In Vivo Measurement of Neuronal Uptake of Norepinephrine in the Human Heart

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Neuronal uptake (Uptake-1) of the sympathetic neurotransmitter norepinephrine from the circulation in the human heart was assessed in vivo with three techniques. 1) Cardiac removal of intravenously infused tracer-labeled norepinephrine was measured before and after Uptake-1 blockade with desipramine; 2) the difference between the fractional extraction of radioactive norepinephrine and of radioactive isoproterenol, which is not a substrate for neuronal uptake, was used to estimate the removal of norepinephrine by Uptake-1 in the heart compared with other vascular beds (arm, leg, brain, and lungs); and 3) regional arteriovenous differences in radioactive and endogenous dihydroxyphenylglycol (DHPG), an exclusively intraneuronal metabolite of norepinephrine, were compared in these beds. In untreated patients, cardiac removal of radioactive norepinephrine averaged 79%, whereas in desipramine-treated patients, cardiac removal of radioactive norepinephrine averaged 19%, a value similar to that of isoproterenol in untreated patients (14%), confirming that in the heart the non-neuronal removals of isoproterenol and norepinephrine were similar. In the heart, 69% of delivered norepinephrine was estimated to be removed by Uptake-1, a much higher percentage than that in the arm (14%), leg (7%), brain (10%), and lungs (4%). The cardiac arteriovenous increment in endogenous DHPG (137%) far exceeded that of the other beds (49%, 26%, 39%, and −19%, respectively), and radioactive DHPG in the great cardiac vein exceeded arterial levels by 113%, whereas in the other beds, arterial radioactive DHPG exceeded venous levels. The results indicate that the human heart is exceptionally dependent on neuronal uptake for in vivo removal of circulating norepinephrine. (Circulation 1988;78:41-48)

Several pathophysiological and therapeutic concepts in cardiology have been based on sympathetic neuroeffector mechanisms in the heart. A limitation in evaluating these concepts has been the inability to measure cardiac sympathetic function in vivo, especially the activity of the neuronal uptake process (Uptake-1), which is the predominant means for terminating the actions of the sympathetic neurotransmitter norepinephrine (NE). The present study introduces three clinical techniques to examine cardiac neuronal uptake of NE.

In the first technique, cardiac removal of tracer-labeled NE was measured before and after Uptake-1 blockade with desipramine, a procedure previously used in laboratory animals.¹-³

A second technique was based on the fact that the synthetic catecholamine isoproterenol (ISO) is not a substrate for neuronal uptake.⁴ A difference in regional removal of NE and ISO therefore would indicate Uptake-1 activity if the removals of these catecholamines did not differ importantly in any other way. This approach was validated in the human forearm⁵ and canine hindlimb⁶ by the observation that the regional removal of tracer-labeled NE in desipramine-treated patients was similar to that of tracer-labeled ISO in untreated patients. In the present study, we determined whether the difference in cardiac removals of NE and ISO would indicate Uptake-1 activity by comparing the cardiac removal of NE in desipramine-pretreated patients with the removal of ISO in untreated patients.

In the third technique, we determined the cardiac rate of production of tracer-labeled dihydroxyphenylglycol (DHPG) during systemic intravenous infusion of tracer-labeled NE. Recent in vitro and in vivo evidence⁶,⁷ has established that DHPG, formed from the deamination of axoplasmic NE, is the predominant intraneuronal metabolite of NE and
that plasma DHPG depends on the intraneuronal metabolism of NE. Axoplasmic NE, in turn, has two main sources: reuptake of endogenously released NE and leakage of NE from vesicular storage sites. Because formation of DHPG from intravenously infused NE is dependent on neuronal uptake, radiolabeled DHPG in plasma during infusion of radiolabeled NE could only be produced after removal of the radiolabeled NE by sympathetic nerves. If Uptake-1 activity were high, then a relatively large arteriovenous increment in tracer-labeled DHPG would be expected. We therefore examined regional production of radiolabeled DHPG from intravenously infused radiolabeled NE.

Patients and Methods

Patients

Eleven patients undergoing cardiac catheterization as part of clinical protocols of the Cardiology Branch, National Heart, Lung, and Blood Institute of the National Institutes of Health (NIH) in the NIH Clinical Center and six patients undergoing cardiac catheterization at the Baker Medical Research Institute (BMRI), Victoria, Australia, were studied.

