Evidence for Functional $\alpha_2$-Adrenoceptors on Vascular Sympathetic Nerve Endings in the Human Forearm

Ehud Grossman, Peter C. Chang, Aaron Hoffman, Marye Tamrat, and David S. Goldstein

The role of $\alpha_2$-adrenoceptors on vascular sympathetic nerve endings in modulating release of the sympathetic neurotransmitter norepinephrine (NE) in humans was examined by measuring the regional rate of appearance of NE in forearm venous plasma (forearm NE spillover [FSO]) in 32 healthy volunteers during intra-arterial infusion of drugs acting at adrenoceptors or directly on vascular smooth muscle. Simultaneous intra-arterial infusions of tracer amounts of $[^3H]NE$ were used to calculate the extraction rate of NE in the forearm. Methoxamine or propranolol with epinephrine (PRO+EPI) was used to stimulate $\alpha_2$-adrenoceptors, yohimbine was used to inhibit $\alpha_2$-adrenoceptors, and sodium nitroprusside (NIP) was used to produce increases in forearm blood flow directly. Sympathetic efferent activity was manipulated by systemic intravenous infusions of NIP or trimethaphan. Yohimbine and NIP increased and PRO+EPI and methoxamine decreased NE FSO, without effects on systemic blood pressure, heart rate, or arterial levels of catechols. Changes in FSO were flow dependent; therefore, the slope of the relation between the changes in FSO and forearm blood flow was used to evaluate the effects of each drug on regional sympathoneural activity. During administration of yohimbine, the mean slope of the relation between the change in estimated FSO and the change in forearm blood flow was about four times that of the mean slope during administration of NIP ($F=6.35, p<0.05$). The slopes of the relations between changes in FSO and forearm blood flow were unaffected by systemic trimethaphan or NIP infusion, indicating that the activity of $\alpha_2$-adrenoceptors was not altered during inhibition or reflexive stimulation of sympathetic outflow. The results suggest that $\alpha_2$-adrenoceptors modulate release of NE from vascular sympathetic nerve endings in humans and that the function of these receptors is unchanged during acute changes in junctional NE concentrations. (Circulation Research 1991;69:887–897)
take into account the regional clearance of NE. Esler et al introduced a method for measuring the clearance of $[^3]$HNE from plasma in humans, and subsequent modifications have allowed estimates of regional spillover of NE that take into account regional NE clearance.

Since $\alpha_2$-adrenoceptor agonists and antagonists are vasoactive drugs, and since changes in regional blood flow can affect regional NE spillover, studies involving intra-arterial infusions of these agents require concurrent assessments of effects of direct-acting vasodilators or vasoconstrictors. If the method of Esler et al. was used, prolonged infusions of $[^3]$HNE would result in unacceptable radiation exposure to the subjects.

The present study applied a different radiotracer method to determine whether $\alpha_2$-adrenoceptors on sympathetic nerve endings modulate release of NE in the forearm vascular bed (forearm NE spillover [FSO]) in humans. By using intra-arterial infusions of $[^3]$HNE, high local concentrations of the tracer could be achieved at slow infusion rates, allowing repeated infusions in the same experiment in the same subjects. Analogously, by intra-arterial infusion of agonists or antagonists of $\alpha_2$-adrenoceptors, high concentrations of the drugs could be attained in the forearm, with much lower concentrations in the systemic circulation. If intra-arterial infusion of an $\alpha_2$-agonist increased and infusion of an $\alpha_2$-agonist decreased regional NE spillover, this would support the existence of functional $\alpha_2$-receptors on vascular sympathetic nerve endings in humans.

To verify that alterations in regional NE spillover during intra-arterial infusion of an adrenoceptor antagonist or agonist actually result from drug actions on adrenoceptors on sympathetic nerve endings, in the present study comparisons were made with effects of a direct-acting vasodilator (sodium nitroprusside [NIP]) and vasoconstrictor (methoxamine [MTX]) on regional NE spillover. NIP is thought to act within vascular smooth muscle cells to inhibit their contraction, and MTX stimulates $\alpha_2$-adrenoceptors, which are thought to be located mainly postsynaptically at vascular neuror-efector junctions.

