Clinical Science (1995) 88, 533–542 (Printed in Great Britain)

Plasma metadrenalines: do they provide useful information about sympatho-adrenal function and catecholamine metabolism?

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(Received 9 November 1994/9 January 1995; accepted 17 January 1995)

1. The clinical utility of plasma metadrenalines for examination of sympatho-adrenal function and catecholamine metabolism was assessed from plasma measurements of these metabolites in a number of clinical conditions (hypertension, cardiac failure, bilateral adrenalectomy and X-chromosomal deletions of the gene for monoamine oxidase), and before and during activation of sympathetic outflow or infusions of noradrenaline and adrenaline.

2. Plasma concentrations of normetadrenaline were less than 25% of those of noradrenaline, concentrations of metadrenaline and adrenaline were similar and those of sulphate-conjugated metadrenalines were 20- to 30-fold higher than free metadrenalines. Hypertensive patients had elevated plasma concentrations of adrenaline, noradrenaline and conjugated but not free metadrenalines. Cardiac failure patients had 2- to 4-fold increases in plasma noradrenaline and free and conjugated normetadrenaline. Adrenalectomy resulted in undetectable plasma concentrations of adrenaline, 91–97% decreases in free and conjugated metadrenaline and a 40% decrease in normetadrenaline relative to noradrenaline. Patients with X-chromosomal deletions of the gene for monoamine oxidase had 6- and 16-fold increases in plasma free and conjugated normetadrenaline and 2- and 4-fold increases in free and conjugated metadrenaline.

3. Infusion of catecholamines increased plasma concentrations of free metadrenalines by less than 6% of increases in precursor amines, indicating that most plasma normetadrenaline (84%) and metadrenaline (90%) is derived from metabolism of catecholamines before their entry into the circulation. Considerable O-methylation of catecholamines within the adrenals explains why sympatho-adrenal activation resulted in smaller proportional increases in plasma metadrenalines than catecholamines.

4. Plasma metadrenalines provide supplementary information about sympatho-adrenal activity to that provided by catecholamines, but are more useful for examination of the extraneuronal inactivation of catecholamines, particularly detection of neurochemical phenotypes in genetic disorders of catecholamine metabolism. Significant formation of metadrenalines within chromaffin tissue explains why measurements of plasma metadrenalines provide an extraordinarily sensitive method for diagnosis of phaeochromocytoma.

INTRODUCTION

The metadrenalines, normetadrenaline and metadrenaline, are produced by O-methylation of noradrenaline and adrenaline, a reaction catalysed by catechol-*O*-methyltransferase (COMT) [1]. Because COMT is localized mainly in non-neuronal tissues, normetadrenaline is produced by extraneuronal metabolism of the noradrenaline released by nerves that escapes reuptake [2]. In contrast, the deaminated metabolites of noradrenaline, dihydroxyphenylglycol (DHPG) and methoxyhydroxyphenylgly-

Key words: adrenal glands, adrenalectomy, adrenaline, catechol-O-methyltransferase, dihydroxyphenylglycol, heart failure, hypertension, metadrenaline, monoamine oxidase, noradrenaline, normetadrenaline, Norrie disease, phaeochromocytoma, sympathetic nervous system.

Abbreviations: COMT, catechol-O-methyltransferase; DHPG, dihydroxyphenylglycol; MAO, monoamine oxidase.

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col, are derived largely from the intraneuronal metabolism of the noradrenaline that leaks from storage vesicles [3, 4]. The above metabolic differences suggest that normetadrenaline, in conjunction with other metabolites, may offer a useful marker of noradrenaline release as distinct from noradrenaline turnover [5]. In rats, measurements of plasma metadrenalines have indeed been shown to offer a useful method for examination of extraneuronal uptake and metabolism of catecholamines [6–8]. Studies of plasma metadrenalines in humans, however, are limited [9–11].

The clinical utility of plasma metadrenalines for examination of sympatho-adrenal function and catecholamine metabolism was therefore explored by studies that answered the following questions. Do the metadrenalines provide useful markers of sympatho-adrenal activity at rest and during sympathetic activation associated with mental stress, lower body negative pressure and cycling exercise? What proportions of plasma normetadrenaline and metadrenaline derive from metabolism of circulating catecholamines? Can plasma metadrenalines provide additional information to plasma catecholamines in clinical conditions affecting sympatho-adrenal function such as adrenalectomy, heart failure, hypertension, angina pectoris, renal artery stenosis and X-chromosomal deletions or mutations affecting monoamine oxidase (MAO)?

METHODS

Subjects

The study included 81 normal control subjects, 50 patients with essential hypertension, 24 patients with angina, 35 patients with heart failure, 11 patients with renal artery stenosis, 11 patients with bilateral adrenalectomies, five Norrie disease patients with X-chromosomal deletions affecting MAO and one patient who underwent inferior vena cava regional blood sampling to localize the site of a suspected phaeochromocytoma. Subjects were studied as part of ongoing protocols at three different institutions: the National Institutes of Health, the University of Göteborg and St Radboud University Hospital. All procedures were approved by the appropriate ethics committee and all patients (and parents of Norrie disease patients) gave informed consent before blood samples were taken or studies began. Blood samples were taken with subjects in the supine position. The physical characteristics (age and sex) and sites of blood sampling for the subjects of the various groups are summarized in Table 1.

Hypertension. A diagnosis of essential hypertension was made if the mean of blood pressure values determined on three separate outpatient visits was higher than 140 mmHg (systolic) or 90 mmHg (diastolic). At each visit, blood pressure was measured as the mean of two readings after at least 5 min of supine rest. Secondary hypertension was excluded according to standard clinical criteria. Hypertensive patients remained unmedicated for 2 weeks before blood samples were taken.

Table 1. Physical characteristics of subjects (age and gender) and site of blood sampling

	Mean age [years (range)]	Gender (M/F)	Sampling site (arterial/venous)
Control subjects ($n=81$)	38 (20–72)	47/34	15/71*
Essential hypertension ($n=50$)	42 (19–66)	26/24	0/50
Angina pectoris ($n=24$)	57 (37–71)	18/6	24/0
Cardiac failure ($n=35$)	55 (34–75)	30/5	31/4
Renal artery stenosis ($n=13$)	51 (25–69)	7/6	8/5
Adrenalectomy ($n=11$)	47 (32–66)	2/9	0/11
Norrie disease ($n=5$)	16 (12–23)	5/0	0/5

*Includes five subjects from whom samples were taken from both arterial and venous sites.

Angina pectoris. Patients with angina pectoris included 11 patients with chest pain and coronary artery disease (angiographic narrowing of more than 50% of the lumen of any major epicardial vessel) and ten patients with chest pain but normal coronary angiograms. Patients were studied after discontinuation of medication (β-adrenoceptor blockers, calcium-channel antagonists, nitrates) at admission.

Renal artery stenosis. Renal artery stenosis was diagnosed on the basis of a stenosis greater than 50% as determined by digital subtraction arteriography of the renal arteries. Two patients had renal failure.

Cardiac failure. Cardiac failure was secondary to coronary artery disease or idiopathic dilated cardiomyopathy. Blood samples were taken 12 h after patients received their last medication, typically a combination of digoxin, calcium-channel blockers, angiotensin-converting enzyme inhibitors, nitrates or diuretics.

