–3 ml of Hypaque. Since the left adrenal drains into the renal vein, blood was drawn from the bifurcation of the renal vein into upper and lower pole veins; adrenal vein contamination was excluded by measurement of cortisol concentration in the renal vein and arterial samples [6, 7]. The arm blood samples were taken by direct venepuncture, the brachial vein being located just medial to the artery. Arterial samples were obtained simultaneously with the venous samples from a catheter in the femoral artery.

Five patients with renal artery stenosis ( angiographically proven on a subsequent occasion) were investigated during the measurement of split renal vein renin concentrations. The patients all had severe hypertension not controlled by triple therapy (BP 150/100–175/115). Most had ECG evidence of left ventricular hypertrophy and mild impairment of renal function ( creatinine clearance 46–78 ml/min). Blood samples were obtained from both renal veins, from the inferior vena cava above and below the orifices of these veins (‘high’ and ‘low’ inferior vena cava), and an arterial sample was drawn from the femoral artery at the end of the study. Adrenal contamination of the renal vein samples was excluded as before. Simultaneous coronary sinus and left ventricular blood samples were obtained from four patients undergoing cardiac catheterization in the investigation of angina who subsequently proved to have normal coronary artery angiograms.

Informed consent was obtained from each patient and the research aspects of the investigations were approved by the Ethics Committee of the Royal Postgraduate Medical School.

Analytical method

Blood for catecholamine estimation was collected into chilled lithium/heparin tubes and the
plasma separated within 30 min at 4°C. This was stored at −80°C and assayed by a double-isotope enzymatic technique [8].

Results

In the suspected phaeochromocytoma group 15 patients were found to have a tumour, of whom eight had peripheral noradrenaline concentrations more than 10-fold above normal. Both in these ‘large phaeochromocytoma’ patients and in the remaining seven ‘small phaeochromocytoma’ patients the A–V differences (Fig. 1) demonstrated a similar extraction of both adrenaline and noradrenaline by all organs studied: 78 ± 9 and 83 ± 10% respectively, by the liver; 58 ± 8 and 54 ± 6% by the kidneys; 68 ± 7 and 68 ± 6% by the legs; 10 ± 9 and 12 ± 9% by the superficial forearm tissue (estimated from arterial and antecubital vein concentrations); 47 ± 9 and 40 ± 10% by deep forearm tissue (estimated from brachial vein concentrations). The pattern was similar for patients with adrenal or extra-adrenal tumours, although the plasma adrenaline concentration in the latter was not elevated. Interestingly, it was possible to detect extraction by the normal adrenal gland in patients with large tumours in the opposite gland, the absence of a positive A–V difference being indicative of a small tumour present in the otherwise apparently normal adrenal. In all these patients there was a marked and similar step-up in the noradrenaline and adrenaline concentrations in the inferior vena cava from below to above the level of the renal and adrenal veins, of 253 ± 31 and 289 ± 37% respectively.

In the nine patients of this group in whom no tumour was found, a very different pattern of A–V differences was observed (Fig. 2). Net hepatic extraction of both catecholamines was not significantly different from that in the phaeochromocytoma patients. Across all other organs studied, this was true only of the adrenaline difference; the A/V difference for noradrenaline was significantly lower or, in some cases, reversed. On the assumption that the A–V difference of adrenaline was a valid marker of noradrenaline, as well as adrenaline, extraction, the percentage contribution of each organ to its venous noradrenaline concentration was calculated; the calculation and the results are shown in Table 1. Only across the adrenal gland was the venous concentration of either catecholamine higher than the arterial value in all patients. The mean venous–arterial difference for adrenaline was 48.4 ± 5.2 nmol/l, which was more than 10-fold higher than that of noradrenaline (4.6 ± 0.28 nmol/l). Since, by contrast, the peripheral (arterial) noradrenaline concentration was more than fivefold that of adrenaline, the contribution of the adrenal medulla to circulating noradrenaline may be estimated to be less than 2% (1/10 × 1/5), assuming that both catecholamines have a similar clearance from plasma. In these non-tumour patients there was no significant step-up in noradrenaline concentration from ‘low’ to ‘high’ inferior vena cava, although that for adrenaline was similar to the increase observed in the phaeochromocytoma patients (264 ± 30%).

