Assessment of sympathetic nervous function in humans from noradrenaline plasma kinetics

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Introduction

Noradrenaline is the neurotransmitter of the sympathetic nervous system. Many attempts have been made, in research spanning two decades [1-4], to utilize tracer methodology and the mathematical techniques of compartmental analysis [5, 6] to elucidate the kinetics of noradrenaline in humans. These studies have often been hampered by technical problems, such as the impracticability of assaying tissue noradrenaline levels in humans, and in earlier experiments, by the unavailability of radio-labelled L-noradrenaline, and the lack of a sufficiently sensitive and specific assay for plasma noradrenaline. The disposition of noradrenaline in fact is complex, defying precise compartmental analysis [4], and in the past this has frustrated attempts to measure noradrenaline turnover.

Noradrenaline in plasma is derived in the main from transmitter released by sympathetic nerves, to a very small degree from the adrenal medulla [7], and probably not at all from the central nervous system [8]. Faced with this lack of an acceptable method for determining noradrenaline turnover, investigators have turned to the plasma concentration of the transmitter as an index of overall sympathetic nervous system 'activity' (nerve firing rate) in studies of human physiology and disease [9-14]. There are several possible objections, however, to this application of plasma noradrenaline values, not the least being that the plasma concentration is determined in part by the rate at which noradrenaline is removed from the circulation, and not solely by the rate of noradrenaline release.

Because plasma noradrenaline measurements provide such an ambiguous guide to sympathetic nervous system activity, kinetic techniques for estimating the rate of release of noradrenaline to plasma have recently been developed [15-19]. These methods for studying plasma noradrenaline kinetics differ from the earlier, more ambitious studies of whole-body noradrenaline kinetics in several respects, in particular in their independence from complex mathematical models of noradrenaline disposition. The central feature of these methods is the determination of the metabolic clearance rate of plasma noradrenaline, clearance being calculated after either a bolus intravenous injection of noradrenaline [17] or intravenous infusion to steady state [15, 16, 18, 19]. Published methods differ in their use of either radiolabelled [15, 17, 18] or unlabelled noradrenaline [16, 19], and if a tracer is used, in the use of either L- or DL-noradrenaline. The use of either pharmacological doses of unlabelled noradrenaline [16, 19] or radiolabelled DL-noradrenaline [15] carries potential disadvantages. If pressor doses of noradrenaline are used, clearance estimates may possibly be distorted by saturation of removal mechanisms or alteration of organ blood flow rates, and reflex suppression of endogenous noradrenaline release could occur [16, 20]. Differences in the body's handling of the D-isomer, particularly for noradrenaline uptake [21], could influence results obtained with racemic radiolabelled noradrenaline.

For infused radiolabelled noradrenaline (NA) the following relationships hold, under steady-state conditions [5, 18]:

\[
\text{NA spillover rate} = \frac{[3\text{H}]\text{NA infusion rate}}{\text{specific radioactivity of plasma NA}} \tag{1}
\]
NA plasma clearance = \[
\frac{[^{3}H]NA \text{ infusion rate in plasma}}{[^{3}H]NA \text{ concn. in plasma}} \tag{2}
\]

Rather than the rate of release of noradrenaline from sympathetic nerve varicosities, 'NA spill-over rate' gives the rate at which noradrenaline released enters plasma. The essential prerequisite is that steady-state conditions hold (constant endogenous plasma noradrenaline concentration and plateau concentration of tracer). An important underlying assumption is that any re-release of tracer from sympathetic nerves is negligible in comparison with the rate of infusion [18]. Sampling from the right atrium will ensure that adequate mixing of venous blood from different organs has occurred, although the results obtained with right atrial and antecubital vein sampling sites are similar (M. Esler, unpublished observations).

Rates of spillover of noradrenaline to plasma

A substantial proportion of the noradrenaline released from sympathetic nerves is inactivated locally, principally by neuronal re-uptake, so that an uncertain, but necessarily small, fraction spills over into the venous drainage of an organ [22]. This rate of spillover has been shown to be directly related to sympathetic nerve firing rates [23].