Adrenalectomy. Subjects with bilateral adrenalectomies included ten patients who had both adrenals removed for treatment of Cushing's syndrome (primary pigmented nodular adrenal disease, $n=5$; ectopic Cushing's syndrome, $n=2$; pituitary Cushing's disease, $n=3$) and one patient who underwent the procedure during removal of phaeochromocytomas on both adrenals. Venous blood samples were collected from patients in the morning, immediately before their normal steroid replacement dose.

Norrie disease. Patients with X-chromosomal deletion-related Norrie disease affecting the genes for both MAO-A and MAO-B [12, 13] included five subjects from three different families described elsewhere [13–16].

Inferior vena cava blood sampling. One patient underwent inferior vena cava sampling of blood to localize a suspected phaeochromocytoma. The patient was subsequently determined not to harbour a phaeochromocytoma, but samples from the infer-

ior vena cava and renal and adrenal veins were analysed to assess the possible contribution of the adrenal medullae to circulating plasma concentrations of metadrenalines.

Activation of sympathetic outflow

Lower body negative pressure. In five control subjects and five hypertensive patients, blood samples were taken simultaneously from a brachial artery and from a deep antecubital vein, before and 15 min after the start of lower body negative pressure (at −40 mmHg).

Mental challenge. In 11 patients with microvascular angina, arterial blood samples were taken in the supine position before and 5 min after subjects started playing a video game, previously demonstrated to elicit increases in plasma catecholamines [17].

Exercise. Twenty-eight patients, including 18 patients with cardiac failure and ten control subjects, performed cycling exercise in the supine position. Arterial blood samples were taken before and during the last minute of cycling exercise, between 10 and 24 min after the start of exercise.

Infusions of ^{3}H-labelled catecholamines

All patients who underwent the manipulations described above received infusions of [^{3}H]noradrenaline (L-2,5,6-[^{3}H]noradrenaline, 40–60 Ci/mmol; New England Nuclear, Boston, MA, U.S.A.) either delivered alone or in combination with [^{3}H]adrenaline (L-*N*-methyl-[^{3}H]adrenaline, 65–75 Ci/mmol; New England Nuclear). The radiotracers were infused into a forearm vein at 1.0–1.5 μCi/min. Blood samples were taken at intervals between 15 and 145 min after the start of infusions. The preparation, storage and handling of the radiotracers has been described in detail elsewhere [18].

Analytical methods

Metadrenaline assays. The metadrenalines (normetadrenaline and metadrenaline) were extracted from 2 ml samples of plasma using solid-phase ion-exchange columns and were quantified by liquid chromatography with electrochemical detection [11]. Samples from subjects who received intravenous infusions of ^{3}H-labelled catecholamines underwent timed collections of the eluant leaving the electrochemical cell, enabling determination of plasma concentrations of ^{3}H-labelled normetadrenaline and metadrenaline by liquid-scintillation spectroscopy. Plasma concentrations of sulphate-conjugated normetadrenaline and metadrenaline were determined from 0.25 ml samples of plasma that were subjected to enzymic deconjugation by incubation with 0.2 unit of sulphatase (Sigma; St Louis, MO, U.S.A.) for 30 min at 37°C. Inter-assay coefficients of variation were 12.2% for normetadrenaline and 11.2% for metadrenaline. Intra-assay coefficients of variation were 4.2% for normetadrenaline and 3.3% for metadrenaline.

Catechol assays. The catechols (noradrenaline, adrenaline and DHPG) were determined by liquid chromatography with electrochemical detection after extraction by alumina adsorption [19]. Inter-assay coefficients of variation were 6.5% for noradrenaline, 11.4% for adrenaline and 8.4% for DHPG. Intra-assay coefficients of variation were 1.9% for noradrenaline, 3.0% for adrenaline and 4.8% for DHPG.

Data analysis

Calculations. The proportion (P) of endogenous free normetadrenaline in plasma formed from metabolism of circulating noradrenaline, or of free metadrenaline formed from circulating adrenaline (i.e. that derived from the catecholamine after its release or entry into the plasma compartment) was estimated according to a previously established method [6], using the equation:

$$P=([^3H]M/[^3H]C)/(M/C)$$

where C is the plasma concentration of endogenous catecholamine precursor (pmol/ml), M is the plasma concentration of endogenous metabolite (pmol/ml), [^{3}H]C is the steady-state plasma concentration of intravenously infused ^{3}H-labelled catecholamine (d.p.m./ml) and [^{3}H]M is the respective plasma concentration of the ^{3}H-labelled metabolite (d.p.m./ml) during infusion of ^{3}H-labelled catecholamine precursor.

Statistical methods. Results are expressed as means ± SEM. Plasma concentrations of normetadrenaline and metadrenaline were not normally distributed. Therefore, for the most part, levels of statistical significance were determined using non-parametric methods: Wilcoxon's signed-rank sum test was used for comparisons of paired data, the Mann–Whitney U-test was used for comparisons of non-paired data, and the significance of relationships was assessed using Spearman's rank correlation coefficient. Where parametric methods were used (i.e. analysis of variance to assess gender–group interactions), levels of significance were determined after logarithmic transformation of the data. Unless stated, statistical significance was defined as $P<0.05$.

RESULTS

Free and conjugated metadrenalines

Forearm venous plasma concentrations of normetadrenaline in control subjects were low, averaging less than 25% of those of noradrenaline and ranging between 0.09 and 0.62 pmol/ml (Table 2). In contrast, venous plasma concentrations of metadrenaline were similar to those of adrenaline, rang-

Table 2. Forearm venous plasma concentrations of catecholamines and free and total metadrenalines in control subjects, essential hypertensive patients, patients with bilateral adrenalectomy and patients with Norrie disease. Values are expressed as means ± SEM. *Significantly higher concentrations ($P<0.05$) compared with control subjects. †Significantly lower concentrations ($P<0.05$) compared with control subjects. Differences were determined by the Mann–Whitney *U*-test.

	Control subjects ($n=71$)	Hypertension ($n=50$)	Adrenalectomy ($n=11$)	Norrie disease ($n=5$)
Normetadrenaline (pmol/ml)	0.29 ± 0.02	0.31 ± 0.02	0.21 ± 0.03	1.77 ± 0.1*
Normetadrenaline sulphate (pmol/ml)	7.68 ± 0.49	8.96 ± 0.46*	8.67 ± 0.53	120.70 ± 28.46*
Noradrenaline (pmol/ml)	1.28 ± 0.08	1.48 ± 0.09*	1.94 ± 0.38	2.76 ± 0.3*
Metadrenaline (pmol/ml)	0.14 ± 0.01	0.15 ± 0.01	0.012 ± 0.003†	0.37 ± 0.15*
Metadrenaline sulphate (pmol/ml)	3.13 ± 0.14	3.90 ± 0.27*	0.081 ± 0.01†	12.67 ± 1.75*
Adrenaline (pmol/ml)	0.11 ± 0.01	0.18 ± 0.02*	<0.02†	0.15 ± 0.05
DHPG (pmol/ml)	5.59 ± 0.23	5.58 ± 0.26	4.87 ± 0.65	<0.02†

ing between 0.03 and 0.36 pmol/ml. Both normetadrenaline and metadrenaline were extensively sulphate-conjugated, so that concentrations of the conjugates were 20- to 30-fold higher than concentrations of free compounds.