In the present study of healthy volunteers, forearm blood flow (FBF) was manipulated by intra-arterial infusions of MTX or NIP, and the results were compared with the effects of the drugs acting at $\alpha_2$-adrenoceptors. Some subjects received propranolol with epinephrine (designated PRO+EPI) or yohimbine (YOH) intra-arterially, and the effects on systemic and forearm hemodynamics and on arterial levels of catechols and FSO were assessed. In some subjects, to examine whether the extent of sympathetic outflow influences $\alpha$-adrenoceptor function, PRO+EPI and YOH were infused before and during systemic intravenous infusions of NIP or trimethaphan (TRI).

**Materials and Methods**

**Subjects**

Thirty-two healthy male volunteers with a mean $\pm$ SEM age of $25 \pm 1$ (range, 18–36) years and a mean body weight of $74 \pm 2$ (range, 56–92) kg participated in the study. In all subjects, the medical history, physical examination, and routine laboratory tests, including complete blood count, serum glucose, renal, liver, and thyroid function tests, plasma cortisol, urinalysis, and electrocardiogram, showed no evidence of cardiovascular or any other diseases. None of the subjects took any medication for at least 2 weeks before the study. Subjects were instructed to refrain from smoking cigarettes or drinking alcohol or caffeine-containing beverages for at least 12 hours before the experiment. The protocol of the study was approved by the Intramural Research Board of the National Heart, Lung, and Blood Institute, and all subjects gave written informed consent before participating in the study.

**Experimental Setup**

All experiments were performed in the morning in a quiet room at a temperature of 22–23°C and with the subjects supine. The brachial artery of the nondominant side was cannulated for intra-arterial blood pressure monitoring and for local infusions and blood sampling. In the same arm, the antecubital vein was cannulated for blood sampling. In 13 subjects, the antecubital vein of the contralateral arm was also cannulated for systemic infusions. Continuous recording of arterial pressure was obtained with a Statham P-23 pressure transducer (Gould, Oxnard, Calif.), and heart rate was measured simultaneously using the electrocardiogram and a cardiometer preamplifier. FBF was measured by strain-gauge, venous occlusion plethysmography (D.E. Hokanson, Issaquah, Wash.). For each determination, five to six measurements were performed, and the results were averaged. The blood pressure, heart rate, and plethysmographic signals were recorded on a polygraph (Gould). Hand blood flow was not excluded during the experiments because of possible artificial effects of prolonged hand ischemia on the neurochemical data. Forearm volume was determined before the study by water displacement.

**Study Protocol**

The experiments started -30 minutes after the cannulations. All subjects received infusions of $[^3]$HNE (100 nCi/min i.a.) into the brachial artery.

To manipulate FBF using substances that have no known important presynaptic effect, in seven subjects MTX was infused at 0.08, 0.4, and 2 $\mu$g/kg/min i.a. to decrease FBF (Figure 1A), and in seven subjects NIP was infused at 5, 15, and 50 ng/kg/min i.a. to increase FBF (Figure 1B). Each dose of each drug was given for 8 minutes.

Intra-arterial PRO+EPI was infused to stimulate $\alpha$-adrenoceptors in 25 subjects (Figure 1C). PRO (0.2 $\mu$g/kg/min) was infused for 8 minutes and was continued while EPI (0.03, 0.1, and 4 ng/kg/min) was infused simultaneously for 8 minutes at each dose. As indicated in Figure 2, the PRO+EPI combination was given as the initial or only treatment in 18
subjects and at least 20 minutes after administration of MTX in seven subjects.

YOH was infused intra-arterially to block $\alpha_2$-adrenoceptors in 20 subjects (Figure 1D). The subjects received YOH at doses of 0.2 and 1 $\mu$g/kg/min for 8 minutes at each dose. Since the effects of YOH can last longer than those of the other drugs that were used, YOH always was given after PRO+EPI (Figure 2).