The NIH patients were being evaluated for chest pain and had angiographically normal coronary arteries. Six of the NIH patients were diagnosed as having a limitation of coronary blood flow at the microvascular level (microvascular angina) based on criteria that included chest pain induced by pacing and responses to ergonovine and diprydamole. The other patients had equivocal or no evidence of microvascular angina. The NIH patients were off all cardiovascular medications for at least 48 hours before testing.

The BMRI patients had mild essential hypertension. They had either been untreated for at least 3 months or else had never been treated for hypertension. Data from these patients were included in the present study because cardiac removal of radiolabeled NE was assessed before and after administration of desipramine to block Uptake-1.

Study Protocol

NIH patients. The NIH patients underwent percutaneous insertion of a Swan-Ganz thermodilution catheter in the pulmonary artery and insertions of a left brachial 20-gauge arterial catheter, a femoral venous catheter, a left brachial vein catheter, and a great cardiac vein thermocatheter, which was used for both blood sampling and measurement of great cardiac vein blood flow.

The infusate contained 50 μCi ring-labeled tritiated l-NE (New England Nuclear, Boston, Massachusetts) and 50 μCi tritiated dl-ISO (Amersham, Arlington Heights, Illinois), which had been tested for sterility, pyrogenicity, and purity by the NIH Radiopharmacy and stored in 50-μCi aliquots (about 5 ml each) at −70°C until thawed just before use. The radionuclides were added to 50 ml 5% dextrose in water to produce about 60 ml infusate, which was infused at 90 ml/hr by a controlled infusion pump into the infusion port of the Swan-Ganz catheter, that is, into the right atrium. At 20 minutes during the infusion, blood samples (8–10 ml) were collected from the above sites.

The specific activities of the radionuclides were sufficiently high that plasma levels of NE and ISO due to the infusate were about 3 pg/ml or less, and no change in vital signs or symptoms could be attributed to the infusion. Dosimetric calculations led to a predicted radiation exposure of less than 0.01 radian as a result of the radioactive infusion, compared with a predicted radiation exposure of at least 1 radian as a result of fluoroscopic imaging for positioning of the vascular catheters.

BRMI patients. The BMRI patients received l-NE tritiated at the seventh position, infused into a systemic vein at a rate of 0.35 μCi/min/m² for at least 60 minutes before the first set of blood samples was taken. Blood samples were obtained from an artery and from the coronary sinus. Desipramine, 0.5 mg base/kg body wt, was then infused intravenously during 30 minutes. Blood samples were obtained 30 minutes after desipramine administration while the tritiated NE infusion continued.

Assay Procedures

NIH patients. The blood samples were transferred into chilled, sterile, evacuated, heparinized glass tubes, and the plasma was separated by refrigerated centrifugation and stored at −70°C until the time of assay. Plasma levels of the catechols, including NE and DHPG, were determined with liquid chromatography with electrochemical detection after batch alumina extraction. Concentrations of radioactive NE, ISO, and DHPG were calculated from the tritium contents of the chromatographic fractions where the elution times corresponded to those of the standards.

BMRI patients. The plasma was assayed with a radioenzymatic procedure for quantifying total NE, and the radioactive NE contents were determined from the total tritium in the eluate after a batch alumina extraction. Radioactive NE and radioactive DHPG therefore were not quantified separately in these samples; instead, the total contents of radioactive catechols were measured.

Pharmacokinetic Variables

Regional removal, clearance, and spillover of NE were quantified from measurements of regional blood flow and arterial and venous concentrations of radioactive and endogenous NE during systemic intravenous infusion of trace amounts of radioactively labeled NE according to the following formulas:
Total NE delivery rate (pg/min) = Q × NEa

(1)

Total [3H]NE delivery rate (dpm/min) = Q × [3H]NEa

(2)

Total NE removal rate (pg/min) = Q × NEa × ([3H]NEa − [3H]NEv)/[3H]NEa

(3)

Regional NE clearance (ml/min) = Q × ([3H]NEa − [3H]NEv)/[3H]NEa

(4)

Regional NE spillover (pg/min) = Q × ((NEv−NEa) + NEa)

(5)

where Q is the regional blood flow (ml/min), NEa and NEv are the arterial and regional venous NE concentrations (pg/ml), and [3H]NEa and [3H]NEv are the arterial and venous concentrations of tritiated NE (dpm/ml).

