# Comparison of High Specific Activity (−) and (+)-6-[$^{18}$F]Fluoronorepinephrine and 6-[$^{18}$F]Fluorodopamine in Baboons: Heart Uptake, Metabolism and the Effect of Desipramine

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(−)-Norepinephrine is the principal neurotransmitter of the mammalian sympathetic nervous system and a major CNS neurotransmitter. The simple ring fluorinated derivatives of (−)- and (+)-norepinephrine [(−)- and (+)6-fluoronorepinephrine] and dopamine (6-fluorodopamine) have been labeled with $^{18}$F in high specific activity (2–5 Ci/$\mu$mol) and evaluated as tracers for (−)-norepinephrine. Comparative PET studies of (−) and (+)-6-[$^{18}$F]fluoronorepinephrine [(−)-6-[$^{18}$F]FNE and (+)-6-[$^{18}$F]FNE] and 6-[$^{18}$F]fluorodopamine (6-[$^{18}$F]FDA) in the same baboon showed strikingly different kinetics in the heart. Analysis of plasma showed more rapid metabolism of 6-[$^{18}$F]FDA with only 1%–2% of $^{18}$F remaining as parent tracer at 10 min after injection of 6-[$^{18}$F]FDA, in contrast to 28% and 17% remaining after injection of (−) and (+)-6-[$^{18}$F]FNE. No changes in vital signs were observed at any time during the study. Pretreatment with desipramine (0.5 mg/kg), a tricyclic antidepressant drug which interacts with a binding site associated with norepinephrine reuptake, markedly decreased cardiac uptake of 6-[$^{18}$F]FDA and (−)-6-[$^{18}$F]FNE. However, a greater blocking effect was observed for (−)-6-[$^{18}$F]FNE. These studies show that (−) and (+)-6-[$^{18}$F]FNE are similar to (−)- and (+)-norepinephrine in their patterns of metabolism and clearance in the heart and that (−)-6-[$^{18}$F]FNE is a promising tracer for endogenous (−)-norepinephrine.

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The principal neurotransmitter of the mammalian sympathetic nervous system and a major CNS neurotransmitter is (−)-norepinephrine (*1,2*). Studies with [$^{3}$H]norepinephrine showing that it is taken up and stored in sympathetic nervous tissue and released by nerve stimulation have formed the basis for its use as an index of sympathetic nervous function (*3*). Accordingly, many studies in animals and humans have used (−)-[$^{3}$H]norepinephrine as a tracer to label the endogenous norepinephrine pool and assess changes in adrenergic activity resulting from disease, stress, drugs and other factors by observing the overflow of labeled norepinephrine and its metabolites into the plasma (*4*). Such studies require the establishment of an equilibrium between (−)-[$^{3}$H]norepinephrine and endogenous (−)-norepinephrine and involve complex, invasive catheterization procedures. Thus, the need for a method to examine directly norepinephrine turnover, as well as other processes associated with the functional activity of the sympathetic neuron in organs like the heart, has been recognized (*5,6*).

One class of compounds that has been studied for this purpose is the simple ring fluorinated derivatives of norepinephrine (*7,8*). Comparative studies of the biological activity of simple ring fluorinated derivatives of catecholamines relative to the parent catecholamines have suggested that fluorine substitution produces compounds which are promising candidates for labeling with $^{18}$F for positron emission tomographic studies of sympathetic innervation and function (*5,9*) (Fig. 1). For example, comparative studies of racemic 6-fluoronorepinephrine and norepinephrine indicate that 6-fluoronorepinephrine is a valid tracer for the presynaptic mechanisms for norepinephrine uptake, storage and release (*5*). Additionally, it has been shown that 6-fluorodopamine is taken up by sympathetic neurons and converted to (−)-6-fluoronorepinephrine by the action of dopamine-$\beta$-hydroxylase (*9*). Even though 6-fluorodopamine is a poorer substrate than

