Comparison of two indices for forearm noradrenaline release in humans

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ABSTRACT

Although there is as yet no method which measures directly the neuronal release of noradrenaline in humans in vivo, the isotope dilution technique with [3H]noradrenaline has been applied to estimate forearm neuronal noradrenaline release into plasma. Two different equations have been developed for this purpose: one to estimate the spillover of noradrenaline into the venous effluent, and a modified formula (often referred to as the appearance rate) which may reflect more closely changes in the neuronal release of noradrenaline into the synaptic cleft, particularly during interventions that alter forearm blood flow. The present study was performed to compare the effects of two interventions known to exert contrasting actions on neuronal forearm noradrenaline release and forearm blood flow. Intra-arterial infusion of sodium nitroprusside at doses without systemic effect increases forearm blood flow, but not neuronal noradrenaline release. In contrast, lower-body negative pressure at –25 mmHg causes forearm vasoconstriction by stimulating neuronal noradrenaline release. During sodium nitroprusside infusion, forearm noradrenaline spillover increased from 1.1±0.3 to 2.2±1.0 pmol min⁻¹·100 ml⁻¹ (P<0.05), whereas the forearm noradrenaline appearance rate was unchanged. Lower-body negative pressure did not affect the forearm noradrenaline spillover rate, but increased the forearm noradrenaline appearance rate from 3.4±0.4 pmol min⁻¹·100 ml⁻¹ at baseline to 5.0±0.9 pmol min⁻¹·100 ml⁻¹ (P<0.05). These results indicate that the noradrenaline appearance rate provides the better approximation of changes in forearm neuronal noradrenaline release in response to stimuli which alter local blood flow.

INTRODUCTION

The isotope dilution technique has been applied in humans to estimate the rate at which neurally released noradrenaline enters the plasma for the entire body, or into the venous effluent of a specific organ [1]. In that the steady-state infusion of tracer amounts of [3H]noradrenaline permits the calculation of its local extraction by neuronal and extraneuronal mechanisms, the rate at which locally released noradrenaline enters the forearm venous effluent can be estimated from formulae with three components: the arterial–venous gradient for noradrenaline, regional plasma flow, and an extraction term [1–3].

Key words: forearm, methodology, noradrenaline kinetics.
Abbreviations: LBNP, lower-body negative pressure; SNP, sodium nitroprusside.
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In the human forearm, changes in blood flow can affect the relationship between the rate of neurotransmitter release from sympathetic nerve endings and the rate at which noradrenaline spills over into the forearm venous effluent. For example, neither sodium nitroprusside (SNP) nor methoxamine affect neuronal noradrenaline release directly, yet, when infused into the brachial artery, both substances change forearm blood flow and forearm noradrenaline spillover [7]. Because many interventions affect neuronal noradrenaline release and forearm blood flow simultaneously, the occurrence of any flow-dependent changes in noradrenaline spillover would exclude the use of the isotope dilution technique to estimate changes in forearm neuronal noradrenaline release. Chang et al. [8] therefore proposed a modified formula as an index of neurally released noradrenaline, as opposed to the original formula that estimates the rate of noradrenaline spillover into the venous effluent. The so-called ‘noradrenaline appearance rate’ can be derived from the original spillover formula by assuming that there exists a fraction of neural noradrenaline that is taken up and/or metabolized by cells at the site of its discharge, and that this equals the forearm fractional extraction of tritiated noradrenaline. Although this assumption may be correct intuitively, it has not been validated.

The purpose of the present investigation was to explore appearance rate as an index for changes in forearm neural noradrenaline release. We compared the effects on forearm noradrenaline spillover rate (according to the original equation developed by Esler et al. [1]), and on forearm noradrenaline appearance rate, of two interventions that differ in their actions on forearm blood flow, forearm fractional noradrenaline extraction and neural release of noradrenaline: (1) brachial artery infusion of SNP, which increases forearm blood flow without augmenting the rate of noradrenaline release from sympathetic nerves within the forearm; and (2) lower-body negative pressure (LBNP), which causes neurogenic vasoconstriction by increasing sympathetic nerve discharge and thus noradrenaline release to forearm skeletal muscles. Our hypothesis was that the noradrenaline appearance rate would provide a better approximation of the anticipated changes in forearm neural noradrenaline release elicited by these interventions. These experiments were performed in two different groups of healthy men, and represent the control segments of two studies that have been published previously [3,9].