The overall rate of noradrenaline spillover to plasma in humans resting supine has been estimated, from steady-state infusion methodology, to be 2.1 nmol min\(^{-1}\) m\(^{-2}\) [16] and 2.1 ± 0.8 nmol min\(^{-1}\) m\(^{-2}\) [18] (mean ± SD). Ghione et al. [17] used bolus methodology to derive their somewhat lower estimate, 1.00 ± 0.78 nmol min\(^{-1}\) m\(^{-2}\). The figure of Coulombe et al. [15], 0.22 nmol min\(^{-1}\) m\(^{-2}\) (approx. 100 µg/day), was calculated from an unvalidated single pool model of noradrenaline disposition, and would seem to be an underestimate since this value is barely adequate to account for urinary noradrenaline excretion, which constitutes but a few per cent of all noradrenaline released [22]. Noradrenaline spillover is low in patients with sympathetic nerve failure (idiopathic peripheral autonomic insufficiency) [18], as would be expected, and substantially reduced by the sympathetic nervous system suppressant, clonidine [24] (Fig. 1).

We found the noradrenaline spillover rate to be elevated in 20% of patients with essential hypertension [25], with a trend towards bimodality (Fig. 1). A simple relationship between noradrenaline spillover and the blood pressure did not exist. Noradrenaline spillover was sometimes elevated in two disorders studied in which blood pressure is typically normal, primary depressive illness [26] and hypothyroidism (\(P < 0.05\), Mann–Whitney U-test) (Fig. 1), the latter finding confirming that of Coulombe et al. [15]. We find noradrenaline spillover to be reduced in thyrotoxicosis (\(P < 0.05\), Fig. 1), but Coulombe et al. do not concur [15].

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**FIG. 1.** Rates of spillover of noradrenaline into plasma. The range of values found in 34 healthy subjects is indicated by the bars.
It should be emphasized that by studying plasma noradrenaline kinetics the rate of entry of noradrenaline to plasma is ascertained, and not actual noradrenaline release by sympathetic nerves. Noradrenaline spillover is influenced not only by sympathetic nerve firing rates, but also by the adequacy of the measures for local inactivation of released noradrenaline [23]. This caveat is underscored by consideration of the noradrenaline kinetics of patients with essential hypertension, among whom those patients with elevated noradrenaline spillover rates seem to have an underlying defect in neuronal noradrenaline re-uptake as the cause [25]. Measurement of actual noradrenaline release in humans will be difficult, perhaps impossible, requiring as it will inhibition of neuronal and extraneuronal noradrenaline uptake, and measurement of the rates of spillover of not only noradrenaline, but in addition all relevant metabolites.

Rates of removal of noradrenaline from plasma
Noradrenaline plasma clearance

Noradrenaline is removed from the circulation rapidly in humans. Noradrenaline plasma clearance has been variously estimated as $1.1 \pm 0.1 \text{ min}^{-1} \text{ m}^{-2}$ [17], $1.3 \pm 0.3 \text{ min}^{-1} \text{ m}^{-2}$ [18], $1.6 \pm 0.1 \text{ min}^{-1} \text{ m}^{-2}$ [16] and $2.1 \pm 0.2 \text{ min}^{-1} \text{ m}^{-2}$ [19]. Clearance values derived from non-tracer infusions appear to be higher [16, 19], possibly a consequence of changes produced in blood flow and its distribution by pressor infusions of noradrenaline. Reflex suppression of the release of endogenous noradrenaline could also lead to an overestimate of noradrenaline clearance, by lowering the baseline plasma noradrenaline value on which the calculations are dependent [16].

Removal of noradrenaline from plasma is thought to be achieved by the combined processes of neuronal uptake into sympathetic nerves, extraneuronal uptake by other tissues, such as vascular endothelium, and metabolic conversion by $O$-methylation, oxidative deamination and conjugation [22]. Studies in cats [27] and rabbits [28] point towards the importance of neuronal uptake, and to a lesser extent, $O$-methylation. Our own experiments in humans reinforce this view. We found that tricyclic antidepressants, at doses producing selective blockade of neuronal noradrenaline uptake, significantly reduced noradrenaline plasma clearance in normal subjects [29] and patients with depressive illness [26], whereas cortisol, a selective extraneuronal blocker of uptake, administered in a dose of 500 mg intravenously, had little effect in normal subjects [29] (Fig. 2). The reduction in noradrenaline plasma clearance observed in patients with idiopathic peripheral autonomic insufficiency (Fig. 2) also probably represents impaired neuronal noradrenaline uptake [29].

It is not known to what extent noradrenaline plasma clearance in humans is dependent on metabolism of noradrenaline. No assessment has yet been made of the contribution to total plasma clearance due to conjugation and $O$-methylation of noradrenaline. Inhibition of monoamine oxidase in normal subjects (by tranylcypromine, in a total dose of 40 mg given over 3 days) was adequate to reduce the plasma concentration of the deaminated metabolite, dihydroxyphenylglycol, by more than 50%, but produced only a modest and inconsistent fall in noradrenaline plasma clearance in the small number of subjects studied (Fig. 2).