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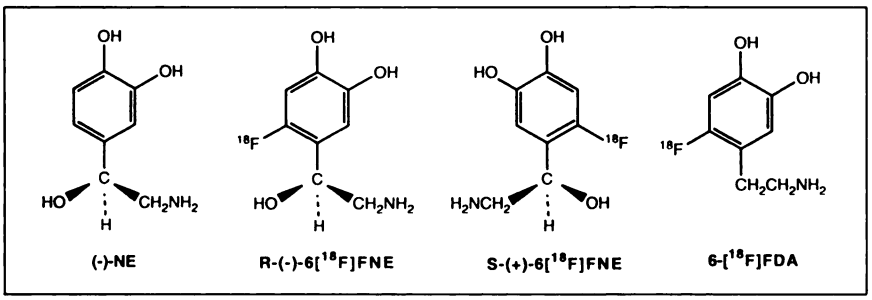


**FIGURE 1.** Structures of (−)-norepinephrine ((−)-NE), (−)-6-[$^{18}$F]fluoronorepinephrine ((−)-6-[$^{18}$F]FNE), (+)-6-[$^{18}$F]-fluoronorepinephrine ((+)-6-[$^{18}$F]FNE) and 6-[$^{18}$F]fluorodopamine (6-[$^{18}$F]FDA).

dopamine for labeling storage pools of norepinephrine (*9*), this observation provided the basis for recent mechanistic PET studies with low specific activity 6-[$^{18}$F]-fluorodopamine (6-[$^{18}$F]FDA), examining its use to image sympathetic innervation and function in the canine heart (*10,11*). While other positron emitters labeled false neurotransmitters such as [$^{18}$F]fluoro-metaraminol (*12–14*) and [$^{11}$C]hydroxyephedrine (*15,16*) have been used to visualize adrenergic innervation in the heart, it has been suggested that the chemical similarity of the ring fluorinated catecholamines to the parent catecholamines may result in tracers whose behavior reflects the turnover rate of endogenous norepinephrine (*6,17*).

We have recently developed a new synthetic route to high specific activity $^{18}$F-labeled catecholamines, (−)- and (+)-6-[$^{18}$F]FNE and 6-[$^{18}$F]FDA, using the nucleophilic aromatic substitution reaction (*18–20*). This reaction provides high specific activity tracers which are devoid of the hemodynamic effects reported for low specific activity 6-[$^{18}$F]FDA (*10*). Since previous studies evaluating the behavior of 6-fluoronorepinephrine as a tracer have been carried out with the unlabeled racemic mixture (*5*), the availability of (−)- and (+)-6-[$^{18}$F]FNE has provided the first opportunity to examine their kinetics and metabolism. Here, we report comparative PET studies of (−) and (+)-6-[$^{18}$F]FNE and of 6-[$^{18}$F]FDA in the same baboon, including heart uptake, tracer metabolism and the effect of desipramine, a tricyclic antidepressant drug which is an inhibitor of norepinephrine reuptake (uptake 1) (*21*). We also have measured the degree of recovery of (−)-6-[$^{18}$F]FNE uptake in the heart after treatment with desipramine.

## METHODS

### Synthesis of NCA (+), (−)-6-[$^{18}$F]FNE, 6-[$^{18}$F]FDA and [$^{13}$N]$H_3$

Fluorine-18-labeled fluorocatecholamines were prepared via the nucleophilic aromatic substitution reaction using an NCA [$^{18}$F]fluoride ion as previously reported (*19,20*). Suitably protected dihydroxyl nitrobenzaldehydes were chosen as precursors. Since 6-[$^{18}$F]FDA is more stable than 6-[$^{18}$F]FNE in the harsh conditions required for hydrolyzing the protected catechol moiety during the synthesis, a commercially available substrate, 6-nitropiperonal, was chosen as the precursor to 6-[$^{18}$F]FDA rather than an isopropylidene protected precursor as was used for 6-[$^{18}$F]FNE. Purification of 6-[$^{18}$F]FDA was accomplished by a semipreparative HPLC (Phenomenex ODS1 column, 25 × 1.0 cm) using 20% methanol in 55 m*M* citric acid (pH 2.9) as the mobile phase (retention time of 6-[$^{18}$F]FDA was 10 min). The residue obtained upon rotary evaporation of the solvent was reconstituted in saline (3 ml), neutralized with sterile 4.2% $NaHCO_3$ to pH 4 and then sterilized by passage through a 0.22 μm filter into a sterile vial for subsequent intravenous injection. Specific activity, determined by HPLC analysis was about 1–2.5 Ci/μmol (EOS). Chemical and radiochemical purities, assayed by radio-HPLC (Phenomenex ODS1 column, 25 × 0.46 cm) and radio-TLC (silica; $CH_3CN$:$H_2O$:HOAc = 18:2:0.4), were >98%.

