Demonstration of Neuronal and Extraneuronal Uptake of Circulating Norepinephrine in the Forearm

PETER C. CHANG, JACQUES A. VAN DER KROGT, AND PETER VAN BRUMMELEN

SUMMARY Disturbances in peripheral norepinephrine release or removal by neuronal and extraneuronal uptake may have pathogenetic significance in cardiovascular disease states. We investigated the mechanisms of removal of norepinephrine in the forearm of healthy subjects under basal conditions, using measurements of arterial and venous plasma norepinephrine concentrations, blood pressure, heart rate, and forearm blood flow. The specific inhibitor of neuronal uptake, desipramine, was infused intra-arterially into the brachial artery of five subjects. Net norepinephrine overflow from the forearm increased markedly, revealing considerable local release of norepinephrine. Six other subjects received four intra-arterial infusions of norepinephrine, 1.18 pmol/kg/min, with various doses of desipramine and the extraneuronal uptake-inhibiting drug hydrocortisone. The forearm extraction rate for circulating norepinephrine decreased with increasing doses of desipramine (from 69.4 ± 3.0 [SEM] to 35.3 ± 8.4%; p<0.001). Increasing doses of hydrocortisone during continued inhibition of neuronal uptake resulted in decreased forearm extraction of norepinephrine (from 63.3 ± 4.9 to 40.6 ± 4.4%; p<0.01). In six other subjects who received the highest dose of hydrocortisone without concomitant inhibition of neuronal uptake, forearm extraction of norepinephrine decreased from 57.1 ± 4.9 to 51.5 ± 4.7% (p<0.05). These results suggest that neuronal uptake contributes markedly to the removal of circulating and endogendusly released norepinephrine in the forearm. For circulating norepinephrine, a corticosteroid-sensitive mechanism of extraneuronal uptake was also demonstrated. These results indicate that neuronal and extraneuronal uptake can be estimated separately in this vascular bed. Similar organ-specific studies in patients may reveal disturbances in mechanisms of norepinephrine removal. (Hypertension 9: 647-653, 1987)

KEY WORDS • neuronal uptake • extraneuronal uptake • norepinephrine • epinephrine • norepinephrine release • desipramine • hydrocortisone • forearm blood flow

A VITAL link between sympathetic nerve activity and cardiovascular responses is the release of the neurotransmitter norepinephrine (NE) from sympathetic nerve endings. After release by exocytosis from storage vesicles, NE activates both postsynaptic and presynaptic adrenergic receptors, which respectively mediate cardiovascular responses and exert a negative feedback on the exocytotic process.1 NE is removed from the synaptic cleft by 1) reuptake into the neuron through an active carrier process (neuronal uptake or U₁) linked to Na⁺,K⁺-adenosine triphosphatase (ATPase), 2) uptake into the effector cells (extraneuronal uptake or U₂), and 3) diffusion into surrounding tissues and blood vessels.2-4 Because of the very effective clearance of released NE, especially by U₁, only a small fraction of the neurotransmitter reaches the intravascular space.4

Disturbances in peripheral NE release or clearance by U₁ or U₂ may have pathogenetic significance in cardiovascular disease states. Indeed, in some hypertensive patients, defective U₁ has been reported.6,7 Interestingly, previous attempts to demonstrate U₂ in humans by means of an intravenous infusion of tracer doses of NE and cortisol, a drug known to possess U₂-inhibiting properties,8 have not been successful.9 Because efferent sympathetic nerve activity is not
uniform, and organ-specific changes in sympathetic outflow can occur in different disease states, studies of local sympathetic nervous function are needed to gain a better insight into sympathetic nervous pathophysiology. 10

Recently, we demonstrated that the removal of circulating NE from the forearm vascular bed is a high-rate extraction process that obeys first-order kinetics and is inversely related to blood flow. 11 In the present study we investigated the mechanism of this extraction process in healthy volunteers, using intra-arterial infusions of a low dose of NE in combination with various doses of the U 1-inhibiting drug desipramine (DMI) 2 and the U 2-inhibiting drug hydrocortisone (HC). 8 As U 1 was anticipated to be the main determinant of local NE removal, U 2 was studied in the absence and in the presence of U 1 inhibition, since in the latter situation more NE is available for U 2.