In the renal artery stenosis patients the renal venous concentration of noradrenaline was higher than arterial on the ischaemic side, but lower than arterial contralaterally (Fig. 3). In all these
TABLE 1. Estimated contribution of various tissues to the noradrenaline in their venous effluent

This was calculated for each tissue (n = number of patients) on the assumption that the percentage extraction of noradrenaline (NA) and adrenaline (AD) were the same, but only NA was simultaneously released. The equation is:

\[
\text{contribution (%) = \frac{\text{venous NA concn.}}{\text{arterial NA concn.}} \times \frac{\text{venous AD concn.}}{\text{arterial AD concn.}} \times 100}
\]

<table>
<thead>
<tr>
<th>Patients (diagnosis)</th>
<th>Tissue</th>
<th>Contribution (means ± sd) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild essential hypertension (n = 9)</td>
<td>Liver</td>
<td>3.7 ± 5.2</td>
</tr>
<tr>
<td></td>
<td>Kidneys</td>
<td>47 ± 6.1</td>
</tr>
<tr>
<td></td>
<td>Legs</td>
<td>68 ± 9.3</td>
</tr>
<tr>
<td></td>
<td>Forearm (deep)</td>
<td>36 ± 7.4</td>
</tr>
<tr>
<td></td>
<td>(superficial)</td>
<td>12 ± 11</td>
</tr>
<tr>
<td>Angina (n = 4)</td>
<td>Heart</td>
<td>21 ± 5.2</td>
</tr>
<tr>
<td>Renal artery stenosis (n = 5)</td>
<td>Kidney (ischaemic)</td>
<td>81 ± 11.6</td>
</tr>
<tr>
<td></td>
<td>(contralateral)</td>
<td>39 ± 6.7</td>
</tr>
</tbody>
</table>

A–V differences of noradrenaline as before, was different on the two sides (Table 1). The cardiac contribution to the noradrenaline concentration in its venous effluent was estimated in four patients (Fig. 4, Table 1). The calculation is based on the same assumption as before, although in this instance no comparable data were available from the phaeochromocytoma patients to confirm that similar extraction of both catecholamines usually occurs.

Discussion

In most subjects the adrenal gland was the only site where the venous noradrenaline concentration was higher than the arterial value, although it was estimated to make little contribution to circulating noradrenaline. The study illustrates how, by contrast, other organs may make an appreciable contribution without any venous–arterial difference in noradrenaline concentration being detectable. In previous studies where A–V differences have been measured, it was not possible to estimate the relative balance of secretion and extraction, although such investigations have been performed in experimental animals [9] as well as man [10, 11]. This difficulty has now been overcome by the simultaneous measurement of noradrenaline and adrenaline differences and by using the percentage extraction of adrenaline as an estimate of noradrenaline extraction. The validity of this estimation depended on (a) showing that the two catecholamines have similar extractions and (b) improved catechol O-methyltransferase assay precision; since the calculation of noradrenaline production requires the simultaneous measure-
Measurement of noradrenaline release

The assumption of similar extraction (of noradrenaline and adrenaline) is apparently justified by the data from the phaeochromocytoma patients. In the eight 'large phaeochromocytoma' patients, in whom all circulating noradrenaline could be assumed to be derived from one source, there was no significant difference in the percentage extraction of noradrenaline and adrenaline (calculated from their A–V differences) across any organ. The data from the 'small phaeochromocytoma' patients has been presented separately since their plasma noradrenaline concentrations were not significantly different from those of the non-tumour group and may have received some contribution from sympathetic nerve endings. That this is unlikely, however, is suggested by their noradrenaline concentration gradient in the inferior vena cava, which was no less than that in the 'large phaeochromocytoma' patients, whereas no inferior vena cava gradient was observed in the non-tumour patients in whom little circulating noradrenaline was derived from the adrenal medulla. It has also been shown recently that a reduction in sympathetic activity by ganglionic blockade does not reduce plasma noradrenaline concentration in phaeochromocytoma patients, even when this is within the normal range [6]. If, then, it is valid to include the data from the 'small phaeochromocytoma' patients, it appears that the similar extraction of noradrenaline and adrenaline occurs independently of their plasma concentration.