To determine if increased sympathetic nerve traffic would affect the function of $\alpha_2$-adrenoceptors, in six subjects PRO (0.2 $\mu$g/kg/min) plus EPI (0.1 and 4 $\mu$g/kg/min) and YOH (1 $\mu$g/kg/min) were administered before and during simultaneous systemic intra-

![Figure 1](image)

**Figure 1.** Intra-arterial drug administration sequences. In all sequences, $l$-[3H]norepinephrine ([3H]-NE) was infused at 100 nCi/min. Panel A: Methoxamine (MTX) was infused at 0.08, 0.4, and 2 $\mu$g/kg/min. Panel B: Nitroprusside (NIP) was infused at 5, 15, and 50 ng/kg/min. Panel C: Propranolol (PRO) was infused at 0.2 $\mu$g/kg/min along with epinephrine (EPI) at 0.03, 0.1, and 4 ng/kg/min. Panel D: Yohimbine (YOH) was infused at 0.2 and 1 $\mu$g/kg/min.

venous infusion of NIP at a dose of 1.3 $\mu$g/kg/min (Figure 2).

To determine if decreased sympathetic nerve traffic would affect the function of $\alpha_2$-adrenoceptors, in seven subjects PRO+EPI and YOH were given, as described above, before and during simultaneous systemic intravenous infusion of TRI at a dose of 1.5 mg/min (Figure 2).

Between infusions, a period of at least 20 minutes was allotted, during which FBF returned to baseline. Arterial blood was drawn before and at the end of each infusion. Venous blood was drawn at the end of each dose. FBF was measured during the last 2 minutes of each dose. When the same drug was given twice (e.g., before and during systemic intravenous infusion of NIP or TRI), at least 1 hour elapsed before the start of the second infusion.

Because each subject served as his own control and since there were several pharmacological manipulations in each study session, the durations of the infusions were limited as much as possible. In a few subjects, blood was drawn at 8 and 15 minutes during infusions of [3H]NE, and no differences in the plasma levels were noted. Therefore, we decided that 8 minutes for each infusion of [3H]NE, including the initial baseline infusion, would be sufficient for the specific purposes of the study.

**Drug Solutions, Sample Collection, and Assays**

L-[2,5,6-$^3$H]NE (specific activity, 38.7 Ci/mmol; radiochemical purity, 95%; New England Nuclear, Boston) was diluted in 5% glucose on the morning of each investigation. Blood samples were collected in ice-chilled, heparinized tubes. Samples were centrifuged at 4°C and 3,500g for 15 minutes, and the plasma was removed and stored at −75°C until assayed.

**Catechol Assays**

The catechols in aliquots of 1 ml plasma were partially purified by batch alumina extraction, separated using high-pressure liquid chromatography, and quantified by the current produced on exposure of the column effluent to oxidizing and then reducing potentials in series.30 Recovery through the alumina extraction step averaged 70–80% for catecholamines and 45–55% for dopa and dihydroxyphenylglycol (DHPG). Catechol concentrations in each sample were corrected for recovery of an internal standard, N-methyl dopamine. Levels of dopa and DHPG were further corrected for differences in recovery of the internal standard and of dopa and DHPG in a mixture of external standards. The limit of detection was about 10 pg/ml for each catechol. The amounts of [3H]NE in plasma and the infusate were measured in fractions of the column effluent corresponding in retention to that of NE standard, with the fractions collected into scintillation vials and the tritium assayed by liquid scintillation spectrometry. Disintegrations per minute were adjusted for background radioactivity and recovery through the alumina extraction. Background values were determined from

![Figure 2](image)

**Figure 2.** Infusion protocols. MTX, methoxamine; PRO+EPI, propranolol with epinephrine; YOH, yohimbine; NIP, sodium nitroprusside; TRI, trimethaphan.
aqueous mixtures of the external standards. Intra-assay and interassay coefficients of variation for catechol assays in this laboratory have been published previously.30

Data Analysis

The fractional extraction of $[^3]$HNE in the forearm (E$_{[^3]$HNE}) was calculated from the equation:

$$E_{[^3]$HNE} = (I^* - QV^*)/I^*$$

where I* is the intra-arterial infusion rate of the tracer, V* is the venous concentration of $[^3]$HNE, and Q is forearm plasma flow, derived from FBF, forearm volume, and hematocrit.