**Subjects and Methods**

Seventeen healthy male volunteers participated in this study after giving informed consent. Their clinical characteristics are listed in Table 1. In all subjects medical history, physical examination, and routine laboratory tests showed no evidence of cardiovascular or other diseases. None of the subjects took any medication either at the time of the study or in the previous 2 weeks. The protocols of the study were approved by the Medical Ethics Committee of the Leiden University Hospital.

**Procedures**

The methods used have been described in detail previously. 11, 12 In short, the brachial artery on the subject's nondominant side was cannulated for intra-arterial (i.a.) infusion of drugs, blood sampling, and blood pressure (BP) monitoring with a Statham P23Id pressure transducer (Gould, Oxnard, CA, USA). A deep cubital vein in the same arm was also cannulated for blood sampling. Forearm blood flow (FBF) values were measured by venous occlusion plethysmography. Each value represents the average of six individual recordings, four to five heartbeats in duration, taken at 15-second intervals before and at the end of each infusion step. Hand blood flow was excluded during all experiments by inflating a small wrist cuff to 40 mm Hg above the systolic BP (SBP). In between each experiment this cuff was deflated for at least 20 minutes. Forearm volume was measured by water displacement. Heart rate (HR), i.a. BP, a one-lead electrocardiogram, and the plethysmographic tracings were registered on a polygraph. All experiments began at least 30 minutes after the cannulation procedure.

**Study Protocols**

**Desipramine Infusion**

Five subjects received a 15-minute i.a. infusion of the U 1 inhibitor DMI, 0.6 nmol/kg/min. Venous blood was sampled at 0, 2, 4, 8, 12, and 15 minutes, and arterial blood was sampled at 0 and 15 minutes for determination of plasma NE (PNE) and plasma epinephrine (PE).

**Norepinephrine Infusions with Desipramine and Hydrocortisone**

Six other subjects received four i.a. infusions of NE, 1.18 pmol/kg/min, each lasting 20 minutes and with at least 20 minutes of rest between infusions. During the first NE infusion DMI, 0.3 nmol/kg/min, was added from the 8th to the 20th minute. During the second NE infusion, DMI, 1.2 nmol/kg/min, was added from the 8th to the 14th minute, and DMI, 4.5 nmol/kg/min, from the 14th to the 20th min. During the third NE infusion, DMI, 0.3 nmol/kg/min, was administered between the -5th and 20th minute and the U 2 inhibitor HC, 1.7 nmol/kg/min, was added from the 8th to 20th minute. During the fourth NE infusion, DMI, 0.3 nmol/kg/min, was again administered between the -5th and 20th minute; HC, 6.6 nmol/kg/min, was added from the 8th to the 14th minute; and HC, 24.8 nmol/kg/min, was given from the 14th to the 20th minute. Venous blood was sampled at 0, 2, 4, 6, 8 (also 9.5 and 11 during the 1st and 3rd infusions), 14 and 20 minutes and arterial blood at 0 and 20 minutes for determination of PNE and PE.

**Norepinephrine Infusion with Hydrocortisone**

Six other subjects received an i.a. infusion of NE, 1.18 pmol/kg/min, for 20 minutes, and HC, 24.8 nmol/kg/min, was added from the 8th to the 20th minute of the infusion. This experiment was performed in the absence of U 1 inhibition. Venous blood was sampled at 0, 2, 4, 8, 10, 14, and 20 minutes and arterial blood at 0 and 20 minutes for determination of PNE and PE.

**Drug Solutions, Sample Collection, and Assay**

l-NE and HC, both prepared according to the *Formularium Nederlandse Apothekers*, and DMI (CIBA-Geigy, Basel, Switzerland) were diluted in saline for i.a. administration. The solutions were freshly prepared on the morning of each investigation. Three-milliliters blood samples were collected into ice-chilled, heparinized tubes containing 60 μl of 0.2 M reduced glutathione. Samples were centrifuged at 4°C and 3500 g for 15 minutes. Plasma was stored at -70°C until assayed. For each sample, PNE and PE were measured in duplicate by a single-isotope, radioenzymatic assay. 13 All samples from a single subject were run in the same assay. Mean intra-assay

<table>
<thead>
<tr>
<th>TABLE 1. Clinical Data for 17 Healthy Subjects</th>
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<tbody>
<tr>
<td>Characteristic</td>
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<tr>
<td>Age (yr)</td>
</tr>
<tr>
<td>Weight (kg)</td>
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<tr>
<td>Systolic blood pressure (mm Hg)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
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<tr>
<td>Forearm blood flow (ml/100 ml/min)</td>
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coefficient of variation for both PNE and PE was 5% in the 0.5 nmol/L range.