### METHODS

**Subjects**

A total of 34 normotensive, non-smoking, healthy male volunteers participated in these studies. The protocols were approved by our local institutional ethics committees, and all subjects provided written informed consent. All studies were performed in the morning in a supine position after 24 h of caffeine abstinence, in a temperature-controlled room (24–25 °C).

**Study 1**

Nine men, whose average (± S.D.) age, weight and height were 32.6 ± 9.4 years, 78.9 ± 6.2 kg and 179.2 ± 7.5 cm respectively, were studied. ECG was recorded continuously. The brachial artery of the non-dominant arm was cannulated [2 inch (50.8 mm)/20 gauge catheter] for the continuous recording of intra-arterial blood pressure [Novakit* pressure monitoring line connected via a Novotrans* pressure transducer (Medex, Hilliard, OH, U.S.A.), to a blood pressure recorder, model Horizon 2000* (Mennen Medical, Clarence, NY, U.S.A.)], infusion of SNP or vehicle (5% w/v glucose) by a syringe pump (model 11; Harvard Apparatus, South Natick, MA, U.S.A.), and for blood sampling. Deep antecubital veins of both arms were cannulated retrogradely (2 inch/20 gauge catheter; Angiocath; Becton Dickinson, Sandy, UT, U.S.A.) for blood sampling. A calf or foot superficial vein was cannulated for infusion of [3H]-noradrenaline by an anaesthesia syringe pump (model P3000; IVAC Canada, Richmond Hill, Ontario, Canada).

Blood flow was measured in both forearms simultaneously using venous occlusion strain gauge plethysmography. For this purpose, a cuff was applied to each upper arm. These were inflated simultaneously at regular intervals to a venous occlusion pressure of 40 mmHg for 6–10 s (Hokanson rapid cuff inflator; D. E. Hokanson, Bellevue, WA, U.S.A.). Changes in forearm volume were detected by strain gauge plethysmography (EC4SB Plethysmograph; D. E. Hokanson). During measurement of forearm blood flow and blood sampling, circulation to the hand was occluded bilaterally by inflation of wrist cuffs to suprasystolic pressures [10]. Both forearms were supported by slings above the level of the right atrium throughout these experiments, to ensure rapid recovery of forearm volume after deflation of the upper arm cuffs.

At least 30 min after these cannulations, [3H]noradrenaline ([levo]-ring-2,5,6-3H)noradrenaline; specific radioactivity 30–60 Ci/mmol; New England Nuclear) was then infused into a superficial leg vein at 1 μCi/min. After 15 min, the wrist cuffs were inflated to 200 mmHg, and 5% (w/v) glucose, followed by 0.02 and then 0.2 μg of SNP·min⁻¹·100 ml⁻¹ forearm, were infused sequentially (5 min per dose). The wrist cuffs were again deflated after blood sampling was complete (see below). After a further 10 min, the wrist cuffs were re-inflated and the highest dose of SNP (0.6 μg·min⁻¹·100 ml⁻¹ forearm) was infused for 5 min.

Blood flow in the experimental and contralateral forearm...
forearms was measured during the 5 min infusion of 5% (w/v) glucose, and during the final 2 min of each drug infusion. At the end of each drug infusion, forearm venous (bilaterally) and arterial blood was sampled for measurement of plasma noradrenaline and [3H]noradrenaline. To prevent any interruption of the intra-arterial drug infusions, these arterial samples were collected only after bilateral venous sampling was complete.

**Study 2**
A total of 25 men, whose average (± S.D.) age, weight and height were 34.6 ± 16.8 years, 76.0 ± 10.4 kg and 181.4 ± 6.6 cm respectively, were studied. The brachial artery and antecubital vein of the non-dominant arm were cannulated as described above. A deep antecubital vein of the dominant arm was cannulated anterogradely for infusion of [3H]noradrenaline. Forearm blood flow was measured bilaterally as described above. The lower body was sealed in an airtight Plexiglas® box for application of LBNP for 15 min at −25 mmHg. The magnitude of negative pressure achieved was registered by an internal manometer.