Forearm arteriovenous differences

In the ten subjects from whom simultaneous arterial and forearm venous blood samples were obtained, arteriovenous differences in plasma concentrations of metadrenalines showed a similar, albeit less dramatic, pattern to the parent amines. Venous concentrations of metadrenaline were 24 ± 9% lower ($P<0.05$) than arterial concentrations, whereas venous concentrations of normetadrenaline were slightly (18 ± 14%), but not significantly, higher than arterial concentrations. Forearm venous concentrations of adrenaline were 75 ± 2% lower ($P<0.01$) than arterial concentrations, whereas venous noradrenaline concentrations were 23 ± 7% higher ($P<0.05$) than arterial concentrations.

Influence of gender and age

Gender. Consistent sex differences in forearm venous plasma concentrations of metadrenaline, metadrenaline sulphate and adrenaline were observed for both hypertensive and control subjects. Plasma adrenaline was higher ($P<0.001$) in males than in females (0.17 ± 0.02 compared with 0.11 ± 0.01 pmol/ml), a difference that was significant for both hypertensive and normotensive groups considered alone. This difference was also reflected in higher ($P<0.02$) concentrations in males than females of both metadrenaline (0.16 ± 0.01 compared with 0.13 ± 0.01 pmol/ml) and metadrenaline sulphate (4.00 ± 0.23 compared with 3.18 ± 0.17 pmol/ml). Except for higher ($P<0.05$) concentrations of normetadrenaline in female compared with male control subjects (0.32 ± 0.02 and 0.26 ± 0.02 pmol/ml, respectively), there were no other gender differences in noradrenaline and its O-methylated metabolites.

Age. There were weak positive relationships between age and plasma concentrations of noradrenaline ($r=0.29$, $P<0.002$), normetadrenaline ($r=0.21$, $P<0.05$) and normetadrenaline sulphate ($r=0.30$, $P<0.002$), but no age-related relationships were apparent for adrenaline or metadrenaline. The observed gender differences in plasma catecholamines and metadrenalines were unrelated to any gender difference in age distribution (39 ± 1 years in males compared with 41 ± 2 years in females).

Comparisons among subject groups

Due to the differences between arterial and forearm venous plasma concentrations of metadrenalines and catecholamines, comparisons among subject groups were made according to the site of blood sampling.

Hypertension. In patients with essential hypertension, venous plasma concentrations of noradrenaline and adrenaline and of conjugated normetadrenaline and metadrenaline were 15–25% higher ($P<0.02$) than in control subjects, whereas concentrations of unconjugated normetadrenaline and metadrenaline were not significantly different (Table 2). Increased plasma concentrations of metadrenaline sulphate and adrenaline in hypertensive patients were independent of gender. In contrast, there was a significant ($P<0.01$) group–gender interaction for plasma normetadrenaline, such that plasma concentrations of this metabolite were higher in hypertensive than normotensive males (0.35 ± 0.03 compared with 0.26 ± 0.02 pmol/ml), but not in females (0.28 ± 0.03 compared with 0.32 ± 0.02 pmol/ml). There was no associated group–gender interaction for plasma noradrenaline.

Bilateral adrenalectomy. In adrenalectomized subjects, forearm venous plasma concentrations of adrenaline were below the detection limits of the assay (<0.015 pmol/ml). Plasma concentrations of free and conjugated metadrenaline were detectable, but were decreased ($P<0.001$) by more than 91% compared with control subjects (Table 2). Adrenalectomy was associated with decreased plasma concentrations of normetadrenaline and increased concentrations of noradrenaline, but these differences did not reach significance. Considered together, however, the ratio of plasma normetadrenaline to noradrenaline concentrations was 43% lower ($P<0.01$) in adrenalectomized subjects than in control subjects (0.144 ± 0.013 compared with 0.254 ± 0.015).

Table 3. Arterial plasma concentrations of catecholamines and free and total metadrenalines in control subjects and patients with angina pectoris, renal artery stenosis and cardiac failure. Values are expressed as means ± SEM. *Significantly higher concentrations ($P<0.05$) compared with control subjects, as determined by Mann–Whitney *U*-test. †Samples from these groups include some taken from venous sampling sites (see Table 1), but observed differences remained significant when comparisons were made with the control subjects from whom venous samples were taken (see Table 2).

	Control subjects ($n=15$)	Angina ($n=24$)	Renal artery stenosis† ($n=13$)	Cardiac failure† ($n=35$)
Normetadrenaline (pmol/ml)	0.20 ± 0.01	0.25 ± 0.02	0.48 ± 0.12*	0.46 ± 0.07*
Normetadrenaline sulphate (pmol/ml)	6.78 ± 0.49	10.80 ± 1.25*	14.40 ± 2.28*	24.25 ± 4.98*
Noradrenaline (pmol/ml)	1.13 ± 0.13	1.45 ± 0.13	3.04 ± 0.66*	3.31 ± 0.56*
Metadrenaline (pmol/ml)	0.24 ± 0.02	0.23 ± 0.02	0.30 ± 0.06	0.25 ± 0.02
Metadrenaline sulphate (pmol/ml)	3.53 ± 0.37	3.62 ± 0.36	5.12 ± 1.24	9.12 ± 2.17*
Adrenaline (pmol/ml)	0.31 ± 0.08	0.28 ± 0.04	0.41 ± 0.11	0.54 ± 0.08*
DHPG (pmol/ml)	4.85 ± 0.22	5.68 ± 0.38	4.74 ± 0.32	6.80 ± 0.32*

Norrie disease. The five patients with Norrie disease showed considerable increases ($P<0.001$) above normal in forearm venous plasma concentrations of free (6.1-fold increase) and conjugated (15.7-fold increase) normetadrenaline (Table 2). Plasma concentrations of free and conjugated metadrenaline were also increased ($P<0.01$), although the extent of these increases was not as large (about a third) as those observed for free and conjugated normetadrenaline. Plasma concentrations of conjugated metadrenaline and normetadrenaline were increased 50 and 160% above normal than the respective concentrations of free metadrenalines. Plasma noradrenaline was increased by 2-fold ($P<0.05$) in Norrie disease patients, whereas plasma adrenaline was unaffected. Plasma concentrations of DHPG were below the limits of detection ($P<0.02$) in all five patients with Norrie disease.

Angina pectoris. Patients with angina pectoris had higher ($P<0.05$) arterial plasma concentrations of conjugated normetadrenaline than control subjects, but apart from this there were no other differences for this subject group (Table 3).

Renal artery stenosis. Plasma concentrations of noradrenaline and free and conjugated normetadrenaline were 2.1- to 2.7-fold higher ($P<0.01$) in patients with renal artery stenosis than in control subjects, whereas concentrations of adrenaline and free and conjugated metadrenaline were not different (Table 3).

Cardiac failure. Cardiac failure was associated with 2.3- to 3.6-fold higher ($P<0.001$) plasma concentrations of noradrenaline and free and conjugated normetadrenaline than in control subjects (Table 3). Plasma concentrations of adrenaline and metadrenaline sulphate were also increased ($P<0.01$) in patients with heart failure, but concentrations of free metadrenaline were not affected.