**Data Analysis**

The half-time ($t_{1/2}$) of venous PNE, defined here as the time course of approach to steady state conditions in the forearm during the i.a. infusions, was derived from the individual venous concentration–time curves by nonlinear least-squares curve fitting, using the one compartmental model: $C_v = A (1 - e^{-kt}) / AT + C_0$, where $C_v$ and $C_0$ are venous PNE at time 0 and $t$ of the infusion, $T$ is the duration of infusion, and $A$ and $\lambda$ are parameters to be estimated by the curve fitting (Procedure NLIN of Statistical Analysis Systems computer package, SAS Institute, Cary, NC, USA). The $t_{1/2}$ is defined as equal to $0.693/\lambda$. The extraction across the forearm (FE) of circulating NE was calculated using the following equation:

$$FE = (A + \lambda FPF - V)/(A + \lambda FPF),$$

where $A$ is arterial PNE, $I$ is i.a. NE infusion rate, $FPF$ (forearm plasma flow) is $FBF \times$ forearm volume (in deciliters) $\times (1 - $ hematocrit), and $V$ is venous PNE. The net overflow of NE or epinephrine is defined as $FBF(V - A)$, where $V$ and $A$ are the respective venous and arterial PNE or PE. The FE of epinephrine was calculated as equal to $(A - V)/A$, where $A$ and $V$ are arterial and venous PE, respectively. Statistical analysis included analysis of variance with correction for repeated comparisons of means and two-tailed Student's $t$ test for paired observations when appropriate. Values are presented as means ± SEM.

**Results**

**Single Desipramine Infusion**

At the start of the single DMI infusion the venous and arterial PNE were nearly equal (Figure 1). During the infusion venous PNE increased from $0.77 \pm 0.11$ to $1.97 \pm 0.46$ nmol/L ($p<0.01$) but did not reach a steady state level (see Figure 1). The arterial PNE did not change significantly. The net overflow of NE from the forearm (FE) of circulating NE was calculated using the following equation:

$$FE = (A + \lambda FPF - V)/(A + \lambda FPF),$$

where $A$ is arterial PNE, $I$ is i.a. NE infusion rate, $FPF$ (forearm plasma flow) is $FBF \times$ forearm volume (in deciliters) $\times (1 - $ hematocrit), and $V$ is venous PNE. The net overflow of NE or epinephrine is defined as $FBF(V - A)$, where $V$ and $A$ are the respective venous and arterial PNE or PE. The FE of epinephrine was calculated as equal to $(A - V)/A$, where $A$ and $V$ are arterial and venous PE, respectively. Statistical analysis included analysis of variance with correction for repeated comparisons of means and two-tailed Student's $t$ test for paired observations when appropriate. Values are presented as means ± SEM.

**Four Norepinephrine Infusions Combined with Desipramine and Hydrocortisone**

**First Norepinephrine Infusion**

Basal arterial and venous PNE were almost equal during the first NE infusion (Figure 2). During the infusion of NE, $1.18$ pmol/kg/min, venous PNE reached a steady state level after approximately 8 minutes (see Figure 2) with an estimated $t_{1/2}$ of 2.1 ± 0.1 minutes. Upon addition of DMI, 0.3 nmol/kg/min, the venous PNE increased further without reaching a steady state level again (see Figure 2). The $t_{1/2}$ of this DMI effect on venous PNE was $7.9 \pm 0.7$ minutes. SBP, diastolic BP (DBP), and HR did not change significantly, whereas FBF markedly decreased ($p<0.01$; see Figure 1).