All experiments began at least 30 min after the intra-arterial cannulation. Venous blood was collected for the measurement of haematocrit. [3H]Noradrenaline (specific radioactivity 30–60 Ci/mmol) was then infused at a constant rate of 1.0 μCi/min (automatic syringe infusion pump, type STC-521; Terumo, Tokyo, Japan). After 10 min, wrist cuffs were inflated and forearm blood flow was recorded. After a further 10 min, following completion of venous and arterial blood collection from the non-dominant arm as described above, the wrist cuffs were deflated and LBNP was applied at −25 mmHg and sustained for 15 min. At 5 min into the period of LBNP, the wrist cuffs were re-inflated and flow measurements were repeated. Venous and arterial blood was sampled, as described above, at the end of the period of LBNP.

**Data collection and statistical analysis**
Mean arterial blood pressure was measured continuously during each recording of forearm blood flow and averaged. Forearm vascular resistance was calculated as the quotient of the average mean arterial blood pressure and forearm blood flow, and expressed in arbitrary units (mmHg · min · 100 ml tissue/ml). For study 1, baseline values were defined as the means of all measurements obtained during the infusion of 5% (w/v) glucose. The response to SNP was calculated as the difference between the mean values obtained over the last 2 min of infusion of each drug dose and this baseline value. For study 2, the haemodynamic parameters were averaged to one value at baseline (8 min before starting LBNP) and during LBNP (last 6 min of LBNP, when forearm blood flow had reached steady state).

The methods used for blood sampling and analysis of plasma concentrations of noradrenaline and [3H]noradrenaline have been described in detail previously [3,9]. Arterial and venous concentrations of [3H]noradrenaline ([3H]NA) and noradrenaline (NA) were used for calculation of the forearm noradrenaline spillover rate (SOR), according to the equation:

\[
\text{SOR} = \frac{\text{NA}_a \times \text{FPF} - \text{NA}_v \times \text{FPF}}{\text{f}_\text{NA}}
\]

FPF and \( f_{NA} \) represent forearm plasma flow and forearm fractional extraction of [3H]noradrenaline respectively. The forearm fractional extraction was calculated using the equation:

\[
\text{f}_{\text{NA}} = \frac{([3H]\text{NA}_a) - ([3H]\text{NA}_v)}{([3H]\text{NA}_a)}
\]

The forearm noradrenaline appearance rate (AR) was derived from the forearm spillover rate according to the equation:

\[
\text{AR} = \frac{\text{SOR}}{1 - f_{\text{NA}}}
\]

Total-body noradrenaline spillover, i.e. the estimated rate of appearance of endogenous noradrenaline in arterial plasma, was derived from the arterial plasma noradrenaline concentration ([NA]a), the arterial steady-state plasma concentration of [3H]noradrenaline ([3H]-NAa), and the infusion rate of [3H]noradrenaline, according to the equation [11]:

\[
\text{Total-body noradrenaline spillover (pmol/min)} = \text{NA}_a \times \frac{\text{infusion rate (d.p.m./min))}}{[3H]\text{NA}_a}\text{ (d.p.m./ml)}
\]

All results are presented as mean ± S.E.M. For study 1, effects on blood pressure, heart rate, forearm blood flow and vascular resistance were assessed by ANOVA for repeated measurements using the dose of SNP as the within-subject factor. Since data on forearm noradrenaline spillover and appearance rate were not normally distributed (P < 0.1; Shapiro–Wilk test for normality), the Wilcoxon matched-pairs signed rank test was used to assess the effects of SNP infusions on baseline values. To avoid multiple comparisons within this non-parametric analysis, statistical tests were performed on the overall response to intra-arterial SNP. This was quantified, for each volunteer, by reducing the responses to SNP (three doses) to one value (area under the curve, standardized by the duration of the infusions) [11]. For study 2, the effect of LBNP on baseline values was analysed by the Wilcoxon matched-pairs signed rank test. A two-sided P value of 0.05 for statistical significance was selected.