An influence of age on noradrenaline plasma clearance was evident in our own studies [30], values being lower in older normal subjects (Fig. 2). Some unexpected effects of drugs on noradrenaline clearance have been encountered, for example the fall in clearance with $\beta$-adrenoceptor blockade reported both in normal subjects [20] and in patients with essential hypertension [25]. Of considerable interest is the claim that noradrenaline regulates its own plasma clearance [20], noradrenaline plasma clearance adjusting to fit the prevailing plasma noradrenaline concentration. Contrary to this view is the finding of reduced clearance in a patient with phaeochromocytoma and high plasma noradrenaline concentration [17], and the observations presented below which indicate that interventions which persistently elevate the plasma concentrations of noradrenaline do not invariably increase noradrenaline plasma clearance, nor do those which lower plasma noradrenaline values necessarily reduce noradrenaline clearance.

Plasma noradrenaline disappearance curve

The conformation of the plasma noradrenaline disappearance curve is a matter of dispute, this disagreement arising from differences in methodology. After intravenous bolus injections of tritiated $L$-noradrenaline the disappearance curve is multieponential, with simple exponential decay beyond 30–60 min after dosing [17, 31]. With tritiated $D,L$-noradrenaline, decay again is multiexponential, but noradrenaline disappearance is considerably slowed, as is achievement of the terminal exponential phase [1, 2]. This slowing presumably is a consequence of the stereo-
selectivity of noradrenaline uptake and metabolism [21].

The noradrenaline disappearance curve after infusion of noradrenaline to plateau plasma concentration is notable in the absence of the early distributive phase seen with bolus injections of noradrenaline. When unlabelled noradrenaline is infused, plasma noradrenaline disappearance post-infusion has been invariably reported as simple monoexponential [16, 19, 32, 33]. Contrasting with this, the post-infusion disappearance of tritiated L-noradrenaline is biexponential [25, 29], with half-time of the first exponential \( t_{1/2} \) in normal subjects of 2.0 ± 0.4 min, and half-time of the second exponential 34 ± 10 min (Fig. 3). The reason for this discrepancy, between post-infusion decay of tritiated and unlabelled noradrenaline, is not clear. One possible explanation is that it may be difficult to differentiate a terminal slope with slow decay from baseline plasma noradrenaline values when the non-tracer methodology is used, especially when possible suppression of endogenous noradrenaline release during the infusion renders plasma noradrenaline values post-infusion rather uncertain. Another possibility, that the terminal exponential is an artifact, representing re-release of tracer, is unlikely because this slope is not influenced by clonidine, which would be expected to slow such release of tritiated noradrenaline (M. Esler, unpublished observations).

The rapid removal phase (first exponential) of
Noradrenaline plasma kinetics in humans

the plasma tritiated noradrenaline disappearance curve, although it is not determined in simple fashion by a single removal process [5, 6], does seem to be related in particular to neuronal uptake of noradrenaline [25, 27, 29]. This is suggested by the observations that the $t_{1/2}$ is lengthened in normal subjects by inhibition of neuronal noradrenaline uptake with desipramine, but not changed with either inhibition of extra-neuronal uptake by cortisol, or monoamine oxidase block with tranylcypromine (Fig. 3). The $t_{1/2}$ value is also prolonged in patients with idiopathic peripheral autonomic insufficiency, in whom neuronal noradrenaline uptake presumably is subnormal, but not in patients with autonomic insufficiency from central nervous disease (Fig. 3), who have normal sympathetic nerves [34]. The rapid removal phase is slowed in a proportion of patients with essential hypertension, providing presumptive evidence that neuronal uptake of noradrenaline is defective [25].

Mismatching of noradrenaline spillover rate and plasma concentration values

It might be expected that alterations in noradrenaline plasma clearance would disturb the relationship between noradrenaline spillover rate and plasma concentration values. Reduced clearance would disproportionately elevate the plasma noradrenaline concentration for a given level of sympathetic nervous activity and noradrenaline release. Conversely, increased noradrenaline clearance would lower the plasma value. Several such instances of mismatching of noradrenaline spillover rate and plasma concentration figures have been reported. One is the existence of elevated plasma noradrenaline concentration in older normal subjects due, not to an increase in sympathetic nervous tone, but rather to diminished noradrenaline plasma clearance [30]. Another is the failure of plasma noradrenaline concentration to be sufficiently lowered in patients with idiopathic peripheral autonomic insufficiency to represent faithfully the existing sympathetic nervous deficit, and subnormal noradrenaline release, present in this condition [18, 35]. This is due to the presence of slowed noradrenaline plasma clearance.