FSO was calculated using the equation:

$$FSO = QV - QA + QAE_{[^3]$HNE}$$

where V is the venous concentration of NE and A is the arterial concentration of NE.

Results

Methoxamine

MTX administered intra-arterially decreased FBF in a dose-related manner (Figure 3, p<0.01). There were no changes in blood pressure, heart rate, or arterial or venous NE levels (Table 1). The forearm extraction percent of $[^3]$HNE increased slightly and was statistically significant only when the lowest dose of MTX was compared with the highest dose (56±4% versus 65±7%, p<0.05). FSO decreased significantly from 2.6±0.5 ng/min at baseline to 1.6±0.2 ng/min at the highest dose of MTX (Figure 3, p<0.005). Changes in FSO were significantly positively correlated with changes in FBF during MTX infusion (y=0.049+0.094x, R=0.48, p=0.05, Figure 4).

Sodium Nitroprusside

NIP administered intra-arterially increased FBF in a dose-related manner (Figure 3, p<0.001). FBF at the 50 ng/kg/min dose of NIP was higher than FBF at the 15 ng/kg/min dose (p<0.05). There were no

<table>
<thead>
<tr>
<th>Table 1. Hemodynamics and Plasma Levels of Norepinephrine During Intra-arterial Infusion of Methoxamine or Nitroprusside</th>
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<tbody>
<tr>
<td>MAP (mm Hg)</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>Baseline</td>
</tr>
<tr>
<td>Methoxamine</td>
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<tr>
<td>Baseline</td>
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<tr>
<td>Nitroprusside</td>
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</tbody>
</table>

Values are mean±SEM for the highest dose of each drug. MAP, mean arterial pressure; HR, heart rate; NEv, venous concentration of norepinephrine; NEa, arterial concentration of norepinephrine.
changes in systemic blood pressure, heart rate, or arterial or venous NE levels during the intra-arterial infusions of NIP (Table 1).

The forearm percent extraction of [H]NE decreased from 61±4% at baseline to 38±10% at the highest dose of NIP (p<0.005), and FSO increased from 4.4±0.8 ng/min at baseline to 7.6±0.9 ng/min at the highest dose of NIP (Figure 3, p<0.005). Changes in FSO were significantly positively correlated with changes in FBF during NIP infusion (y = -0.57 ± 0.44x, R = 0.79, p<0.05, Figure 4).

PRO+EPI

EPI administered intra-arterially with PRO did not alter mean arterial pressure or heart rate (Table 2). FBF was unchanged at the low dose of EPI but decreased significantly at the second and third doses (Figure 5, p<0.001). EPI infusion increased venous plasma EPI levels significantly (Table 2, p<0.001). Venous plasma levels of DHPG, dopa, and dopamine were unchanged (Table 2); however, venous plasma NE levels decreased significantly during the infusion of the high dose of EPI (p<0.05). Arterial levels of NE remained unchanged during the infusion of EPI.

The extraction percent of [H]NE did not change during the two low-dose EPI infusions but increased significantly during the high-dose infusion (Table 2, p<0.001). FSO decreased from 3.3±0.3 ng/min at baseline to 1.1±0.1 ng/min during the high-dose EPI infusion in the second and third dose EPI infusions.

Table 2. Hemodynamics and Plasma Levels of Catechols During Intra-arterial Infusion of Propranolol and Epinephrine