Relationships among plasma catecholamines and metabolites

There were significant ($P<0.001$) positive relationships between plasma concentrations of noradrenaline and normetadrenaline or DHPG, as well as between plasma concentrations of adrenaline and metadrenaline (Fig. 1). Although all linear regression lines intersected the *y*-axis (metabolite axis) above the origin, this was most apparent for the regression line describing the relationship between plasma noradrenaline and DHPG.

Positive linear relationships ($P<0.001$) were also observed between plasma concentrations of free and sulphate-conjugated normetadrenaline or metadrenaline (data not shown).

Infusion of ^{3}H-labelled catecholamines

Steady-state plasma concentrations of [^{3}H]noradrenaline were reached within 20 min of the start of the intravenous infusion of [^{3}H]noradrenaline (Fig. 2). Plasma concentrations of free [^{3}H]normetadrenaline increased during the first 30 min of the [^{3}H]noradrenaline infusion, reaching steady-state concentrations by 60 min after the start of the infusion that were 3.1 ± 0.2% of those of [^{3}H]noradrenaline concentrations. In contrast, plasma concentrations of [^{3}H]DHPG continued to climb throughout the infusion so that, at 90 min after the start of infusion, concentrations of [^{3}H]DHPG were higher than those of [^{3}H]normetadrenaline. Between 60 and 90 min after the start of the [^{3}H]noradrenaline infusion, the specific activity of free [^{3}H]normetadrenaline was 89 ± 5 d.p.m./pmol, significantly lower than the specific activity of [^{3}H]noradrenaline (527 ± 53 d.p.m./pmol), but significantly higher than that of [^{3}H]DHPG (7.4 ± 0.5 d.p.m./pmol).

During simultaneous infusion of ^{3}H-labelled noradrenaline and adrenaline, plasma concentrations of free [^{3}H]metadrenaline increased to 6.2 ± 0.5% of steady-state plasma [^{3}H]adrenaline concentrations, about 2-fold higher than the increase in [^{3}H]normetadrenaline relative to [^{3}H]noradrenaline concentrations (Fig. 3*a*).

Comparison of the ratio of steady-state arterial plasma concentrations of free [^{3}H]normetadrenaline to [^{3}H]noradrenaline with the ratio of arterial plasma concentrations of endogenous normetadre-

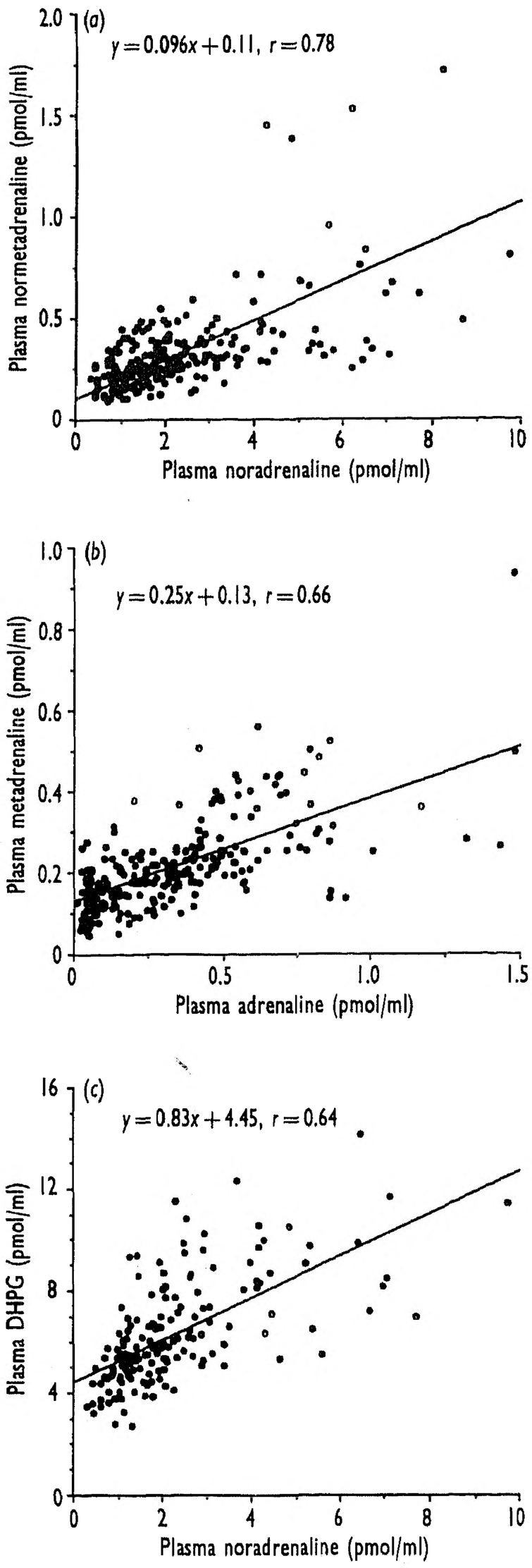

Fig. 1. Relationships between plasma normetadrenaline and noradrenaline (*a*), plasma metadrenaline and adrenaline (*b*) and plasma DHPG and noradrenaline (*c*). Regression equations for each relationship are shown at the top left-hand corner of each panel. Data are from all patient groups with the exclusion of Norrie disease patients and adrenalectomized subjects.