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Basal arterial and venous PNE were almost equal during the first NE infusion (Figure 2). During the infusion of NE, 1.18 pmol/kg/min, venous PNE reached a steady state level after approximately 8 minutes (see Figure 2) with an estimated $t_{1/2}$ of 2.1 ± 0.1 minutes. Upon addition of DMI, 0.3 nmol/kg/min, the venous PNE increased further without reaching a steady state level again (see Figure 2). The $t_{1/2}$ of this DMI effect on venous PNE was 8.3 ± 1.8 minutes. During this first NE infusion arterial PNE (see Figure 2), SBP, DBP, and HR did not change (Table 2). The single infusion of NE did not significantly influence FBF, but after addition of DMI, FBF markedly decreased ($p<0.01$) concomitant with a decrease in all subjects in the FE of NE from a mean value of 69.4 ± 3.0 to 65.4 ± 2.8% ($p<0.05$; Figure 3).
TABLE 2. Forearm Blood Flow, Intra-arterial SBP and DBP, and HR Determined Before and at the End of Four Intra-arterial Infusions of Norepinephrine (1.18 pmol/kg/min) with Addition of Desipramine and Hydrocortisone in Six Healthy Subjects

<table>
<thead>
<tr>
<th>Infusion</th>
<th>FBF (ml/100 ml/min)</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
<th>HR (beats/min)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>End</td>
<td>Before</td>
<td>End</td>
</tr>
<tr>
<td>DMI Dose 1</td>
<td>2.7±0.4*</td>
<td>1.8±0.2</td>
<td>129.5±3.7</td>
<td>128.8±3.0</td>
</tr>
<tr>
<td>Dose 2</td>
<td>1.8±0.2</td>
<td>1.8±0.2</td>
<td>137.8±3.4</td>
<td>135.8±2.7</td>
</tr>
<tr>
<td>Dose 3</td>
<td>2.5±0.2†</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>DMI + HC Dose 1</td>
<td>1.6±0.2*</td>
<td>1.7±0.1</td>
<td>135.8±3.3</td>
<td>135.5±3.2</td>
</tr>
<tr>
<td>Dose 2</td>
<td>1.6±0.1</td>
<td>1.7±0.1</td>
<td>139.2±3.3$</td>
<td>136.8±3.2</td>
</tr>
<tr>
<td>Dose 3</td>
<td>2.1±0.1*</td>
<td>—</td>
<td>138.5±3.0$</td>
<td>71.2±3.7</td>
</tr>
</tbody>
</table>

Values are means ± SEM. FBF = forearm blood flow; DMI = desipramine; HC = hydrocortisone; DMI Dose 1 = 0.3 nmol/kg/min; DMI Dose 2 = 1.2 nmol/kg/min; DMI Dose 3 = 4.5 nmol/kg/min; DMI + HC Dose 1 = DMI, 0.3 nmol/kg/min, +HC, 1.6 nmol/kg/min; DMI + HC Dose 2 = DMI, 0.3 nmol/kg/min, +HC, 6.6 nmol/kg/min; DMI + HC Dose 3 = DMI, 0.3 nmol/kg/min, +HC, 24.8 nmol/kg/min.

*p<0.01, †p<0.001, ‡p<0.05, compared with the initial value.

§p<0.01, ||p<0.05, compared with values for DMI Dose 1.

**Third Norepinephrine Infusion**

Because of the concomitant DMI infusion, 0.3 nmol/kg/min, which was started 5 minutes before the NE infusion, and also as a result of carry-over from the previous infusions, the initial venous PNE again was higher than the arterial PNE (p < 0.05; Figure 4). After approximately 8 minutes of the combined NE-DMI infusion, venous PNE had reached a steady state level again (see Figure 4) with an estimated t½ of 2.2 ± 0.3 minutes. The addition of HC, 1.7 nmol/kg/min, did not change venous PNE significantly (see Figure 4). The FBF, SBP, DBP, HR, and the FE of NE also did not change significantly (see Table 2 and Figure 3).