**RESULTS**

**Study 1 (SNP infusion)**
Intra-arterial SNP did not affect significantly blood pressure, heart rate or total-body noradrenaline spillover;
the latter was $3.0 \pm 0.8 \text{nmol/min at baseline and } 3.6 \pm 1.1$, $3.1 \pm 0.8$ and $2.9 \pm 0.8 \text{nmol/min during the three increasing SNP doses (0.02, 0.2 and } 0.6 \mu\text{g min}^{-1}\cdot100 \text{ml}^{-1})$. In the non-infused arm, intra-arterial SNP had no effect on forearm blood flow ($2.7 \pm 0.4 \text{ml min}^{-1}\cdot100 \text{ml}^{-1} 	ext{ at baseline and } 2.7 \pm 0.4$, $2.9 \pm 0.4$ and $2.6 \pm 0.4 \text{ml min}^{-1}\cdot100 \text{ml}^{-1}$ respectively during the three doses). These observations indicate that these intra-arterial infusions had no systemic effect. In the infused arm, forearm blood flow increased from $3.2 \pm 0.7 \text{ml min}^{-1}\cdot100 \text{ml}^{-1}$ at baseline to $4.9 \pm 1.0$, $9.9 \pm 2.2$ and $14.0 \pm 3.5 \text{ml min}^{-1}\cdot100 \text{ml}^{-1}$ respectively during the three increasing SNP doses ($P < 0.01$) (Figure 1).

In the non-infused arm, SNP had no effect on fractional extraction ($0.65 \pm 0.02$ at baseline, and $0.62 \pm 0.04$, $0.62 \pm 0.02$ and $0.61 \pm 0.04$ respectively during the three doses of SNP), whereas in the infused arm the fractional extraction of $[^{3}H]\text{noradrenaline was } 0.68 \pm 0.03$ at baseline and fell to $0.59 \pm 0.04$, $0.39 \pm 0.04$ and $0.36 \pm 0.05$ respectively during the three SNP infusions ($P < 0.05$). These observations indicate that local changes in noradrenaline extraction were related to vasodilation caused by intra-arterial SNP infusion. In the infused arm the noradrenaline spillover rate doubled, from $1.1 \pm 0.3 \text{pmol min}^{-1}\cdot100 \text{ml}^{-1}$ at baseline to $1.9 \pm 0.4$, $2.8 \pm 0.6$ and $2.2 \pm 1.0 \text{pmol min}^{-1}\cdot100 \text{ml}^{-1}$ respectively during the three SNP doses ($P < 0.05$). On the other hand, when this estimate of forearm noradrenaline release into plasma was recalculated as appearance rate, there was no significant change from baseline during the course of these infusions: $5.0 \pm 1.2$, $4.7 \pm 1.3$ and $4.2 \pm 1.8 \text{pmol min}^{-1}\cdot100 \text{ml}^{-1}$ respectively for the three SNP doses, compared with $3.5 \pm 0.8 \text{pmol min}^{-1}\cdot100 \text{ml}^{-1}$ at baseline. There was a significant difference ($P < 0.05$) in the response to SNP as estimated by these two methods (Figure 1). As expected, neither spillover nor appearance rate in the contralateral arm changed significantly during the SNP infusions.

### Study 2 (LBNP)

Systolic blood pressure was not significantly affected by LBNP at $-25 \text{mmHg (121} \pm 2 \text{mmHg, compared with } 122 \pm 2 \text{mmHg at baseline)}$. Diastolic blood pressure increased slightly, from $67 \pm 1 \text{mmHg at baseline to } 68 \pm 1 \text{mmHg (} P < 0.05$). Heart rate increased from $57 \pm 1$ to $60 \pm 2 \text{beats/min (} P < 0.01$).

As anticipated, both total-body noradrenaline spillover and forearm vascular resistance increased significantly, indicating successful activation of the sympathetic nervous system and neurogenic vasoconstriction in these subjects (Table 1). This decrease in forearm blood flow was accompanied by a small increase in the fractional extraction of noradrenaline by the forearm, from

<table>
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<th>Conditions</th>
<th>FBF</th>
<th>FVR</th>
<th>TBS</th>
<th>SOR</th>
<th>AR</th>
<th>$\zeta_{FA}$</th>
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<td>Baseline</td>
<td>$2.6 \pm 0.3$</td>
<td>$42 \pm 3$</td>
<td>$2.2 \pm 0.2$</td>
<td>$0.9 \pm 0.1$</td>
<td>$3.4 \pm 0.4$</td>
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<tr>
<td>LBNP</td>
<td>$1.5 \pm 0.2^*$</td>
<td>$87 \pm 13^*$</td>
<td>$2.7 \pm 0.2^*$</td>
<td>$1.0 \pm 0.1$</td>
<td>$5.0 \pm 0.9^*$</td>
<td>$0.75 \pm 0.03^*$</td>
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Table 1 Effects of LBNP ($-25 \text{mmHg}$) on forearm noradrenaline appearance rate and spillover rate as indices for local noradrenaline release into plasma.

