Estimation of Intrasympathetic Norepinephrine Concentrations in Humans

DAVID S. GOLDSTEIN, REUVEN ZIMLICHMAN, ROBIN STULL, HARRY R. KEISER, AND IRWIN J. KOPIN

SUMMARY Levels of synaptic cleft norepinephrine associated with pressor responses were estimated in humans by measuring blood pressure and arterial plasma norepinephrine during norepinephrine infusion and during yohimbine-induced release of endogenous norepinephrine. Linear pressor response-log norepinephrine concentration relationships were observed during the infusions. At a pressor response of 20 mm Hg, arterial norepinephrine averaged 3647 pg/ml. The pressor-log norepinephrine relationship was shifted more than fivefold to the left during combined ganglionic, α₂-adrenergic receptor, and Uptake, (neuronal norepinephrine uptake) blockade: arterial norepinephrine averaged 684 pg/ml at a 20 mm Hg pressor response. During yohimbine-induced release of endogenous norepinephrine in desipramine-pretreated subjects, arterial norepinephrine averaged 467 pg/ml at a 20 mm Hg pressor response. Since the norepinephrine concentration in the synaptic clefts must have been between the values for plasma norepinephrine during its infusion and during its endogenous release, we estimated that in healthy people, a 20 mm Hg sympathetically mediated pressor response is associated with about a 560 pg/ml (3.3 nM) concentration of norepinephrine in the average neuroeffector junction. (Hypertension 8: 471-475, 1986)

KEY WORDS • sympathetic nervous system • blood pressure • norepinephrine • adrenergic receptors • yohimbine • trimethaphan • desipramine

THE important role of the sympathetic nervous system in circulatory homeostasis has led many investigators to hypothesize that abnormal sympathetic function causes or contributes to essential hypertension. Evaluating this possible contribution requires a method for estimating norepinephrine (NE) levels in the synaptic cleft; this has only been accomplished in vitro and in pithed rats. We present here a method that permits estimation of cleft NE levels and their associated pressor responses in humans.

Subjects and Methods

The method we used is based on relationships between pressor responses and plasma NE in arterial blood during NE infusion and during stimulation of endogenous NE release. In the general circulation NE derives mainly from sympathetic nerve endings. Mixed venous or arterial concentrations of NE reflect the averaged contributions from the several sympathetically innervated vascular beds. The relationships among sympathetic neural activity, NE release, and the NE concentration in the general circulation are complex, however, because several removal processes for NE intervene between the synapse and the general circulation. The most prominent is Uptake, where NE is taken up into the presynaptic terminal. Because of these processes, during an infusion of NE to achieve a given pressor response, the steady state NE concentration in the plasma must exceed that in the average vascular neuroeffector junction. Similarly, during stimulation of endogenous NE release to achieve the same pressor response, the steady state NE concentration in the plasma must be less than that in the average vascular neuroeffector junction. The cleft NE concentration associated with a given pressor response must be somewhere between the plasma concentrations measured during NE release and during NE infusion. This "window" for cleft NE is quite large. To produce an increment of 20 mm Hg by infusing NE in humans, the venous NE concentration often must exceed 2000 pg/ml, whereas during pressor responses of similar magnitude induced by release of endogenous NE, the venous NE level increases by only a few hundred picograms or less per milliliter.

The window for cleft NE can be made much smaller by blocking Uptake. This reduces the concentration...
gradient for NE between the synapse and plasma. We
have found that blockade of Uptakei by desipramine
results in a marked shift to the left in the pressor-log
plasma NE relationship during NE infusion and a sym-
metrical shift to the right in this relationship during
sympathetic stimulation in pithed, adrenal-demedul-
lated, yohimbine pretreated rats. Meaningful estimates
of cleft NE can be obtained by blocking reflexive cir-
culatory controls (Uptake), and a2-adrenergic recep-
tors, which appear to be situated extrasynaptically.