**Second Norepinephrine Infusion**

At the start of the second NE infusion venous PNE had not yet returned to the baseline level and was higher than arterial PNE (p<0.05; see Figure 2), indicating carry-over of the DMI effect from the previous infusion. During the infusion of NE, 1.18 pmol/kg/min, venous PNE did not reach a steady state level. On addition of the two highest doses of DMI, venous PNE showed a further rise (see Figure 2). Arterial PNE, SBP, and HR did not change significantly, but DBP increased (p<0.05; see Table 2). The NE infusion alone did not change FBF; after the addition of the highest dose of DMI, however, FBF increased (p<0.001; see Figure 3). The FE of NE decreased from 66.6 ± 3.1 to 60.4 ± 5.1% (NS; see Figure 3) and 35.3 ± 8.4% (p<0.001; see Figure 3) on addition of DMI, 1.2 and 4.5 nmol/kg/min, respectively.

**FIGURE 3.** Changes in forearm extraction rate (FE; ○) for circulating norepinephrine (NE) and forearm blood flow (FBF; □) during the i.a. infusions of NE combined with desipramine (DMI) and hydrocortisone (HC) in six healthy subjects (see Figures 2 and 4). Values are means ± SEM. Single (p<0.05), double (p<0.01), and triple asterisks (p<0.001) indicate significant difference from initial values.

**FIGURE 4.** Arterial (●, ■) and venous (○, □) plasma norepinephrine concentration (PNE) during i.a. infusions of norepinephrine (NE) with desipramine (DMI) and three doses of hydrocortisone (HC) in the same subjects as in Figure 2. Values are means ± SEM. Doses and time of infusions are indicated by the bars.
Fourth Norepinephrine Infusion

The initial venous PNE during the fourth NE infusion was again higher than the arterial PNE (p<0.02; see Figure 4) but not significantly different from the initial venous PNE of the third NE infusion. After 8 minutes of the combined NE-DMI infusion, venous PNE had reached a steady state level (see Figure 4) with an estimated t½ of 1.9 ± 0.3 minutes. Upon addition of the two highest doses of HC, venous PNE increased further (see Figure 4), but arterial PNE (see Figure 4), SBP, DBP, and HR did not change (see Table 2). FBF increased during the highest HC dose (p<0.05; see Table 2 and Figure 3). The FE of NE decreased from 61.5 ± 2.5 to 53.8 ± 4.2% (NS; see Figure 3) and 40.6 ± 4.4% (p<0.01; see Figure 3) upon addition of HC, 6.6 and 24.8 nmol/kg/min, respectively. Between the first and the fourth infusion, SBP, DBP, and HR increased (p<0.01, p<0.05, and p<0.05, respectively; see Table 2) while arterial PNE did not change significantly.

Norepinephrine Infusion Combined with Hydrocortisone

At the start of the combined NE-HC infusion in six other subjects, the venous and arterial PNE did not differ significantly (Figure 5). During the first 8 minutes venous PNE reached a steady state level (see Figure 5) with an estimated t½ of 2.0 ± 0.2 minutes. Upon addition of HC, 24.8 nmol/kg/min, venous PNE did not change significantly (see Figure 5), while the FE of NE decreased in all subjects, from a mean value of 57.1 ± 4.9 to 51.5 ± 4.7% (p<0.05; see Figure 3). During this infusion, arterial PNE, SBP, and FBF did not change significantly while DBP increased 5.3 ± 1.8 mm Hg (p<0.05) and HR increased 3.5 ± 1.1 beats/min (p<0.05).

Epinephrine

During all infusions the initial arterial PE was higher than the venous PE (p<0.05 for all infusions) and the arterial PE did not change significantly. During the highest DMI dose, venous PE increased from 0.17 ± 0.04 to 0.33 ± 0.03 nmol/L (p<0.02) and the FE of epinephrine decreased from 59.0 ± 10.6 to 24.0 ± 6.0% (p<0.05). The net removal of epinephrine in the forearm circulation decreased from -2.7 ± 0.6 to -1.8 ± 0.7 pmol/min (NS). During the combined NE-HC infusion, the FE of epinephrine decreased after the addition of HC from 61.7 ± 4.8 to 40.1 ± 7.5% (p<0.05) and the net removal of epinephrine decreased from -4.3 ± 1.0 to -3.2 ± 1.0 pmol/min (p<0.05). During all other infusions venous PE and the FE of epinephrine did not change significantly.