The cardiac rate of removal of circulating NE by Uptake-1 was estimated by comparing the cardiac proportionate removal of radioactive NE in patients with Uptake-1 blockade with that of unblocked patients.

The magnitude of the difference between the regional removal of NE and that of ISO provided a second means to estimate Uptake-1 activity. If ([3H]NEa − [3H]NEv)/[3H]NEa is the regional proportionate removal of NE, fxRemNE, and if ([3H]ISOa − [3H]ISOv)/[3H]ISOa is the regional proportionate removal of ISO, fxRemISO, then

Neuronal uptake rate (pg/min) = Total delivery rate × (fxRemNE − fxRemISO)

(6)

and

Neuronal uptake rate (dpm/min) = Total [3H]NE delivery rate × (fxRemNe − fxRem ISO)

(7)

In a bed where blood flow was not measured, the neuronal uptake rate could not be calculated; however, the proportion of delivered NE that was removed by Uptake-1 could be quantified by the value for fxRemNE − fxRemISO.

A third technique was based on the regional release of radioactive DHPG during infusion of labeled NE until a steady state was achieved. Regional release of radioactive DHPG during infusion of radioactive NE would require uptake of the NE into sympathetic nerve endings. Because a substantial proportion of NE in the cytoplasm is removed by translocation into storage vesicles rather than by deamination to form DHPG, the regional rate of release of radioactive DHPG would be expected to be less than the regional neuronal uptake rate. The relative activity of Uptake-1 among the beds could be assessed by measuring the arteriovenous increments in tracer-labeled DHPG, that is, by comparing values for [3H]DHPGv − [3H]DHPGa.

The total body clearance of NE and the total body rate of spillover of NE, the sum of regional contributions to NE in arterial blood, were quantified according to the following formulas:

TB clearance (ml/min) = [3H]NEinf × Inf Rate/[3H]NEa

(8)

TB spillover (pg/min) = TB clearance × NEa

(9)

where [3H]NEinf is the infusate concentration of tritiated NE (dpm/ml), Inf Rate is the infusion rate (ml/min), [3H]NEa is the arterial concentration of tritiated NE (dpm/ml), and NEa is the arterial total concentration of NE (pg/ml).

Data Analysis

The results were analyzed with paired and unpaired t tests. Values were mean ± SEM. A p value less than 0.05 defined statistical significance.

Results

Arterial and regional venous levels of plasma NE and DHPG in the NIH patients are summarized in Figure 1. At baseline, there was a small, statistically nonsignificant arteriovenous increment in NE across
ISO in the heart was entered into Equations 6 and 7, it was estimated that $82 \pm 5\%$ ($9.7 \pm 1.4$ ng/min) of the NE removed in the heart was removed by Uptake-1. The percentage of total delivered NE that was removed by Uptake-1 was estimated to be $69 \pm 4\%$ in the heart (Figure 2), which was significantly higher than in the arm ($14 \pm 2\%$), leg ($7 \pm 3\%$), lungs ($4 \pm 5\%$), and brain ($10 \pm 4\%$).

Levels of arterial and regional venous NE and DHPG and the regional removals of tritiated NE and of tritiated ISO did not differ significantly between the subgroup of patients with microvascular angina and the subgroup with equivocal or no evidence of microvascular angina.

As indicated in Figure 3, the arteriovenous increment in DHPG was significantly larger in the heart ($137 \pm 37\%$) than in the arm ($49 \pm 14\%$), leg ($26 \pm 15\%$), lungs ($19 \pm 12\%$), and brain ($39 \pm 9\%$). Whereas the great cardiac venous concentration of radioactive DHPG exceeded the arterial level by $113 \pm 46\%$ ($p<0.05$), no significant arteriovenous increments in radioactive DHPG were noted in the other beds. The $113\%$ value was higher than predicted from the mean arteriovenous increment divided by the mean arterial level of radioactive DHPG because of a high percent increment (540%) in one patient. Without the data point, the arteriovenous increment in radioactive DHPG averaged $70 \pm 19\%$.