Since 6-[$^{18}$F]FNE contains a chiral center at the benzylic position, two HPLC purifications were required at the end of synthesis after nucleophilic substitution, cyanohydrin formation, reduction and deprotection. Semipreparative HPLC (Phenomenex ODS1 column, 25 × 1 cm, eluting with 2.5% $CH_3COOH$) was first used to purify the crude, racemic 6-[$^{18}$F]FNE. Chiral HPLC (Daicel Crownpak CR(+), 15 × 0.46 cm, eluting with 0.02 *M* $HClO_4$) was used to resolve the racemic mixture to obtain enantiomerically pure (−) and (+)-6-[$^{18}$F]FNE (*19*). Rotary evaporation was prohibited for the two enantiomer fractions collected from the chiral HPLC column since racemization occurred upon heating and evaporation of the solvent. Therefore, each fraction (about 2 ml) was directly neutralized with 4.2% $NaHCO_3$ to pH 4 and followed by passage through a sterile 0.22 μm filter into a sterile, pyrogen-free injection vial. Specific activity was about 0.7–1.9 Ci/μmol (EOS). Chemical and radiochemical purities, as well as enantiomeric purity determined by radio-HPLC using a Crownpak CR(+) column, were >99%. For example, from 160 mCi of $^{18}$F, we obtained 5.5 mCi of each enantiomer at the end of a 150 min synthesis.

Nitrogen-13-labeled ammonia was prepared and quality control performed as described previously (*22*).

### PET Studies in Baboons

Adult female baboons were anesthetized and prepared for PET studies as described previously (*23*). Scanning was performed in a Computer Technology Imaging positron tomograph (model 931-08/12; 15 slice, 6.5 mm slice thickness, FWHM with an in-plane resolution of 6.0 × 6.0 mm). The relative myocardial distribution of $^{13}$N-ammonia was measured to determine the homogeneity of blood flow. In the case of the desipramine pretreatment experiment, $^{13}$N-ammonia scanning was carried out before and after injection of desipramine. The same scanning protocol as previously described for racemic 6-[$^{18}$F]FNE (*19*) was followed for all the tracers, including (−)-6-[$^{18}$F]FNE,

(+)-6-[$^{18}$F]FNE and 6-[$^{18}$F]FDA. Vital signs were monitored and recorded throughout the study.

Regions of interest (ROIs), appropriate in size for the resolution of the tomograph, were drawn directly on the transaxial PET images and included the septum and left ventricular wall. These regions were copied, using software supplied with the tomograph, onto the same transaxial slice on every dynamic frame in order to determine if any animal movement had occurred during the scanning interval. This multiplanar method of analysis reduces differences due to changes in position from one study to the next.

### Pretreatment with Desipramine

An injection of (−)-6-[$^{18}$F]FNE, (+)-6-[$^{18}$F]FNE or 6-[$^{18}$F]-FDA was made into the same baboon (n = 2) on different days without drug pretreatment (injection dose: 2–9 mCi; chemical mass: ≈0.17 μg/mCi). These studies also served as controls to determine whether pretreatment with desipramine prior to the administration of the tracers affected the uptake and kinetics of the tracers in the heart. The same two baboons were pretreated intravenously with desipramine (0.5 mg/kg) at 40 min before the injection of either 6-[$^{18}$F]FDA or (−)-6-[$^{18}$F]FNE.