Discussion

This study in healthy men demonstrates that U₁ and U₂ mechanisms are involved in the removal of NE from the forearm vascular bed. In addition, considerable local release of NE under basal conditions was revealed by the increase in net overflow of NE from the forearm during the infusion of DMI, a specific inhibitor of U₁.

Inhibition of Neuronal Uptake by Desipramine

U₁ appeared to be the main mechanism responsible for the removal of NE from the forearm circulation, since at least 50% of the total removal of circulating NE could be inhibited by DMI. Although a progressive decrease in the rate of extraction for circulating NE was found with increasing doses of DMI, it cannot be concluded from these experiments that the U₁ inhibition by DMI is dose-dependent over the dose range used. First, a steady state venous PNE concentration was not reached during the infusion of DMI. The response to DMI developed slowly, with an estimated t½ of 7.9 ± 0.7 minutes, resulting in marked carry-over effects during the consecutive infusions. Second, during U₁ inhibition NE release also is likely to be inhibited by presynaptic α₂-adrenergic receptors, because of the increased NE concentration in the synaptic cleft. This inhibition of uptake and, indirectly, of release cannot be estimated separately in the forearm circulation. Third, the FE rate of circulating NE inversely relates to local blood flow. Vasodilation increases the removal rate of circulating NE in the forearm circulation probably because of a prolonged exposure of NE to the uptake systems. Thus, the measured decrease in the rate of extraction of NE, induced by the lowest dose of DMI, is likely to be an underestimate of the actual degree of U₁ inhibition by DMI, as it was accompanied by a marked decrease in FBF. Conversely, vasodilatation, which occurred during the highest DMI dose, could have contributed to the decrease in the rate of NE extraction. Since the
FBFs measured just before the lowest DMI dose and at the end of the highest DMI dose were practically equal, the accompanying decrease in the rate of NE extraction (from 69 to 35%) may be fully ascribed to the inhibiting effect of DMI on the U₁ process. As the local removal of circulating NE obeys first-order kinetics, the rate of extraction may be the best parameter for evaluation of changes in the local removal processes.

The only other study (to our knowledge) of mechanisms governing the removal of circulating NE in this vascular bed reported that only 15% of the removal of intravenously infused radiolabeled NE was inhibited by DMI. There may be several reasons for this discrepancy. First, in our experiment hand blood flow was excluded. Therefore, venous PNE concentration was less likely to be subject to arterialization by arteriovenous shunting of blood in the hand. Second, the estimated blood concentration of DMI reached in the forearm in our experiment was approximately 12 \( \mu \text{mol/L} \), which is likely to be much higher than reached 3 hours after an oral dose of 125 mg of DMI (470 \( \mu \text{mol DMI} \)).

Third, a single, 100-mg oral dose of DMI has been shown to induce increases in HR, BP, and PNE concentration in healthy subjects. Therefore, in the experiment by Goldstein et al., local release of NE could have been influenced by baroreceptor reflex-mediated changes in sympathetic nerve activity and, possibly, also by a central action of DMI.

The removal of circulating NE in the forearm can be studied by administering systemic infusions of radiolabeled NE or, as in the present study, by intra-arterial infusion of a low dose of "cold" NE. Neither method directly measures the fate of endogenously released NE. However, Kopin et al. have argued that the routes for diffusion of NE from the region of the synapse to the blood vessel lumina and vice versa are probably identical, and it seems unlikely that any removal process operating along these pathways can distinguish between NE released from nerves and that originating in blood. An influence of the intra-arterial, low dose NE infusion on the local NE release is unlikely, as the highest PNE concentration reached is much lower than the estimated effective concentrations in peripheral sympathetic synaptic clefts.

Also, an influence on the rate of extraction of circulating NE in the forearm is unlikely, as local blood flow was unaffected and the uptake processes are not saturated.

Our finding of an unchanged U₁ in the forearm circulation of venous PNE before and after U₁ inhibition contrasts with the prolongation in rapid disappearance of NE from the central plasma pool, which was found after orally administered DMI in healthy subjects by Esler et al. The reason for this discrepancy is not clear, but it is questionable whether it is appropriate to compare our short-term experiments in the forearm with the results of whole body experiments, which are composed of contributions from all organs.