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Dose 1</th>
<th>Dose 2</th>
<th>Dose 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>84±2</td>
<td>84±2</td>
<td>85±1</td>
<td>85±2</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>61±2</td>
<td>60±1</td>
<td>61±2</td>
<td>60±2</td>
</tr>
<tr>
<td>FBF (ml/dl/min)</td>
<td>6.1±0.7</td>
<td>6.0±0.7</td>
<td>5.0±0.6*</td>
<td>1.6±0.2†</td>
</tr>
<tr>
<td>DHPGv (pg/ml)</td>
<td>841±43</td>
<td>826±51</td>
<td>819±42</td>
<td>856±44</td>
</tr>
<tr>
<td>DOPAv (pg/ml)</td>
<td>1,831±147</td>
<td>1,748±120</td>
<td>1,760±119</td>
<td>1,895±108</td>
</tr>
<tr>
<td>NEv (pg/ml)</td>
<td>146±9</td>
<td>139±9</td>
<td>136±9</td>
<td>110±8*</td>
</tr>
<tr>
<td>NEa (pg/ml)</td>
<td>144±11</td>
<td>. .</td>
<td>. .</td>
<td>. .</td>
</tr>
<tr>
<td>EPIv (pg/ml)</td>
<td>30±8</td>
<td>60±11</td>
<td>140±20*</td>
<td>2,378±165†</td>
</tr>
<tr>
<td>DAv (pg/ml)</td>
<td>16±11</td>
<td>14±8</td>
<td>20±8</td>
<td>21±14</td>
</tr>
<tr>
<td>NE extraction (%)</td>
<td>51±3</td>
<td>46±3</td>
<td>55±3</td>
<td>87±1†</td>
</tr>
<tr>
<td>FSO (ng/min)</td>
<td>3.3±0.3</td>
<td>2.8±0.3</td>
<td>2.5±0.4*</td>
<td>1.1±0.1†</td>
</tr>
</tbody>
</table>

Values are mean±SEM. PRO+EPI, infusion of 0.2 µg/kg/min propranolol (PRO) with varying doses of epinephrine (EPI); Dose 1, Dose 2, and Dose 3, 0.03, 0.1, and 4 ng/kg/min EPI, respectively; MAP, mean arterial pressure; HR, heart rate; FBF, forearm blood flow; DHPGv, venous concentration of dihydroxyphenylglycol; DOPAv, venous concentration of dopa; NEv, venous concentration of norepinephrine (NE); NEa, arterial concentration of NE; EPIv, venous concentration of epinephrine; DAv, venous concentration of dopamine; FSO, forearm spillover of NE into venous drainage.

*p<0.05 and †p<0.001 vs. baseline.
infusion (Figure 5, p<0.001). During EPI infusion, changes in FSO were correlated significantly positively with changes in FBF (y=-0.73+0.29x, R=0.45, p<0.05, Figure 4). The relation between changes in FBF and changes in FSO during EPI infusion was not different from the relation during MTX infusion (F=1.56, p=0.21 by ANCOVA for comparison between slopes, Figure 4).

Yohimbine

YOH administered intra-arterially did not affect systemic hemodynamic parameters or arterial levels of NE (Table 3). FBF was increased by 18% at the high dose of YOH (Figure 5, p<0.05). Venous plasma levels of DHPG, dopa, and dopamine were unchanged (Table 3). The venous plasma NE level increased significantly during the high-dose YOH infusion (Table 3, p<0.05). The extraction percent of [3H]NE in the forearm was decreased at the high dose of YOH (Table 3, p<0.05). FSO increased by 83% at the high dose of YOH (Figure 5, p<0.01). During YOH infusion, changes in FSO were correlated significantly positively with changes in FBF (y=0.80+1.74x, R=0.52, p<0.05, Figure 4). For the same change in FBF, YOH increased FSO more than did NIP (F=6.35, p=0.015 by ANCOVA for comparison between slopes, Figure 4). The slope of the spillover-flow relation was about four times steeper during the YOH infusion than during the NIP infusion.

Systemic NIP Infusion

Systemic intravenous infusion of NIP decreased mean arterial pressure by 5 mm Hg (Table 4, p=NS) and increased heart rate by 13 beats/min (Table 4, p<0.05). FBF and arterial levels of DHPG, dopa, and dopamine were unchanged, whereas plasma NE levels were increased more than threefold and plasma EPI levels were increased approximately two-fold. The forearm percent extraction of [3H]NE was unchanged, whereas FSO approximately doubled (Table 4, p=0.07).