Cardiac production of endogenous DHPG (arteriovenous increment in DHPG $\times$ blood flow, 57 $\pm 10$ ng/min) far exceeded the cardiac removal of circulating endogenous NE by Uptake-1 ($9.7 \pm 1.4$ ng/min, $p<0.001$). In contrast, the cardiac removal of circulating radioactive NE from arterial blood ($64,027 \pm 9,098$ dpm/min) was more than 30 times the cardiac production of radioactive DHPG ($1,957 \pm 757$ dpm/min).

The total body clearance of NE was estimated to be $2.3 \pm 0.2$ l/min, and the total body spillover rate was estimated to be $482 \pm 58$ ng/min. The latter would correspond to about $694 \mu g/24$ hr. The cardiac spillover rate of NE was only 3% of the total body spillover rate.

**Discussion**

In the present study, we found that removal of circulating NE by the heart exceeded that of ISO,
which is not a substrate for neuronal uptake (Uptake-1), by more than fivefold; treatment with desipramine to block Uptake-1 decreased cardiac NE removal by at least threefold (probably more, as discussed below) to a value similar to that of ISO in untreated patients; and only in the heart was there detectable release of radioactive DHPG, an exclusively intraneuronal metabolite of NE,\(^6\)\(^7\) during systemic intravenous infusion of radioactive NE. These findings indicate that the heart is mainly dependent on neuronal uptake for removal of circulating NE.

This conclusion differs from those for other vascular beds and for the body as a whole. Early studies of urinary excretion of deaminated and O-methylated radioactive metabolites after intravenous administration of radioactive catecholamines showed that mainly O-methylated metabolites were excreted,\(^14\) and because catechol-O-methyltransferase appears to be concentrated in non-neural cells,\(^15\) the inference was drawn that NE is removed from the circulation mainly by extraneuronal uptake and metabolism. Similarly, neuronal uptake blockade prolonged the clearance of radiolabeled NE from arterial blood by about 30%,\(^5\) indicating the proportion of circulating NE removed by Uptake-1. In the arm, only about 15% of NE removed from the circulation was estimated to be taken up into nerve endings,\(^5\) and a substantial proportion of circulating ISO was found to be removed in the kidney\(^3\) and viscera.\(^16\) In the present study, the proportion of regional delivery of NE that was removed by Uptake-1 was much larger in the heart than in the arm, leg, lungs, and brain.

Results from several animal studies, with a variety of techniques, have also suggested that the heart depends importantly on neuronal uptake for removal of circulating NE. In the perfused rabbit heart, Mann and Yudivilevich\(^17\) found that Uptake-1 blockade with desipramine decreased cardiac removal of tritiated NE by over 90%, whereas blockade of extraneuronal uptake of NE had practically no effect. Other studies\(^1\)\(^2\)\(^18\) have reported that cardiac responses to exogenous NE were prolonged significantly by Uptake-1 blockade but not by blockade of extraneuronal uptake, indicating that neuronal uptake was the more important determinant of NE removal in this preparation. Also, these studies reported that blockade of neuronal uptake with desipramine decreased cardiac removal of exogenously administered NE from about 80% to 20%,\(^1\) which are values remarkably similar to those reported here and that cardiac decreased cardiac removal of exogenous NE from 69% to 24%.\(^2\) Cousineau et al,\(^19\) applying a complex kinetic analysis in anesthetized dogs, found that desipramine treatment decreased cardiac removal of tracelabeled NE by about 60%. In a study of pithed rats, where reflexive circulatory controls are abolished, administration of NE causes tachycardia, and Docherty and McGrath\(^20\) reported that cardiac chronotropic responses to sympathetic stimulation, as well as to intravenous NE, were potentiated by Uptake-1 blockade with cocaine or desipramine, whereas blockade of extraneuronal uptake with corticosterone had no potentiating effect. As would be expected from the fact that isoproterenol is not a substrate for uptake-1, desipramine treatment did not affect cardiac chronotropic responses to isoproterenol in the pithed rats. Kirby and Stewart\(^21\) prevented cardiac neuronal development by ablating the neural crest at various somite levels in embryonic chicks. Cardiac atrial uptake of tritiated NE was decreased by over 60% in embryos with somite ablation at levels corresponding with cardiac innervation. A similar deficit in NE uptake was observed in embryos treated with the sympatholytic drug 6-hydroxydopamine.\(^22\) Thus, evidence from rabbits, rats, dogs, and chicks suggests that Uptake-1 is the main removal mechanism for circulating NE in the heart, and the present evidence confirmed this suggestion in humans.