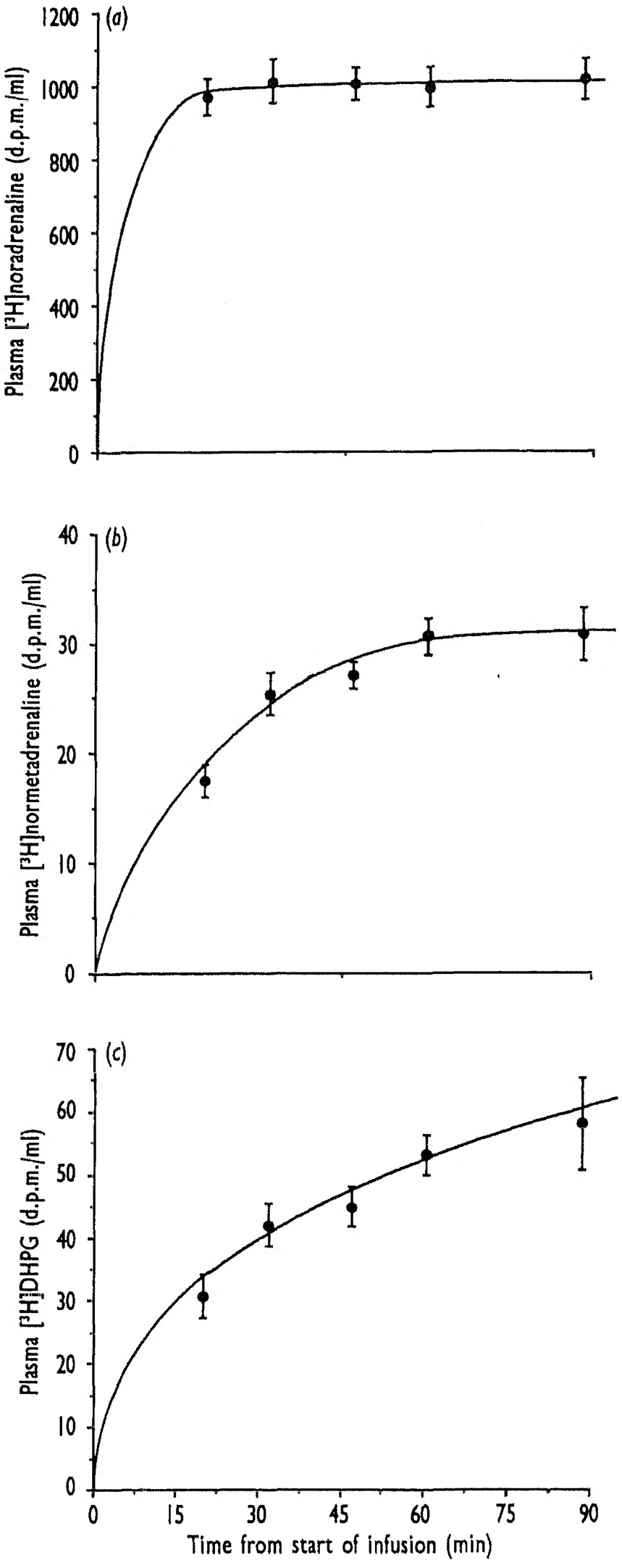

Fig. 2. Plasma concentrations of ^{3}H-labelled noradrenaline (*a*), normetadrenaline (*b*) and DHPG (*c*) as a function of time from the start of intravenous infusions of [^{3}H]noradrenaline. Data were derived from samples taken at different times after the start of infusions and partitioned into 15 min collection periods.

naline to noradrenaline, indicated that 15.8 ± 1.4% of the endogenous free normetadrenaline in plasma was derived from metabolism of noradrenaline after it entered the circulation (Fig. 3*b*). Surprisingly, ratios of ^{3}H-labelled and endogenous metadrenaline to adrenaline indicated that only 10.1 ± 1.9% of the endogenous free metadrenaline in plasma was derived from adrenaline after it was released into the circulation, a proportion that was actually less ($P < 0.01$) than that for the endogenous normetadrenaline produced from circulating noradrenaline.

Inferior vena cava blood sampling

In the patient who underwent inferior vena cava regional blood sampling, the plasma concentration of adrenaline in the right adrenal vein was 45-fold higher than in the downstream vena cava sampling site (Table 4). The plasma concentration of metadrenaline was 12-fold higher in the adrenal vein

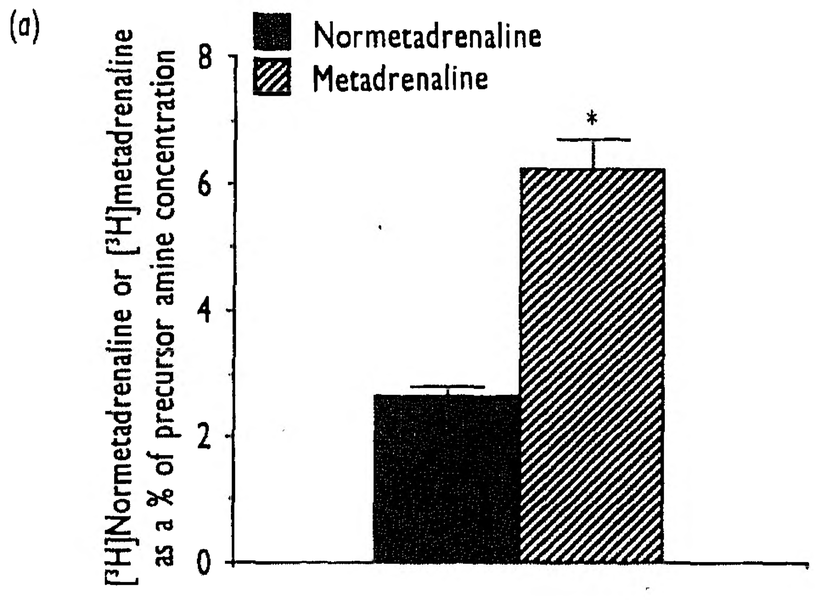

Fig. 3. Plasma concentrations of ^{3}H-labelled normetadrenaline or metadrenaline as a percentage of steady-state plasma concentrations of intravenously infused [^{3}H]noradrenaline or [^{3}H]adrenaline (*a*). These ratios were used in conjunction with ratios of endogenous normetadrenaline to noradrenaline, or metadrenaline to adrenaline, to estimate (see Data analysis in the Methods section) the percentage amounts of endogenous normetadrenaline or metadrenaline in plasma that were derived from metabolism of noradrenaline or adrenaline after release of precursor amines into the circulation (*b*). Results represent means with SEMs represented by vertical bars. *Denotes a significant difference between metadrenaline and normetadrenaline as determined by Wilcoxon's signed rank sum test.

than in the vena cava. The plasma normetadrenaline concentration was also higher in the adrenal vein than at other sampling sites. Plasma concentrations of adrenaline and metadrenaline were higher in the left renal vein, which drains both the left kidney and adrenal gland, than in the right renal vein, which drains only the right kidney.

Activation of sympathetic outflow

Application of −40 mmHg of lower body negative pressure, mental challenge and supine cycling exercise all increased ($P<0.01$) plasma normetadrenaline and plasma noradrenaline. Percentage increases in plasma normetadrenaline were considerably and consistently lower ($P<0.01$) than increases in plasma noradrenaline in response to all stimuli (Fig. 4*a*).

Lower body negative pressure, mental challenge and cycling exercise also increased ($P<0.001$) plasma adrenaline, but only lower body negative pressure increased plasma metadrenaline. Again, metadrenaline responses were considerably and consistently smaller than adrenaline responses to all stimuli (Fig. 4*b*).

DISCUSSION

The results show that measurement of plasma metadrenalines provides information about sympatho-adrenal activity that supplements that provided by the parent amines. More importantly, these metabolites provide information about catecholamine metabolism in extraneuronal tissues, including the adrenal medullae, that is not available from measurements of the parent amines alone.

Other reports about the clinical utility of plasma metadrenalines for assessment of sympatho-adrenal activity are largely limited to those of DeQuattro and colleagues [9, 10, 20–23], who used a radioenzymic assay to measure plasma concentrations of normetadrenaline in hypertension and during various manipulations of sympathetic outflow. The radioenzymic assay depends on N-terminal methylation of normetadrenaline, so that metadrenaline cannot be measured. The present use of liquid chromatography with electrochemical detection allows measurements of both metadrenaline and normetadrenaline.

The much lower plasma concentrations of free normetadrenaline than of noradrenaline, but higher concentrations of normetadrenaline sulphate, the age-dependence of plasma noradrenaline and normetadrenaline, higher plasma concentrations in hypertensive than in normotensive subjects and increases in concentrations during sympathetic activation agree well with previous findings [9, 10, 20–25]. The parallel increases in plasma normetadrenaline and noradrenaline in heart failure also support the view that plasma normetadrenaline reflects sympathetic outflow. Thus, measurement of normetadrenaline as an index of sympathetic outflow provides supplementary information that may strengthen the conclusions derived from measurements of noradrenaline. However, the proportionally smaller changes in plasma concentrations of free normetadrenaline than of noradrenaline during mental challenge, lower body negative pressure and exercise, indicate that normetadrenaline is a less sensitive index of acute changes in sympathetic outflow than noradrenaline.