PRO+EPI Before and During Systemic NIP Infusion

Intra-arterial PRO+EPI decreased FBF to the same extent before and during systemic intravenous NIP infusion (Figure 6). FSO was decreased similarly before

<table>
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<tr>
<th>Table 3. Hemodynamics and Plasma Levels of Catechols During Intra-arterial Infusion of Yohimbine</th>
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<td></td>
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<tr>
<td>MAP (mm Hg)</td>
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<tr>
<td>HR (beats/min)</td>
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<tr>
<td>FBF (ml/dl/min)</td>
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<tr>
<td>DHPGv (pg/ml)</td>
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<td>DOPAv (pg/ml)</td>
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<td>NEv (pg/ml)</td>
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<tr>
<td>NEa (pg/ml)</td>
</tr>
<tr>
<td>EPIv (pg/ml)</td>
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<tr>
<td>DAv (pg/ml)</td>
</tr>
<tr>
<td>NE extraction (%)</td>
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<tr>
<td>FSO (ng/min)</td>
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</tbody>
</table>

Values are mean±SEM. Dose 1 and Dose 2, 0.2 and 1 µg/kg/min yohimbine, respectively; MAP, mean arterial pressure; HR, heart rate; FBF, forearm blood flow; DHPGv, venous concentration of dihydroxyphenylglycol; DOPAv, venous concentration of dopa; NEv, venous concentration of norepinephrine (NE); NEa, arterial concentration of NE; EPIv, venous concentration of epinephrine; DAv, venous concentration of dopamine; FSO, forearm spillover of NE into venous drainage.

*<p<0.05 and tp<0.01 vs. baseline.
Table 4. Hemodynamics and Plasma Levels of Catechols During Intravenous Infusion of Nitroprusside

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<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Nitroprusside</th>
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</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>83.3±3.0</td>
<td>78.6±3.0</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>59±3</td>
<td>72±5*</td>
</tr>
<tr>
<td>FBF (ml/dl/min)</td>
<td>4.5±0.7</td>
<td>4.6±0.5</td>
</tr>
<tr>
<td>DHPGa (pg/ml)</td>
<td>814±63</td>
<td>937±98</td>
</tr>
<tr>
<td>NEa (pg/ml)</td>
<td>124±16</td>
<td>395±60†</td>
</tr>
<tr>
<td>DOPaa (pg/ml)</td>
<td>1,343±130</td>
<td>1,473±145</td>
</tr>
<tr>
<td>EPIa (pg/ml)</td>
<td>37±23</td>
<td>62±29</td>
</tr>
<tr>
<td>DAA (pg/ml)</td>
<td>6±4</td>
<td>11±9</td>
</tr>
<tr>
<td>NE extraction (%)</td>
<td>63±5</td>
<td>65±6</td>
</tr>
<tr>
<td>FSO (ng/min)</td>
<td>3.9±1.0</td>
<td>6.9±0.1</td>
</tr>
</tbody>
</table>

Values are mean±SEM. MAP, mean arterial pressure; HR, heart rate; FBF, forearm blood flow; DHPGa, arterial concentration of dihydroxyphenylglycol; NEa, arterial concentration of norepinephrine (NE); DOPaa, arterial concentration of dopa; EPIa, arterial concentration of epinephrine; DAA, arterial concentration of dopamine; FSO, forearm spillover of NE into venous drainage.

*p<0.05 and †p<0.005 vs. baseline.

and during NIP infusion. The slope of the relation between changes in FSO and changes in FBF was larger during than the slope before NIP infusion (F=6.00, p=0.02 by ANCOVA for comparison between slopes, Figure 6). However, one subject had extremely large falls in FSO and FBF during NIP infusion; when this subject was discounted, the slopes of the regression lines were congruent (F=0.049, p=0.83 by ANCOVA for comparison between slopes).

**YOH Before and During Systemic NIP Infusion**

YOH increased FBF more during than before NIP infusion (84% versus 12%, p<0.05, Figure 7). FSO also was increased by YOH to a larger extent during than FSO before NIP infusion (110% versus 58%, p<0.005, Figure 7). The slope of the relation between changes in FSO and changes in FBF was unchanged by systemic NIP infusion (F=0.06, p=0.8 by ANCOVA for comparison between slopes, Figure 7).