Removal of radioactive arterial NE by the heart averaged 79% in the NIH patients, whereas cardiac removal of tritiated total catechols averaged only about 65% in the BMRI patients. This discrepancy, and the applicability of the desipramine results in
the BMRI patients, requires further consideration. In the NIH patients, the plasma was first subjected to alumina extraction, which is a highly efficient means of separating catechols from other substances but which does not separate NE from other catechols. Liquid chromatography was then used to separate tritiated NE from other tritiated catechols and liquid scintillation spectrometry was used to quantify the tritium contents of the eluent fraction corresponding in retention to that of the NE standard. In contrast, in the BMRI patients, the plasma was subjected to alumina extraction, and the tritium contents of the alumina extract were measured without the liquid chromatography step. Thus, in the BMRI patients, the total content of tritiated catechols, rather than tritiated NE specifically, was measured. This technical difference appeared at first to be unimportant because we previously had found that during systemic intravenous infusions of tritiated NE, only a small proportion of total tritiated catechols in arterial plasma was attributable to other than tritiated NE.11 In the heart, however, this technical difference proved to be important because of the exceptionally large arteriovenous increments in levels of both endogenous and radio-labeled plasma DHPG, which is a catechol. Because of the cardiac production of radiolabeled DHPG, the regional cardiac removal of total arterial tritiated catechols underestimated the regional cardiac removal of tritiated NE in the BMRI patients. These considerations lead us to suggest that in studies of cardiac NE release and metabolism using infusions of tracer-labeled NE, radioactive NE must be quantified separately from radioactive DHPG to avoid underestimation of regional NE spillover into the bloodstream.

Because Uptake-1 blockade probably abolished regional production of radioactive DHPG from radioactive NE,6 the proportionate removal of total catechols would have been similar to that of NE after desipramine administration in the BMRI patients. Thus, the finding that the cardiac removal of tritiated catechols in the desipramine-treated BMRI patients was similar to the cardiac removal of tritiated ISO in the NIH patients confirmed that cardiac removal of ISO did not differ importantly from cardiac removal of NE except in terms of the relative susceptibility of the two catechols to removal by Uptake-1.

The results indicate that in the heart and in the body as a whole, a much larger proportion of endogenously released NE was removed locally and metabolized than diffused unchanged into the circulation. The total body spillover of NE was estimated to be 694 μg/day. Because the total daily urinary excretion of NE and its metabolites is about 5 mg/day,23 at least 80–90% of the NE released from sympathetic nerves in the body as a whole must have been removed locally and metabolized before entering the bloodstream. Similarly, the cardiac spillover rate of NE (12 ng/min) was far smaller than the cardiac net production of endogenous DHPG (57 ng/min).

Considering the small arteriovenous production of NE by the heart, cardiac release of NE into the bloodstream accounts for only a small proportion of circulating NE. Cardiac NE spillover averaged only about 3% of total body NE spillover. Nevertheless, expressed per volume of tissue, the cardiac release of NE into the bloodstream (or, more accurately, the release of NE from interseptal myocardium into the bloodstream because the great cardiac vein drains the region perfused by the left anterior descending artery) exceeded by far the NE spillover rate of the arm. If the left ventricular mass were 175 g24 and the specific gravity of myocardial tissue about 1.1,25 the myocardial spillover of NE expressed in nanograms per milliliter per 100 ml would have been about 7.8 ng/min/100 ml. 20 times the forearm NE spillover rate per 100 millimeters tissue.