Similarly, the much smaller increases in plasma metadrenaline than adrenaline during sympatho-adrenal activation also indicate that changes in plasma metadrenaline are a less sensitive index of acute changes in adrenal medullary secretion of adrenaline than the parent amine itself. Nevertheless, the findings of higher plasma concentrations of adrenaline in hypertensive than in normotensive subjects and in males than in females, while consistent with other studies [26, 27], are further supported and strengthened by similar differences in plasma concentrations of free or sulphate-conjugated metadrenaline.

Table 4. Plasma concentrations of catecholamines and metadrenalines during regional sampling of renal and adrenal veins in a single patient

	Plasma concn. (pmol/ml)			
	Adrenaline	Metadrenaline	Noradrenaline	Normetadrenaline
Vena cava (inferior lower)*	0.34	0.20	1.91	0.43
Right renal vein	0.52	0.14	4.37	0.38
Left renal vein	2.01	0.27	4.81	0.43
Right adrenal vein	15.30	2.45	3.46	0.67
Vena cava (inferior upper)*	0.49	0.20	2.50	0.49

*The inferior vena cava was sampled at two places, downstream (inferior lower) and upstream (inferior upper) from renal and adrenal venous sampling sites.

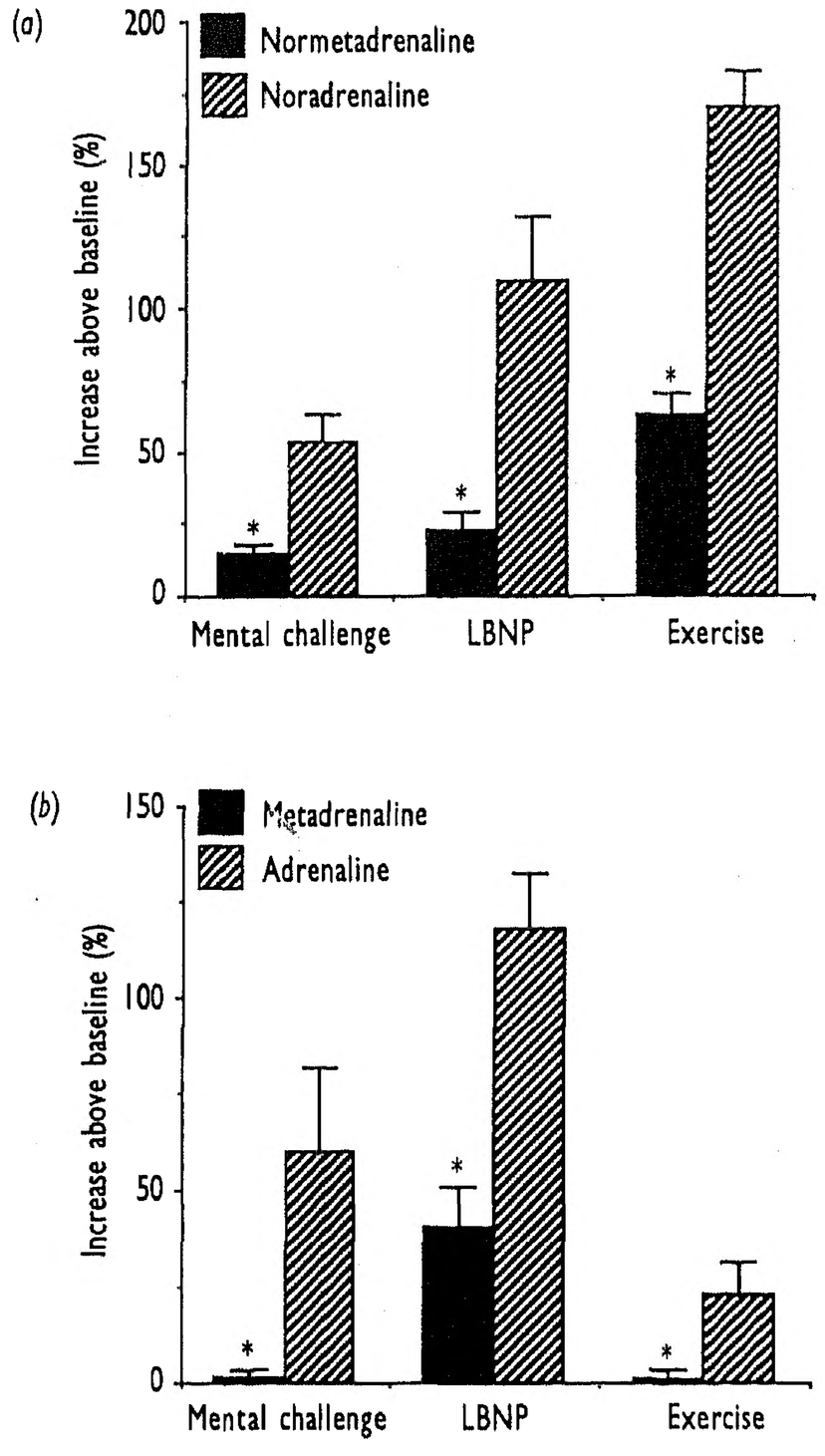

Fig. 4. Percentage increases in plasma concentrations of normetadrenaline compared with noradrenaline (*a*) and of metadrenaline compared with adrenaline (*b*) in response to mental challenge (i.e. playing a video game), application of −40 mmHg lower body negative pressure (LBNP) and supine cycling exercise. Values are expressed as means with SEMs represented by vertical bars. *Denotes a significant difference between normetadrenaline and noradrenaline responses, or metadrenaline and adrenaline responses, as determined by Wilcoxon's signed rank sum test.

An explanation for the insensitivity of plasma metadrenalines to acute changes in sympathoadrenal activation is provided by the observations about production of free metadrenalines from infused catecholamines. Similar plasma concentrations of endogenous metadrenaline to adrenaline, despite increases in plasma metadrenaline that were only 6% of those of infused adrenaline, indicated that most (90%) of the free metadrenaline in plasma is derived from metabolism of adrenaline before its release into the circulation, and is therefore unlikely to be responsive to acute changes in adrenaline release. The relative independence of metadrenaline production on adrenaline release is further reflected by the regression line for the relationship between plasma metadrenaline and adrenaline that intersected the *y*-axis well above the origin. Comparison of the *y*-intercept value of metadrenaline (0.13 pmol/ml) with mean plasma concentrations of metadrenaline in control subjects (0.14 pmol/ml) indicates that 90% of metadrenaline in plasma is independent of adrenaline release into the circulation.

Other findings in adrenalectomized rats showing normal plasma concentrations of metadrenaline despite undetectable adrenaline concentrations, suggested that much of the metadrenaline in plasma might be derived from adrenaline synthesized at extra-adrenal sites [6]. The results in patients with bilateral adrenalectomy indicate that, in humans, at least 91% of the metadrenaline in plasma is derived from adrenaline synthesized in the adrenals. This could reflect metabolism of adrenaline within the adrenals or local metabolism of adrenaline taken up and released by extra-adrenal tissues after synthesis and initial release by the adrenals. The former source is suggested by the high metadrenaline concentrations in plasma draining the adrenal veins.