**Systemic TRI Infusion**

Systemic infusion of TRI decreased mean arterial pressure by 7 mm Hg (Table 5, p<0.05) and increased heart rate by 29 beats/min (Table 5, p<0.001), whereas FBF remained unchanged. Plasma arterial levels of EPI, DHPG, dopa, and dopamine remained unchanged, whereas arterial NE levels were decreased by more than 50% (Table 5, p<0.01). The extraction percent of [3H]NE and FSO were unchanged (Table 5).

**PRO+EPI Before and During Systemic TRI Infusion**

Combined infusion of EPI and PRO decreased FBF to the same levels before and during systemic TRI infusion (Figure 8). FSO also decreased similarly before and during TRI infusion. The slope of the relation between changes in FSO and changes in FBF was unchanged during the TRI infusion (F=0.64, p=0.43 by ANCOVA for comparison between slopes, Figure 8).

**YOH Before and During Systemic TRI Infusion**

YOH increased FBF slightly more before than during TRI infusion (26% versus 11%, Figure 9). The response of FSO also tended to be higher before than during TRI infusion (89% versus 48%). The slope of the relation between changes in FSO and changes in FBF was unchanged during the TRI infusion.
**FIGURE 7.** Top panel: Graph showing change in forearm blood flow (FBF) during yohimbine infusion before and during administration of intravenous nitroprusside (NIP, n=6). The increase in FBF during intravenous NIP administration was larger than that before NIP administration (p<0.05). Middle panel: Graph showing change in forearm norepinephrine spillover (FSO) during yohimbine infusion before and during intravenous NIP administration. The increase in FSO during intravenous NIP administration was larger than that before NIP administration (p<0.05). Bottom panel: Graph showing relation between FSO and FBF during yohimbine infusion before and during intravenous NIP administration. *p<0.05 vs. baseline.

*(F=0.54, p=0.47 by ANCOVA for comparison between slopes, Figure 9).*

**Discussion**

The present study applied a unique technique, based on intra-arterial infusions of vasoactive drugs and simultaneous assessments of regional tracer kinetics of intra-arterial [3H]NE, to test whether α2-adrenoceptors on vascular sympathetic nerve endings modulate release of the sympathetic neurotransmitter NE in humans.

This technique allowed a distinction between changes in NE release resulting from alterations in presynaptic α2-adrenoceptor activity from changes in NE release resulting from effects mediated by central neural α2-adrenoceptors or reflexive adjustments to direct hemodynamic effects of the drugs. Since none of the intra-arterial infusions, including those of the potent vasodilator NIP and the vasoconstrictors MTX and PRO+EPI, affected systemic blood pressures, heart rate, or arterial levels of catechols, the effects of the drugs administered intra-arterially on regional NE spillover were probably due to local actions in the forearm rather than reflexive adjustments in sympathetic outflow related to central or systemic actions of the drugs.

The FSO was decreased during intra-arterial infusion of PRO+EPI to stimulate α1-adrenoceptors and was increased during intra-arterial infusion of the α2-adrenoceptor blocker YOH. Therefore, the results indicated that in the human forearm α2-adrenoceptors on vascular sympathetic nerve endings do modulate release of the sympathetic neurotransmitter.

As expected, intra-arterial administration of PRO+EPI decreased FBF, and intra-arterial infusion of YOH increased FBF. When FBF was decreased by intra-arterial infusion of MTX, FSO decreased, and when FBF was increased by intra-arterial infusion of NIP, FSO increased. These findings confirm that regional NE spillover is dependent on regional blood flow.25 To assess the extent to which alterations in regional NE spillover during intra-arterial administration of the PRO+EPI combination and of YOH could be attributed to the concurrent regional flow changes, the slope of the relation between changes in FSO and changes in FBF during administration of PRO+EPI was compared with the slope during administration of MTX, and the slope during ad-
ministration of YOH was compared with the slope during administration of NIP.