We applied this approach in modified form to esti-
mate synaptic NE concentrations in humans. We com-
pared pressor response-log NE concentration relation-
ships in healthy volunteers during stimulation of endogenous NE release and during NE infusion, in a
setting where 1) reflexive circulatory controls, which
might modify the pressor-NE relationship, were
blocked; 2) a2-adrenergic receptors, which would dis-
proportionately contribute to pressor responses to in-
fluenced NE, were blocked; and 3) Uptake [was blocked].

The relatively specific a2-adrenergic receptor ago-
nist yohimbine causes increases in blood pressure and
plasma NE in humans presumably by blocking toni-
cally active a2-adrenergic receptors or stimulating cen-
tral sympathetic outflow. We used yohimbine to
enhance release of endogenous NE.

Eighteen male normotensive volunteers ranging in
age from 21 to 53 years participated in this study,
which was approved by the National Heart, Lung, and
Blood Institute's Institutional Review Board.

Each study began with the subject reporting to a
patient observation room in the morning, about 1 to 3
hours after having eaten a light breakfast (juice and
toast). Electrocardiographic leads were attached. An
intravenous catheter was inserted in each arm, one
catheter for administering drugs and the other for
drawing blood. A brachial or radial arterial catheter for
blood sampling and pressure monitoring was inserted
percutaneously after infiltrating the overlying skin
with lidocaine. After the subject had been supine for at
least 20 minutes, baseline blood samples and hemody-
namic measurements were obtained.

Three infusion rates of NE (5 mg base/L 5% dex-
trose in water) were then used to increase mean arterial
pressure by 10, 20, and 30 mm Hg for at least 10
minutes each. At the end of each infusion, blood was
drawn approximately simultaneously from the arterial
and venous catheters for determination of plasma cate-
cholamines.

At least 10 minutes after the last NE infusion, when
blood pressure and heart rate had returned to baseline
values, trimethaphan (1 gm/L 5% dextrose in water)
and yohimbine (0.125 mg/kg bolus followed by a
0.001 mg/kg/min infusion) were administered intrave-
nously to produce ganglionic and a2-adrenergic recep-
tor blockade. The sequence of administration of these
drugs was varied to assess their individual effects. The
infusion rate of trimethaphan was progressively in-
creased until no heart rate response was obtained in
response to the Valsalva maneuver. In selected sub-
jects, phentolamine, 25 to 50 mg, was injected as an
intravenous bolus before and after yohimbine to deter-
mine if yohimbine was producing a1-blockade. Yo-
himbine was obtained from Sigma Chemical Corp.
(St. Louis, MO, USA) and administered under Investi-
gational New Drug Authorization 21220.

After establishing ganglionic and a2-adrenergic re-
ceptor blockade, the NE infusions were repeated, with
the same 10, 20, and 30 mm Hg pressor criteria. In five
subjects, the study took place 1 to 3 hours after oral
treatment with 125 mg of desipramine to block Up-
take..

Arterial and venous concentrations of plasma cate-
cholamines were determined using liquid chroma-
tography with electrochemical detection, the validity
and reliability of which were established in this lab-
oratory.

Statistical testing included independent-means and
dependent-means t tests. A p value less than 0.05 de-
defined statistical significance. All mean values were
expressed ± 1 standard deviation (SD).

**Results**

Infusion of NE produced dose-related increases in
mean arterial pressure in all subjects (Table 1). The
pressor response-log arterial plasma NE relationships
were approximately linear (Figures 1 and 2). Figures 1