FBF, forearm blood flow (ml min$^{-1}\cdot100 \text{ml}^{-1}$); FVR, forearm vascular resistance (arbitrary units); TBS, total body spillover (nmol/min); SOR, forearm noradrenaline spillover rate (pmol min$^{-1}\cdot100 \text{ml}^{-1}$); AR, forearm noradrenaline appearance rate (pmol min$^{-1}\cdot100 \text{ml}^{-1}$); $\zeta_{FA}$, forearm noradrenaline fractional extraction. Values are means ± S.E.M. ($n = 25$); * indicates significant differences from baseline ($P < 0.05$).
0.71 ± 0.02 to 0.75 ± 0.03 (P < 0.05). The forearm noradrenaline appearance rate increased significantly, whereas forearm noradrenaline spillover was unchanged (Table 1).

**DISCUSSION**

The main findings of this analysis were: (1) during a local vasodilator-mediated increase in forearm blood flow, the calculated forearm noradrenaline spillover rate increased, whereas the forearm noradrenaline appearance rate remained constant; and (2) an increased sympathetic drive to the forearm, induced by the application of LBNP at −25 mmHg, was detected as a rise in the forearm noradrenaline appearance rate; in contrast, the forearm noradrenaline spillover rate did not increase in response to this intervention. Together, these findings indicate that changes in appearance rate provide a better approximation of changes in forearm noradrenaline release in response to stimuli which also alter forearm blood flow.

Thus far, SNP has not been demonstrated to facilitate noradrenaline release from sympathetic nerve endings by a pre-junctional mechanism. Indeed, SNP, an NO donor, might actually reduce the local release of noradrenaline via an NO-mediated pathway [12–14]. Local infusion of SNP in the present study did not activate the sympathetic nervous system reflexively. Intra-arterial SNP had no effect on blood pressure, heart rate, total-body noradrenaline spillover, or forearm blood flow and noradrenaline kinetics in the contralateral forearm.

At a dose of 0.2 μg min⁻¹ 100 ml⁻¹, SNP increased forearm blood flow by 100% and reduced forearm noradrenaline extraction by 40%. Grossman et al. [7] observed similar effects on forearm noradrenaline extraction at their highest dose of SNP (50 ng min⁻¹ kg⁻¹), which increased forearm blood flow by 100%. This decrease in the fractional extraction of noradrenaline has been attributed to the more rapid transit of blood across the forearm, allowing circulating noradrenaline to escape from extraction [8].

The forearm noradrenaline spillover rate increased during the SNP-induced augmentation of forearm blood flow. This confirms previous observations that venous spillover of locally released noradrenaline will change with forearm blood flow, even in the absence of a stimulus to noradrenaline release [7]. This finding has been attributed to flow-dependent wash-out of locally released noradrenaline, a phenomenon that is not incorporated in the spillover-rate equation. In the formula for noradrenaline appearance rate, it is presumed that alterations in local blood flow will affect the neural and non-neural extraction of circulating arterial and locally released noradrenaline similarly. Therefore, for convenience, it is assumed that local changes in blood flow will have similar actions on the extraction of tritiated and neurally released noradrenaline. This assumption is supported by experimental evidence showing similar kinetics for locally released noradrenaline (as induced by intra-arterial infusion of tyramine) and circulating [3H]-noradrenaline [8].

The calculation of noradrenaline appearance rate addresses this issue. This formula contains an additional term to account for the extraction of neurally released noradrenaline before it enters the venous effluent [8]. Our observation that the forearm noradrenaline appearance rate did not increase significantly during forearm vasodilation supports the use of noradrenaline appearance rate to minimize any flow-induced bias in this estimate of the rate of noradrenaline release.