During the administration of YOH, the mean slope of the relation between changes in FSO and changes in FBF was about four times that of the mean slope during administration of NIP (Figure 4, right panel). Thus, YOH administered intra-arterially resulted in large increases in FSO that could not be attributed to local hemodynamic effects.

Jie et al.\(^2\) reported that YOH administered intravascularly did not increase the NE arteriovenous production rate (i.e., the arteriovenous increment in the NE concentration, multiplied by the blood flow) in the forearm under similar conditions. Since their study did not include \(^3\)H]NE infusions, the effects of YOH on regional NE extraction were not assessed, and the spillover estimates were based only on the NE arteriovenous difference and on FBF. In the present study, estimates of NE spillover took into account regional NE extraction, and the results led to entirely different conclusions about the existence of functional \(\alpha_2\)-adrenoceptors on sympathetic nerve endings.
Whereas YOH relatively selectively blocks \( \alpha_2 \)-adrenoceptors, no selective \( \alpha_2 \)-adrenoceptor agonist was available in injectable form. The PRO+EPI combination stimulated \( \alpha \)-adrenoceptors nonselectively. For a given amount of fall in FBF due to this combination, inspection of Figure 4 (left panel) suggests that there was a tendency for a larger fall in FSO when PRO+EPI was given when than MTX, a selective \( \alpha_1 \)-adrenoceptor agonist, was given. If this had been verified statistically, one would have concluded that stimulation of \( \alpha_2 \)-adrenoceptors during PRO+EPI administration attenuated NE release. Because of the larger fall in FBF with PRO+EPI than with MTX, however, the ranges of data clearly differed in the two scatter plots relating FSO to FBF. The larger fall in FBF with PRO+EPI than with MTX was consistent with the view that \( \alpha \)-adrenoceptors of both subtypes are located on vascular smooth muscle cells.26,27 The difference in ranges of the data, as well as interindividual variability, can explain why the differences between PRO+EPI and MTX in the slopes for the relations between FSO and FBF did not achieve statistical significance. A study including a selective \( \alpha_2 \)-adrenoceptor agonist could test this explanation. It should be noted, however, that PRO+EPI combination decreased venous NE significantly, a finding that cannot be explained by decreased FBF. The main conclusion of the present study, that YOH treatment augments FSO for a given amount of sympathetic nerve traffic, was confirmed recently in a study in which skeletal muscle sympathetic activity was measured directly while YOH was administered systematically.18

The present study also examined the possibility that the function of \( \alpha_2 \)-adrenoceptors on sympathetic nerve endings might be altered when sympathetic postganglionic activity is manipulated. Bevan et al31 suggested that high concentrations of NE in the synaptic cleft would desensitize presynaptic \( \alpha \)-adrenoceptors. In the present study, when sympathetic activity was stimulated reflexively by systemic intravenous administration of NIP, the slopes of the relations between changes in FSO and changes in FBF during infusion of YOH were unchanged; and the mean slope of the relation between changes in FSO and changes in FBF during YOH infusion was not affected significantly when postganglionic sympathetic activity was blocked by TRI (Figures 8 and 9). Therefore, the results suggest that acute alterations in sympathetic outflow do not affect importantly the function of \( \alpha_2 \)-adrenoceptors on sympathetic nerve endings. Consistent with these findings, Szemeredi et al32 found that in conscious rats the effects of clonidine administration on plasma NE levels are similar, with or without reflexive stimulation of sympathetic outflow. Although there was an increase in the slope for the relation between changes in spillover and flow when NIP was coadministered with the PRO+EPI combination, the values for FBF were very low at the highest dose of EPI; therefore, values of FSO were subject to relatively large errors of measurement.

In conclusion, since intra-arterial infusion of YOH produced much larger increments in FSO than could be attributed to local or systemic hemodynamic effects of the drug, the results support the existence of functional \( \alpha_2 \)-adrenoceptors on vascular sympathetic nerve endings that modulate release of NE in humans. The activity of these receptors does not appear to be augmented during reflexive stimulation of sympathetic outflow, casting doubt on the hypothesis that presynaptic inhibitory \( \alpha_2 \)-adrenoceptors are downregulated in response to increased junctional NE concentrations.

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