<table>
<thead>
<tr>
<th>Condition</th>
<th>Arterial (pg/ml)</th>
<th>Venous (pg/ml)</th>
<th>MAP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No blockers (n = 10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>236±168</td>
<td>291±171</td>
<td>86±9</td>
</tr>
<tr>
<td>+ 10</td>
<td>2092±1284</td>
<td>1044±645</td>
<td>96±11</td>
</tr>
<tr>
<td>+ 20</td>
<td>4344±3178</td>
<td>2018±1334</td>
<td>108±11</td>
</tr>
<tr>
<td>+ 30</td>
<td>7674±5800</td>
<td>3363±2308</td>
<td>121±10</td>
</tr>
<tr>
<td>TRI + YOH, no DMI (n = 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>171±110</td>
<td>260±222</td>
<td>78±16</td>
</tr>
<tr>
<td>+ 10</td>
<td>1133±533</td>
<td>639±122</td>
<td>93±13</td>
</tr>
<tr>
<td>+ 20</td>
<td>1973±760</td>
<td>1152±363</td>
<td>100±12</td>
</tr>
<tr>
<td>+ 30</td>
<td>3227±1523</td>
<td>1514±833</td>
<td>118±12</td>
</tr>
<tr>
<td>DMI (n = 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>108±16</td>
<td>123±14</td>
<td>90±7</td>
</tr>
<tr>
<td>+ 10</td>
<td>774±836</td>
<td>431±279</td>
<td>102±9</td>
</tr>
<tr>
<td>+ 20</td>
<td>1235±1360</td>
<td>797±876</td>
<td>113±8</td>
</tr>
<tr>
<td>+ 30</td>
<td>1687±1303</td>
<td>1189±1136</td>
<td>125±8</td>
</tr>
<tr>
<td>DMI + TRI + YOH (n = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>173±64</td>
<td>238±99*</td>
<td>92±12</td>
</tr>
<tr>
<td>+ 10</td>
<td>478±129</td>
<td>427±118</td>
<td>102±12</td>
</tr>
<tr>
<td>+ 20</td>
<td>793±241</td>
<td>611±483</td>
<td>114±13</td>
</tr>
<tr>
<td>+ 30</td>
<td>1055±229</td>
<td>774±276</td>
<td>127±9</td>
</tr>
</tbody>
</table>

Values are means ± 1 SD.

MAP = mean arterial pressure; DMI = pretreatment with desipra-
mine; TRI + YOH = concurrent administration of trimethapa-
phan and yohimbine during norepinephrine infusion; + 10, +20,
and +30 = target increments in MAP.

*1 outlying data point not counted.
FIGURE 1. Mean arterial pressure (MAP) and arterial plasma norepinephrine concentration ([NE]A) at baseline (M) and during NE infusions (•) and yohimbine-induced NE release. X shows estimated cleft NE concentration at 110 mm Hg (about 20 mm Hg pressor response). DMI = desipramine; YOH = yohimbine; TRI = trimethaphan.

FIGURE 2. Change in mean arterial pressure (AMAP) and in arterial plasma norepinephrine concentration ([NE]A). X shows estimated increment in cleft NE concentration. See Figure I for key to abbreviations.

TABLE 2. Calculated Increment in Arterial Norepinephrine Associated with a 20 mm Hg Pressor Response

<table>
<thead>
<tr>
<th>Condition</th>
<th>EC20 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No DML, no TRI or YOH (n = 10)</td>
<td>3067 ± 1774</td>
</tr>
<tr>
<td>No DML, +TRI + YOH (n = 4)</td>
<td>1712 ± 945</td>
</tr>
<tr>
<td>DML, no TRI or YOH (n = 4)</td>
<td>915±1062*</td>
</tr>
<tr>
<td>DML, +TRI + YOH (n = 5)</td>
<td>511±205t</td>
</tr>
</tbody>
</table>

Values are means ± 1 SD.

EC20 values were calculated for each subject from the linear regression line relating increments in mean arterial pressure to increments in log arterial norepinephrine during norepinephrine infusions with target pressor responses of 10, 20, and 30 mm Hg.

ECM = estimated concentration of arterial norepinephrine at a 20 mm Hg pressor response; DMI = oral pretreatment with 125 mg of desipramine; TRI = intravenous infusion of trimethaphan; YOH = intravenous infusion of yohimbine.