To further substantiate these observations during SNP infusion, we calculated both noradrenaline spillover and appearance rate in response to LBNP, an intervention that increases efferent sympathetic nerve discharge to forearm skeletal muscle [15]. Indeed, studies with α-adrenoceptor antagonists have demonstrated that an important component of the vasoconstrictor response to LBNP is mediated by the local release of noradrenaline [16]. As expected, LBNP reflexively increased total-body noradrenaline spillover. Forearm blood flow fell and vascular resistance rose, reflecting this increase in sympathetic outflow. Despite this evidence for neurogenic vasoconstriction, the calculated rate of spillover of noradrenaline did not increase in response to LBNP. The decrease in forearm blood flow was accompanied by an increase in the fractional extraction of [3H]noradrenaline. Similar changes in the fractional extraction of [3H]-noradrenaline have been observed during pharmacologically induced vasoconstriction by the intra-arterial infusion of methoxamine [7]. If this increase in the fractional extraction of circulating noradrenaline is accompanied by an increase in the fractional extraction of neurally released noradrenaline, the calculated noradrenaline spillover into the venous effluent will be reduced. Thus any increase in forearm neuronal noradrenaline release under conditions of LBNP is likely to be underestimated when the spillover rate formulation, which does not account for the extraction of locally released noradrenaline, is applied. On the other hand, the calculated noradrenaline appearance rate was increased. This observation is consistent with both the forearm vasoconstriction and the increase in total-body noradrenaline spillover evoked by LBNP. Based on this observation, we conclude that changes in the appearance rate approximate more closely the LBNP-induced increases in forearm noradrenaline release than do changes in the spillover rate.

Our results contrast with those of some investigators, who did not detect an effect of a change in forearm blood flow on the fractional extraction of [3H]noradrenaline, and described increases in both the noradrenaline appearance rate and the noradrenaline spillover rate during...
infusion of SNP into the brachial artery [17,18]. Differences between these studies, the present study and the studies of Grossman et al. [7] and Chang et al. [8] are summarized in Table 2. Of note is the fact that those studies that did not detect such flow-dependent changes in the fractional extraction of noradrenaline are characterized by rather low fractional extractions at baseline. Possibly, these differences in baseline fractional extraction reflect the contribution of the cutaneous vascular bed to the measured forearm blood flow and fractional extraction of noradrenaline. This concept is supported by results from other studies suggesting a relationship between baseline fractional extraction of noradrenaline and inclusion of the hand in the experimental preparation: Eisenhofer et al. [19] excluded the hand circulation and reported a fractional noradrenaline extraction of 0.72, whereas Goldstein et al. [20] included the hand circulation and reported a fractional noradrenaline extraction of 0.54. The assumption for the calculation of appearance rate is not met if the hand is included in the experimental preparation, since arteriovenous shunts in the cutaneous vasculature would decrease the extraction of 0.54. The assumption for the calculation of appearance rate is not met if the hand is included in the experimental preparation, since arteriovenous shunts in the cutaneous vasculature would decrease the extraction rate of 0.54. This concept is supported by results from other studies suggesting a relationship between baseline fractional extraction of noradrenaline and inclusion of the hand in the experimental preparation: Eisenhofer et al. [19] excluded the hand circulation and reported a fractional noradrenaline extraction of 0.72, whereas Goldstein et al. [20] included the hand circulation and reported a fractional noradrenaline extraction of 0.54. The assumption for the calculation of appearance rate is not met if the hand is included in the experimental preparation, since arteriovenous shunts in the cutaneous vasculature would decrease the extraction of [3H]noradrenaline without affecting the extraction of locally released endogenous noradrenaline.

On the basis of comparisons of the responses to LBNP and the local infusion of SNP in the present study, we prefer the use of appearance rate rather than spillover rate as an index of changes in the forearm neural release of noradrenaline under conditions that also alter forearm blood flow. Whether these observations and this conclusion can be extrapolated to measurements across other organs, such as heart or kidney, remains to be determined, since fractional extraction varies, and therefore uptake processes and their dependency on blood flow may differ between these organs [19]. Furthermore, it should be emphasized that definitive validation of this technique in vivo is hampered by a lack of methods for the measurement of neurally released noradrenaline directly in humans.

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REFERENCES

Noradrenaline appearance versus spillover


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