**Other Effects of Desipramine**

In humans, no data are available concerning direct effects of DMI on presynaptic or postsynaptic adrenergic receptors. DMI has been shown to have some affinity for \( \alpha \)-adrenergic receptors in a rat brain preparation and was found to be a noncompetitive antagonist of NE-induced contractions in rat anococcygeus muscle. Direct antagonistic effects of DMI on presynaptic \( \alpha \)-adrenergic receptors were absent in the pithed rabbit and in rat and guinea pig atria, but conflicting results have been reported in the perfused dog heart model and the rat vas deferens.

The vasoconstriction during infusion of DMI alone in our experiment can be explained by an increased NE concentration in, and overflow from, neuromuscular synapses, resulting in greater activation of intrasynaptic and, possibly, extrasynaptic \( \alpha \)-adrenergic receptors. The highest dose of DMI used, however, was accompanied by vasodilatation, which may be due to postsynaptic \( \alpha \)-adrenergic receptor antagonism. High doses of amitriptyline, administered intra-arterially into the forearm of healthy subjects, resulted in vasodilatation, which was also ascribed to \( \alpha \)-adrenergic receptor blockade.

During the combined infusions of NE with DMI and HC, we observed gradual increases in BP and HR. This response is likely to be due to systemic uptake-inhibiting effects of DMI and HC overflowing from the forearm. As a result, a baroreceptor reflex-mediated decrease in sympathetic nerve activity may have occurred, leading to some underestimation of the local uptake-inhibiting effects of DMI and HC. Interestingly, these increases in BP and HR were not accompanied by significant changes in the arterial PNE concentration, indicating that even the arterial PNE concentration does not necessarily reflect physiologically significant changes in NE concentration in the region of the receptors.

**Inhibition of Extraneuronal Uptake by Hydrocortisone**

In vitro experiments have demonstrated several extraneuronal NE uptake mechanisms, some of which are corticosteroid-sensitive. For instance, the effect of HC on U₁ was much greater in cat nictitating membrane than in an isolated rat heart preparation. It therefore seems that, in contrast to the U₁ mechanism for NE, the U₂ system exhibits a pronounced degree of organ and species specificity.

In humans, intravenously administered HC had no effect on extraneuronal removal of NE. HC also did not affect the U₁ of NE in isolated human venous tissue. In the present study a significant decrease in the rate of extraction of NE was induced by the highest dose of HC. This decrease was found both in the presence of DMI, given to increase the amount of NE available for U₁, and in the absence of DMI. The decrease in the rate of NE extraction during infusion of HC with DMI could partly be explained by the increase in blood flow that occurred. However, in the experiment without DMI, a small but significant decrease in the rate of NE extraction also was observed without changes in FBF. This finding demonstrates that at least part of the extraneuronal removal of NE in this vascular bed is sensitive to inhibition by HC.
Uptake Mechanism of Epinephrine

The FE rate of circulating epinephrine in this study was influenced only by the highest doses of DMI and HC used. Although data for human tissues are lacking, this may be due to a higher \( K_u \) of the U, process for NE than for epinephrine. Indeed, in rat heart the \( K_u \) for U, of NE was 0.67 \( \mu \text{M} \) and for epinephrine, 1.40 \( \mu \text{M} \). The influence of the highest dose of HC on the epinephrine extraction rate was evident, in both the presence and absence of U, inhibition. However, statistical significance was only reached in the absence of U, inhibition.

In conclusion, we found that the removal of circulating NE from the forearm is mainly due to U,. The presence of a corticosteroid-sensitive U, mechanism was also demonstrated. Under basal conditions, considerable local release of NE became apparent when U, was inhibited by DMI, even though indirect pressynaptic inhibition of release may have been present. Epinephrine was also subject to U,, in the forearm, but apparently to a lesser extent than was NE. Evidence was provided that even arterial PNE does not necessarily reflect physiologically significant changes in NE concentration in the region of postsynaptic adrenergic receptors. The forearm seems to be a suitable model for the study of NE release and clearance mechanisms and of disturbances in these processes.

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