*p < 0.05, t < 0.01, compared with values in no DML, no TRI/YOH group.
Table 3. Effects of Ganglionic and α2-Adrenergic Receptor Blockade on Blood Pressure and Plasma Catecholamines

<table>
<thead>
<tr>
<th>Condition</th>
<th>MAP (mm Hg)</th>
<th>Norepinephrine (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arterial</td>
<td>Venous</td>
</tr>
<tr>
<td>Baseline (n = 9)</td>
<td>85 ± 1</td>
<td>231 ± 181</td>
</tr>
<tr>
<td>Trimethaphan (n = 4)</td>
<td>71 ± 8</td>
<td>155 ± 71</td>
</tr>
<tr>
<td>Yohimbine (TI = 5)</td>
<td>96 ± 8</td>
<td>320 ± 60</td>
</tr>
<tr>
<td>Trimethaphan + yohimbine (n = 9)</td>
<td>76 ± 12</td>
<td>193 ± 112</td>
</tr>
</tbody>
</table>

Values are means ± 1 SD. MAP = mean arterial pressure; A-V = arteriovenous increment in norepinephrine.

Table 4. Percent Decrease in Norepinephrine Between Arterial and Venous Plasma During Norepinephrine Infusions

<table>
<thead>
<tr>
<th>Condition</th>
<th>(% decrease)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No blockers (n = 10)</td>
<td>51 ± 15</td>
</tr>
<tr>
<td>Desipramine (n = 4)</td>
<td>30 ± 12</td>
</tr>
<tr>
<td>Trimethaphan + yohimbine (n = 4)</td>
<td>40 ± 26</td>
</tr>
<tr>
<td>Desipramine + trimethaphan + yohimbine</td>
<td>18 ± 25</td>
</tr>
</tbody>
</table>

Values are means ± SD. For each subject, the ratio was averaged for the three infusion rates. NE = norepinephrine.

Discussion

During an NE infusion to levels eliciting a pressor response, the circulating level of NE must exceed the average level at vascular neuroeffector junctions. Conversely, during stimulation of endogenous NE release from sympathetic nerve endings, the circulating level of NE must be less than the average level at vascular neuroeffector junctions. The circulating NE levels in these settings therefore provide a window within which the synaptic cleft NE concentration must lie.

This window usually is quite large, but our results demonstrate that the window can be made much smaller by blocking Uptake, α2-adrenergic receptors, and ganglionic neurotransmission. The combination of α2-blockade (using yohimbine) and ganglionic blockade (using trimethaphan) resulted in about a twofold shift to the left of the pressor-log NE relationship during NE infusion; blockade of Uptakei (using desipramine) resulted in more than a threefold shift to the left of this relationship; and the combination of all three blockers resulted in about a sixfold shift.

We reasoned that for a 20 mm Hg pressor response, the cleft NE concentration was between 467 pg/ml (2.8 nM), the value for yohimbine-induced increases in arterial NE and in mean arterial pressure in subjects pretreated with desipramine, and about 680 pg/ml (4 nM), the value for the same pressor response during NE infusion in subjects pretreated with desipramine and the trimethaphan-yohimbine combination. If in humans, as in the rat, the desipramine-induced shifts in the pressor-log NE curves during NE infusion and during sympathetic stimulation were reciprocal and equal, then the estimated cleft NE concentration for a 20 mm Hg pressor response would be about 560 pg/ml (3.3 nM), the geometric mean of the 2.8 and 4.0 nM values.

During an infusion of NE, the product of the circulating NE concentration ([NE]c) and the fraction if, the mean proportionate concentration gradient between the synapse and the plasma, equals the cleft NE concentration ([NE]c); and during sympathetically mediated NE release, the circulating NE concentration ([NE]c) is related to the cleft concentration by the equation [NE]c = [NE]c x /f. If pressor responses result only from stimulation of postsynaptic adrenergic receptors (i.e., extrasynaptic receptors are blocked), then for the same pressor response, whether induced by NE infusion or by NE release from sympathetic nerve endings, [NE]c is the same. Therefore, f = [NE]c/[NE]c, and 2 log f = log [NE]c - log [NE]c. From Figure 1, for a pressor response of 20 mm Hg, the cleft NE concentration was about 560 pg/ml. For the same pressor response induced by infused NE in ganglion-blocked, α2-adrenergic receptor-blocked subjects, the arterial NE concentration was 1973 pg/ml. The value for the pressor response of 20 mm Hg would therefore be about 1/3.6 = 0.28. This value agreed well with the value of 1/3.4 =