Other examples of disparity between noradrenaline spillover and plasma concentration values are contained in Fig. 4. Single doses of the tricyclic antidepressant, desipramine (125 mg orally), lowered the noradrenaline spillover rate in seven normal subjects, but the plasma concentration of noradrenaline was unchanged because noradrenaline clearance was reduced [29]. Changing dietary calorie intake also altered noradrenaline plasma clearance, invalidating plasma noradrenaline measurements as an index of sympathetic tone and noradrenaline release [35] (Fig. 4). Six healthy lean subjects were placed on two diets, each adhered to for 10 days, providing 1674 kJ (400 kcal) daily, and 4184 kJ/m² daily in excess of energy requirements [35]. The noradrenaline spillover rate was 81% higher on the high-calorie diet ($P < 0.05$, paired $t$-test), but the plasma concentration of noradrenaline rose much less (25% higher, difference not significant) owing to the concurrent increase in noradrenaline plasma clearance with overeating (Fig. 4). $\beta$-Adrenergic blockade in six patients with essential hypertension, treated for 1 month in a double-blind drug trial setting with oxprenolol, 320 mg daily, also led to disagreement between noradrenaline spillover and concentration values (Fig. 4). The plasma concentration of noradrenaline rose on oxprenolol by 46%, this rise being due in large part to the fall in noradrenaline.

**Fig. 3.** (a) Tritiated plasma noradrenaline disappearance curve in normal subjects (means ± SEM). (b) Half-time of the rapid removal component ($t_{1/2}$) before and after desipramine (Des), cortisol (Cort) and tranylcypromine (MAOI). (c) $t_{1/2}$ in normal subjects, compared with that of patients with peripheral, and with central, autonomic insufficiency. **$P < 0.01$.**
clearance, noradrenaline spillover rate rising by only 19% (change not significant).

The difficulty of knowing, a priori, whether plasma noradrenaline measurements can be validly used as an index of sympathetic nervous system activity in a particular situation is illustrated by contrasting these results with those obtained in six patients with essential hypertension treated with oxprenolol, plasma noradrenaline concentration values were not indicative of noradrenaline release rate, because of alterations produced in noradrenaline plasma clearance. In contrast, clonidine and chlorthalidone did not alter noradrenaline clearance, so that changes in noradrenaline spillover and plasma concentration were in harmony. *P < 0.05; **P < 0.01.

**Fig. 4.** Mismatching of noradrenaline spillover rate and plasma concentration values. In normal subjects given desipramine, or changed from a low- to a high-calorie diet, and in patients with essential hypertension treated with oxprenolol, plasma noradrenaline concentration values were not indicative of noradrenaline release rate, because of alterations produced in noradrenaline plasma clearance. In contrast, clonidine and chlorthalidone did not alter noradrenaline clearance, so that changes in noradrenaline spillover and plasma concentration were in harmony. *P < 0.05; **P < 0.01.

To what extent noradrenaline entering plasma is derived from sympathetic nerves of organs directly involved in cardiovascular regulation, such as the heart and kidney, to what degree from other sites with no special cardiovascular function, such as exocrine glands and the wall of the gut, and to what extent from the relatively
massive skeleto-muscular system is not known. Studies of regional noradrenaline spillover may
perhaps provide explanations for existing anomalies with overall noradrenaline spillover
measurements, such as the finding that approximately 50% of patients with depressive illness
have elevated overall noradrenaline spillover, but entirely normal heart rates and blood pressures
[26].

At which sites noradrenaline is removed from the circulation also has been little studied. Circulating noradrenaline is probably extracted by all organs, removal of noradrenaline having been demonstrated directly in humans for both the lungs [38] and liver [39]. Counterbalancing noradrenaline release, however, coupled with pulmonary extraction, maintains a net positive mixed venous–arterial plasma noradrenaline concentration difference.

Conclusion

Biochemical methods have been used extensively in an attempt to quantify overall sympathetic nervous system activity in humans. The present review demonstrates that plasma noradrenaline measurements are a fallible guide to sympathetic tone, being dependent as they are on noradrenaline plasma clearance. The measurement of the noradrenaline spillover rate provides a better index of sympathetic activity than does the plasma noradrenaline concentration. The overall noradrenaline spillover rate measurement, however, is not without its limitations, the chief being that the sources of the released noradrenaline are not identified. Measurement of regional noradrenaline release may be more pertinent than overall release rate measurements in some instances, such as measuring renal noradrenaline release in essential hypertension, perhaps, or cardiac noradrenaline release in patients with coronary artery spasm, and is now feasible in humans with little modification of existing tracer methodology.

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