Higher plasma concentrations of normetadrenaline in the adrenal vein than the vena cava, the lower ratio of normetadrenaline to noradrenaline concentrations in adrenalectomized patients than control subjects, and other findings of higher left renal than right renal venous plasma normetadrenaline concentrations [9], indicate that some circulating normetadrenaline is also derived from metabolism of noradrenaline within the adrenals. A contribution of the adrenals to plasma normetadrenaline is consistent with the regression line for the relationship between plasma noradrenaline and normetadrenaline that intersected the *y*-axis above the origin. Comparison of the *y*-intercept value of normetadrenaline (0.11 pmol/ml) with mean plasma concentrations of normetadrenaline in control subjects (0.29 pmol/ml), suggests a 38% contribution of the adrenals to plasma normetadrenaline. Similarly, comparison of the ratio of plasma normetadrenaline to noradrenaline in adrenalectomized (0.144) and

control (0.254) subjects, indicates a 45% [(0.254−0.144)/0.244] contribution of the adrenals to plasma concentrations of normetadrenaline. Formation of metadrenalines within the adrenals is further supported by immunohistochemical findings of COMT-positive cells in cortical layers of the adrenal gland [28, 29].

Although the metadrenalines provide little new information about sympatho-adrenal activity to that provided by the parent amines, the source of these metabolites makes them particularly useful for examination of catecholamine metabolism in extraneuronal tissues, including the adrenal medullae and tumours of chromaffin-cell origin. Comparison of plasma concentrations of catecholamines and metadrenalines in a large group of phaeochromocytoma patients showed that plasma metadrenalines were increased more consistently, and considerably more above normal, than plasma catecholamines (J. Lenders et al., unpublished work). In particular, patients with Von Hippel–Lindau's disease, a genetically inherited disorder associated with susceptibility to develop phaeochromocytomas, show increased plasma metadrenalines well before other tests (e.g. plasma catecholamines, clonidine suppression, glucagon stimulation) provide a positive diagnosis of the tumour. The extraordinarily high sensitivity of plasma metadrenalines for diagnosis of phaeochromocytoma can be explained by the present findings of active O-methylation of catecholamines within the adrenal gland, and presumably therefore in tumours derived from chromaffin tissue. High concentrations of normetadrenaline in the venous drainage of phaeochromocytomas [22] support this possibility. Even when quiescent or not releasing catecholamines, the tumours appear to be actively metabolizing catecholamines to the O-methylated derivatives.

Although as much as 40% of plasma normetadrenaline is derived from metabolism of noradrenaline within the adrenal medullae, most (60%) is derived from extraneuronal metabolism of neuronally released noradrenaline before or after entry of the transmitter into the circulation. The increases in plasma concentrations of free normetadrenaline from infused noradrenaline indicate that 16% of the free normetadrenaline in plasma is derived from metabolism of circulating noradrenaline. Thus, 73% [(60−16)/60] of the normetadrenaline that is derived from neuronally released noradrenaline is formed by extraneuronal metabolism before entry of the transmitter into the circulation, leaving 27% formed from metabolism after entry of noradrenaline into the circulation. This compares with the neuronal metabolite of noradrenaline, DHPG, of which less than 2% is derived from circulating noradrenaline [7].

The extraneuronal source of normetadrenaline, as distinct from the neuronal source of DHPG, is reflected by the relationships of normetadrenaline and DHPG with noradrenaline and production of both metabolites from infused [^{3}H]noradrenaline. The regression line for the relationship between plasma noradrenaline and DHPG intersected the *y*-axis well above the origin, consistent with formation of most DHPG from metabolism of noradrenaline leaking from vesicles and not released by nerves [3, 4, 30]. In contrast, the relationship between normetadrenaline and noradrenaline intersected the *y*-axis closer to the origin, consistent with formation of most (60%) normetadrenaline from neuronally released noradrenaline.

During infusion of [^{3}H]noradrenaline, the progressive increase in plasma [^{3}H]DHPG with time reflects continuous loading of neuronal vesicular stores with [^{3}H]noradrenaline and the substantial contribution by leakage of noradrenaline from these stores to DHPG production [18]. In contrast, attainment of steady-state plasma concentrations of [^{3}H]normetadrenaline indicates production of this metabolite from extraneuronal sites, where loading of vesicular stores exerts no influence. Similarly, the much lower specific activities of [^{3}H]DHPG compared with [^{3}H]normetadrenaline reflect the different sources of these metabolites from neuronal and extraneuronal sites and the much larger contribution of circulating noradrenaline to production of normetadrenaline than DHPG.

The potential use of plasma metadrenalines for the clinical examination of extraneuronal catecholamine metabolism is clearly demonstrated in the patients with Norrie disease, where the deficiency of MAO was reflected not only by the complete absence of intraneuronal DHPG production, but also by considerably increased plasma concentrations of metadrenalines. Plasma normetadrenaline was increased about 3-fold more than metadrenaline, consistent with findings in rats where pharmacological blockade of MAO increased plasma normetadrenaline 3-fold more than metadrenaline, a difference due to both increased formation and decreased plasma clearance of normetadrenaline and only decreased clearance of metadrenaline [8]. These findings indicated that little adrenaline or metadrenaline is deaminated in the compartment where metadrenaline is formed, a result explained by differences in rate constants for deamination of adrenaline and noradrenaline. The present findings, showing that most metadrenaline is formed within the adrenals, offer a more likely explanation for the observation that little adrenaline or metadrenaline is deaminated in the compartment where metadrenaline is formed [8].

The marked changes in metabolite profiles in Norrie disease patients illustrate how measurements of plasma metadrenalines in relation to other metabolites, such as DHPG, may be particularly useful for identification of disorders affecting catecholamine metabolism. Specifically, the pattern of increased plasma concentrations of metadrenalines and decreased concentrations of deaminated metabolites is characteristic of MAO-deficiency states.

The use of a ratio of these two metabolite groups, while not necessary for identification of the complete absence of MAO gene expression in the Norrie disease patients studied here, might provide the most sensitive index for detection of partial MAO-deficiency states. Recent identification of a point mutation of the MAO-A gene, associated with bizarre disturbances of behaviour [31, 32], raises the possibility that other as yet unrecognized deficits in MAO function may exist, associated with more subtle neuropsychological manifestations. Also, recent mapping of the COMT gene to the same site on chromosome 22 [33] where deletions are responsible for DiGeorge's syndrome [34], suggests that defective expression of the COMT gene may contribute to the psychosis associated with this syndrome [35]. Measurements of plasma metadrenalines in conjunction with deaminated metabolites, provide a straightforward approach to detect and quantify neurochemical phenotypes in genetic disorders of catecholamine metabolism and turnover.