FIGURE 3. Arterial (A) and venous (V) plasma norepinephrine concentration ([NE]) during infusion of yohimbine or trimethaphan. Numbers in parentheses show the number of subjects. BL = baseline. See Figure 1 for key to other abbreviations.
0.29 obtained in pithed, adrenal-demedullated, yohimbine-treated rats. The effect of desipramine was to shift to the left the pressor-log NE relationship, as would be expected if the plasma-cleft NE relationship were decreased. Based on Figure 1, the calculated value for/after desipramine pretreatment was about 550/680 = 0.81; in the pithed rat this value was 0.67.2

The large shifts in the pressor-log NE curves resulting from desipramine pretreatment mean that in humans, as in the rat, neuronal uptake accounts for a large proportion of the removal of infused NE. In the present study, 51% of NE in arterial blood was removed in the arm, a value that agrees with results of studies involving infusions of tracer-labeled NE.10 In subjects pretreated with desipramine, the average amount of NE removal in the arm was significantly less (30%). We estimated that about two-fifths of the NE removed in the arm was removed by Uptakei.

We found virtually identical shifts in the pressor-log NE relationship after desipramine pretreatment regardless of combined ganglionic and a2-adrenergic receptor blockade. This finding is consistent with effects of Uptakej blockade, which are independent of those resulting from combined ganglionic and a2-adrenergic receptor blockade in modulating pressor responses to infused NE. Similarly, the effects of Uptakei blockade and of combined ganglionic and a2-adrenergic receptor blockade on NE removal in the arm appeared to be additive. In the presence of all three blockers, only about 20% of brachial arterial NE was removed in the arm.

As shown in Table 1, baseline arterial NE concentrations were lower than venous, which is consistent with several other reports. In contrast, during the NE infusions, arterial NE levels consistently exceeded venous levels: the extent of the arteriovenous difference was a bit over 50%. This finding is in agreement with results based on the removal of tracer-labeled NE in the arm.10

In considering our approach for estimating intrasynaptic NE concentrations, the objection may be raised that vascular responses to sympathetic stimulation may differ from responses to exogenous NE because co-transmitters may influence the effect of sympathetic stimulation on vascular tissue or because the density of vasodilator /3-adrenergic receptors in immediate contact with neuronally released NE may be different from that in immediate contact with luminal NE. The design of the present study cannot exclude these possibilities, but the results of the study of pithed rats2 apply to this issue. As previously discussed,2 if transmitters other than NE are coreleased with NE during sympathetic stimulation (adenosine 5‘-triphosphate, neuropeptide Y, chromogranin A, and enkephalins all are candidates to play such a cotransmitter role) and do in fact influence the vascular response, then the pressor response would be augmented (or attenuated) by the cotransmitter in a manner dependent on the frequency of stimulation. This would mean that during sympathetic stimulation, the curve relating the magnitude of the pressor response to the arterial NE concentration would have a slope different from that during NE infusion. This was not the case. Similarly, if the number of vasodilator adrenergic receptors in proximity to the synaptic clefts was different from the number of receptors more closely in contact with circulating NE, then during desipramine-induced inhibition of neuronal NE removal, the pressor-log arterial NE concentration relationships would have been shifted asymmetrically during sympathetic stimulation compared with NE infusion, because a greater or lesser number of these receptors would have been exposed to endogenously released as opposed to infused NE. This also was not the case.

Our model for estimating cleft NE concentrations associated with pressor responses circumvents the problem of possible distortion of the junctional-plasma NE relationship by variability of NE removal mechanisms and so makes more accurate the interpretation of circulating NE levels in terms of sympathetically mediated NE release. The model can now be used to determine if patients with essential hypertension have excessive pressor responses for given concentrations of NE in the synaptic cleft.

Acknowledgments
We gratefully acknowledge the assistance of Ms. Carol Joan Folio, R.N., and Mr. Harold Smith.

References
Estimation of intrasynaptic norepinephrine concentrations in humans.
D S Goldstein, R Zimlichman, R Stull, H R Keiser and I J Kopin

Hypertension. 1986;8:471-475
doi: 10.1161/01.HYP.8.6.471

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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