Although both catecholamines are substrates for the neuronal and extraneuronal uptake processes and for metabolism by catechol O-methyltransferase and monoamine oxidase, there are no assumptions implicit in the present model regarding the relevant importance of these pathways in the tissue extraction of the catecholamines. None will be saturated, since even the lowest $K_m$ (that for neuronal uptake) is in the micromolar range and higher than the plasma concentration of even the 'large phaeochromocytoma' patients [4]. Similarly, none of the patients had plasma catecholamine concentrations approaching the apparent threshold for extraneuronal accumulation. Nor is the model inconsistent with the variation in plasma catecholamine clearance with plasma concentration [12]; this is probably a $\beta$-adrenoceptor-mediated effect, more pronounced with adrenaline, and most likely due to an increased cardiac output rather than a specific effect on any of the kinetic processes [13]. No variation in the relative extraction of the two catecholamines would be anticipated; this is supported by the 'large phaeochromocytoma' patients with extra-adrenal tumours, in whom a normal plasma adrenaline concentration was not associated with any difference in the pattern of extraction.

The value of the model lies in its ability to obtain physiological information from purely clinical data, since such invasive studies could not readily be performed on normal volunteer subjects. The arguments for its validity are indirect admittedly, but have spared the need for administering labelled catecholamines to the subjects to measure directly their extraction by each organ. Clinically, it is of some interest that all phaeochromocytoma patients proved to have the same pattern of A–V differences, different from that of non-tumour patients; the latter pattern, comprising slight (or absent) A–V difference of noradrenaline across the legs and kidneys, provides a positive diagnostic feature showing that the circulating noradrenaline is derived from sympathetic nerves rather than a tumour [14].

Further studies with measurement of organ blood flow and cardiac output would be required to assess the relative contribution of each organ to circulating noradrenaline. It is possible, however, to estimate these approximately from the present data, by using tables of normal values [15], and it is immediately obvious that density of sympathetic innervation of a tissue correlates poorly with its contribution to plasma noradrenaline. This may be calculated, for each organ, from the product of its contribution to its venous noradrenaline concentration (Table 1) and its blood flow as a fraction of cardiac output. The estimated results for heart and legs are 3 and 11% respectively: the reverse of the relative density of their sympathetic innervation [16, 17]. By a similar calculation the kidneys contribute 12% of circulating noradrenaline.

That cardiac sympathetic nervous activity may have little effect on peripheral plasma noradrenaline concentrations has been previously suggested [18, 19]. Nevertheless, this has not been widely agreed, and the heart has been considered to be responsible for the increased plasma noradrenaline concentration reported in some patients with essential hypertension [20, 21]. If sympathetic nervous activity varied uniformly in all organs, it would not necessarily affect the interpretation of peripheral plasma noradrenaline concentrations if some organs made little contribution to these (relative to the degree of sympathetic activity in these organs). But the
data from the renal artery stenosis patients suggest that sympathetic activity to an organ may vary independently of that elsewhere (and be undetectable by peripheral measurements of plasma noradrenaline). Although the increased contribution of the ischaemic kidney to its venous noradrenaline concentration may partly be due to reduced flow, the marked step-up in the concentration of noradrenaline from ‘low’ to ‘high’ inferior vena cava suggests a significant abnormality of renal sympathetic activity; yet the peripheral plasma noradrenaline concentration was not elevated.

Finally, the relation between peripheral venous and arterial noradrenaline concentrations are variable. In the phaeochromocytoma patients all forearm venous concentrations of both catecholamines were lower than arterial; this was especially true of deep forearm (brachial vein) levels. In the non-tumour patients this was true only for adrenaline, suggesting a local contribution to forearm venous concentrations of noradrenaline. The greater extraction of catecholamines by deep than superficial forearm tissue may not affect the interpretation of endogenous noradrenaline concentrations. But investigations of noradrenaline clearance with infused noradrenaline should measure changes in either arterial or superficial venous noradrenaline concentration to avoid the risk of measuring local variations in clearance.

References