REFERENCES

1. Guldberg HC, Marsden CA. Catechol-O-methyltransferase: pharmacological aspects and physiological role. Pharmacol Rev 1975; **27:** 135–206.
2. Graefe K-H, Henseling M. Neuronal and extraneuronal uptake and metabolism of catecholamines. Gen Pharmacol 1983; **14:** 27–33.
3. Maas JW, Benensohn H, Landis DH. A kinetic study of the disposition of circulating norepinephrine in normal male subjects. J Pharmacol Exp Ther 1970; **174:** 381–7.
4. Eisenhofer G, Goldstein DS, Ropchak TG, Nguyen HQ, Keiser HR, Kopin IJ. Source and physiological significance of plasma 3,4-dihydroxyphenylglycol and 3-methoxy-4-hydroxyphenylglycol. J Auton Nerv Syst 1988; **24:** 1–14.
5. Manji HK, Hsiao JK, Risby ED, Oliver J, Rudorfer MV, Potter WZ. The mechanisms of action of lithium. Arch Gen Psychiatry 1991; **48:** 505–12.
6. Lenders JWM, Kvetnansky R, Pacak K, Goldstein DS, Kopin IJ, Eisenhofer G. Extraneuronal metabolism of endogenous and exogenous norepinephrine and epinephrine in rats. J Pharmacol Exp Ther 1993; **266:** 288–93.
7. Eisenhofer G. Plasma normetanephrine for examination of extraneuronal uptake and metabolism of noradrenaline in rats. Naunyn-Schmiedeberg's Arch Pharmacol 1994; **349:** 259–69.
8. Eisenhofer G, Finberg JPM. Different metabolism of norepinephrine and epinephrine by catechol-O-methyltransferase and monoamine oxidase in rats. J Pharmacol Exp Ther 1994; **268:** 1242–51.
9. Kobayashi K, DeQuattro V, Bornheimer J, Kolloch R, Miano L. Plasma normetanephrine: a biochemical marker of sympathetic nerve function in man. Life Sci 1980; **26:** 1821–6.
10. Foti A, Adachi M, DeQuattro V. The relationships of free to conjugated normetanephrine in plasma and spinal fluid of hypertensive patients. J Clin Endocrinol Metab 1982; **55:** 81–6.
11. Lenders JWM, Eisenhofer G, Armando I, Keiser HR, Goldstein DS, Kopin IJ. Determination of metanephrines in plasma by liquid chromatography with electrochemical detection. Clin Chem 1993; **39:** 97–103.
12. de la Chapelle A, Sankila E-M, Lindlof M, Aula P, Norio R. Norrie disease caused by a gene deletion allowing carrier detection and prenatal diagnosis. Clin Genet 1985; **28:** 317–20.
13. Sims KB, de la Chapelle A, Norio R, et al. Monoamine oxidase deficiency in males with an X-chromosome deletion. Neuron 1989; **2:** 1069–76.
14. Murphy DL, Sims KB, Karoum F, et al. Marked amine and amine metabolite changes in Norrie disease patients with an X-chromosome deletion affecting monoamine oxidase. J Neurochem 1990; **54:** 242–7.
15. Collins FA, Murphy DL, Reiss AL, et al. Clinical, biochemical, and neuropsychiatric evaluation of a patient with contiguous gene syndrome due to a microdeletion Xp11.3 including the Norrie disease locus and monoamine oxidase (MAOA and MAOB) genes. Am J Med Genet 1992; **42:** 127–34.
16. Bleeker-Wagemakers EM, Zweije-Hofman I, Gal A. Norrie disease as part of a complex syndrome explained by a submicroscopic deletion of the X chromosome. Ophthalmic Paediatr Genet 1988; **9:** 137–42.
17. Goldstein DS, Eisenhofer G, Sax FL, Keiser HR, Kopin IJ. Plasma norepinephrine pharmacokinetics during mental challenge. Psychosom Med 1987; **49:** 591–605.
18. Eisenhofer G, Esler MD, Goldstein DS, Kopin IJ. Neuronal uptake, metabolism, and release of tritium-labeled norepinephrine during assessment of its plasma kinetics. Am J Physiol 1991; **261:** E505–15.
19. Holmes C, Eisenhofer G, Goldstein DS. Improved assay for plasma dihydroxyphenyl-acetic acid and other catechols using high-performance liquid chromatography with electrochemical detection. J Chromatogr 1994; **653:** 131–8.
20. Kobayashi K, Kolloch R, DeQuattro V, Miano L. Increased plasma and urinary normetanephrine in young patients with primary hypertension. Clin Sci 1979; **57** (Suppl. 5): 173–6.
21. Sullivan PA, DeQuattro V, Foti A, Curzon G. Effects of clonidine on central and peripheral nerve tone in primary hypertension. Hypertension 1986; **8:** 611–17.
22. DeQuattro V, Sullivan P, Foti A, et al. Central and regional normetadrenaline in evaluation of neurogenic aspects of hypertension: aid to diagnosis of phaeochromocytoma. Clin Sci 1980; **59** (Suppl. 6): 275–7.
23. DeQuattro V, Sullivan P, Minagawa R, et al. Central and peripheral noradrenergic tone in primary hypertension. Fed Proc Fed Am Soc Exp Biol 1984; **43:** 47–51.
24. Andrew R, Best SA, Watson DG, Midgley JM, Reid JL, Squire IB. Analysis of biogenic amines in plasma of hypertensive patients and a control group. Neurochem Res 1993; **18:** 1179–82.
25. Pagliari R, Cottet-Emard JM, Peyrin L. Determination of free and conjugated normetanephrine and metanephrine in human plasma by liquid chromatography with electrochemical detection. J Chromatogr 1991; **563:** 23–36.
26. Floras JS. Epinephrine and the genesis of hypertension. Hypertension 1992; **19:** 1–18.
27. Davidson L, Vandongen R, Rouse IL, Beilin LJ, Tunney A. Sex-related differences in resting and stimulated plasma noradrenaline and adrenaline. Clin Sci 1984; **67:** 347–52.
28. Kaplan GP. Immunohistochemical localization of catechol-O-methyltransferase in the mammalian central nervous system and in peripheral tissues. Thesis: Department of Anatomy and Neurobiology, Washington University, St Louis, Missouri, 1980, pp. 13–14.
29. Creveling CR, Hartman BK. Relationships between the cellular localization and the physiological function of catechol-O-methylation. In: Usdin E, Borchardt, Creveling CR, eds. Biochemistry of S-adenosylmethionine and related compounds. London: Macmillan, 1982; 479–86.
30. Ludwig J, Gerlich M, Halbrügge T, Graefe K-H. Plasma noradrenaline and dihydroxyphenylglycol in essential hypertension. Hypertension 1990; **17:** 546–52
31. Brunner HG, Nelen MR, van Zandvoort P, et al. X-linked borderline mental retardation with prominent behavioural disturbance: phenotype, genetic localization, and evidence for disturbed monoamine metabolism. Am J Hum Genet 1993; **52:** 1032–9.
32. Brunner HG, Nelen M, Breakfield XO, Ropers HH, van Oost BA. Abnormal behavior associated with a point mutation in the structural gene for monoamine oxidase A. Science (Washington DC) 1993; **262:** 578–80.
33. Winqvist R, Lundstrom K, Salminen M, Laatikainen M, Ulmanen I. The human catechol-O-methyltransferase (COMT) gene maps to band q11.2 of chromosome 22 and shows a frequent RFLP with BglI. Cytogenet Cell Genet 1992; **59:** 253–7.
34. Driscoll DA, Budarf ML, Emanuel BS. A genetic etiology for DiGeorge syndrome: consistent deletions and microdeletions of 22q11. Am J Hum Genet 1992; **50:** 924–33.
35. Dunham I, Collins J, Wadey R, Scambler P. Possible role for COMT in psychosis associated with velo-cardio-facial syndrome. Lancet 1992; **340:** 1361–2.