DHPG released by the heart was derived mainly from cardiac NE, not from uptake of circulating NE. This conclusion is based on the finding that although most of arterial NE was removed in the heart and although most of the NE that was removed was taken up into sympathetic nerve endings in the heart, the cardiac release of DHPG into the bloodstream (57.0 ng/min) exceeded by far the uptake of circulating NE into nerve endings (11.9 ng/min). The large amount of DHPG production by the heart could have indicated a high rate of release and uptake of endogenous NE or a high rate of leakage of NE into the cytoplasm from storage sites, or both.

Because of several intervening processes that affected the relation between cardiac DHPG production and neuronal reuptake of endogenously released NE, the arteriovenous increment in regional DHPG should not be used as a simple indicator of regional Uptake-1 activity. Only a very small percentage of NE removed from the bloodstream by Uptake-1 was recovered as DHPG released into the bloodstream because the cardiac rate of neuronal uptake of radioactive NE from arterial blood was more than 30 times the cardiac rate of production of radioactive DHPG. It is possible that a proportion of arterial radioactive DHPG was removed in the heart, resulting in an underestimation of regional DHPG production based only on the arteriovenous increment in DHPG. Even if all the arterial DHPG were removed, however, the cardiac rate of production of radioactive DHPG still would have been far less than the neuronal uptake rate of arterial radioactive NE. It is likely that a proportion of DHPG released in the heart was taken up extraneuronally before reaching the bloodstream and converted to methoxyhydroxyphenyglycol (MHPG). Also, most of the NE taken up into the cytoplasm could have been taken up into storage vesicles. Consistent with this explanation, we recently found in rat vas deferens tissue in which most of the NE that was taken up into nerve endings is translocated into storage vesicles rather than metabolized by monoamine
oxidase. A less likely explanation was that circulating levels of radioactive DHPG had not reached a steady state by the time of blood sampling. Because arterial and venous samples were obtained approximately simultaneously, error due to lack of attainment of steady-state conditions should not have been large.

Because neither the NIH nor the BMRI patients were healthy volunteers, one may question whether the obtained results were representative of a normal situation. The BMRI patients were healthy except for mild essential hypertension, and the NIH patients with atypical chest pain all had normal cardiac function, normal coronary arteries, and no chest pain during the study. Further, a subgroup of the NIH patients had little or no evidence of limited coronary vasodilator reserve, and the results of regional NE kinetics were the same as in the subgroup with definite microvascular angina.

Because extraneuronal uptake of catecholamines was not inhibited in the present study and because a proportion of circulating NE was removed at extraneuronal sites before reaching the neuroeffector regions, the calculated proportionate removal of circulating NE by Uptake-1 (about 70%) must have underestimated substantially the proportionate removal of endogenously released NE by this mechanism, which therefore must have been extremely large. Because of this, any drug that would simultaneously release endogenous NE and inhibit Uptake-1 would be expected to increase markedly the concentration of NE at postsynaptic adrenoceptors. Cocaine is one such drug. The cardiac toxicity of cocaine, even in patients without organic heart disease, recently has received increasing attention. The mechanism of cocaine cardiotoxicity is unknown. This local anesthetic agent enters the central nervous system to produce well-known effects including behavioral activation and euphoria. Central sympathetic outflow is enhanced, increasing rates of release of endogenous catecholamines. Cocaine also is well known to be a potent inhibitor of Uptake-1. In a region such as the heart, which appears to be particularly dependent on Uptake-1 for removal of NE, it seems reasonable to speculate that cocaine could cause cardiotoxic effects due to excessive accumulation of catecholamines at myocardial adrenoceptors. A similar explanation may apply for some manifestations of digitalis cardiotoxicity because cardiac glycosides inhibit Uptake-1 and because patients with myocardial infarction or congestive heart failure often have high circulating levels of catecholamines.

In conclusion, three techniques were used to assess neuronal uptake of circulating NE in the heart and other vascular beds: the regional removal of radiolabeled NE with and without desipramine treatment, the difference in regional removals of radiolabeled NE and radiolabeled ISO, and the regional production of radiolabeled DHPG from infused radiolabeled NE. By all three techniques, it was found that the heart was exceptionally dependent on Uptake-1 for removal of NE. This dependence may be particularly relevant in clinical conditions associated with the combination of increased circulating catecholamine levels and inhibition of Uptake-1.

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