### Plasma Analysis

Arterial blood was sampled every 2.5 sec using an automatic blood sampling device (Ole Dich, Denmark) for the first 2 min and then at 5, 10, 30, 60 min and at the end of study. All samples were centrifuged to obtain plasma which was counted. Duplicate samples at 0, 1, 5, 10, 3z0 and 60 min were assayed for the presence of 6-[$^{18}$F]FDA, (−)-6-[$^{18}$F]FNE or (+)-6-[$^{18}$F]FNE using a slight modification of a solid phase extraction technique (*24,25*). Briefly, plasma was added to 0.5 ml of sodium acetate buffer (0.2 *M*, containing EDTA (500 mg/l) and $Na_2SO_3$ (635 mg/l), pH 8.2) and the mixture was kept on ice before the assay. The entire sample was added to an alumina column (200 mg of activity III alumina, prewashed with sodium acetate buffer). The effluent and subsequent washings from the column (using 1 ml of sodium acetate buffer followed by 1 ml of water) contained all the methylated metabolites [products from reaction with catechol-O-methyltransferase (COMT) or reaction with both COMT and monoamine oxidase (MAO)]. All the metabolites containing the catechol moiety were then eluted from the alumina column by the addition of 3 × 1 ml of 0.5 N acetic acid. The acid eluents were applied to an Alltech cation exchange column. The products from the reaction with monoamine oxidase (MAO) were recovered from both the effluent and first washing (2 ml water) from the Alltech column. The catecholamine (unchanged parent tracer) remained on the cation exchange column.

### Data Analysis

The kinetics of (−)- or (+)-6-[$^{18}$F]FNE and 6-[$^{18}$F]FDA in the myocardium were determined by a number of processes, including neuronal and extraneuronal uptake, metabolism, vesicular storage and release. Since a model including all important processes would be too complex to yield identifiable model parameters, time-activity data for the different tracers and the arterial plasma input function have been analyzed in terms of a response function. The response function is a model independent quantity which represents the tissue response to a unit impulse of tracer (*26–28*). Assuming that the kinetics of tracer doses of 6-[$^{18}$F]-FDA and 6-[$^{18}$F]FNE can be described by compartmental analysis—a series of linear, first order coupled differential equations—the predicted total radioactivity A(t) (the solution to the differential equations) for an ROI can be written as

$$A(t) = g(t) \otimes Cp(t), \qquad \text{Eq. 1}$$

where Cp is the concentration of the tracer in plasma and g(t) [referred to as the response function (RF)] is given by

$$RF = g(t) = \Sigma_i \beta_i e^{-\alpha_i t}, \qquad \text{Eq. 2}$$

and ⊗ denotes convolution. The parameters $\alpha_i$ and $\beta_i$ are the eigen values (exponents) and coefficients, respectively, and are functions of the transfer constants which define the compartmental model (see Reference *29* for the relationship between eigen values and model parameters in a three-compartment model). The response function was approximated as a sum of exponentials (2 or 3) where the exponent and coefficient ($\alpha_i$ and $\beta_i$, respectively) were optimized to give the best fit [A(t)] to the measured tissue radioactivity [ROI(t)]. Since a variable number of exponential terms in Equation 2 were required to fit data from different experiments over the entire time course of the experiment, values for the response function were calculated at discrete times (0.5, 1, 10, 30, 60 and 90 min) after injection to facilitate the comparison of tracer kinetics between experiments. The relationship of the parameter $\alpha_i$ to the half-life of the tracer in tissue is given in Equation 3:

$$t_{1/2} = 0.693/\alpha_i. \qquad \text{Eq. 3}$$

## RESULTS

### Response Function Analysis

Values for the response function and exponents for the PET experiments described in this manuscript are given in Tables 1, 2 and 3. The time versus the natural log of the value of the response function [ln(RF)] are inserted in the plots of time-activity curves (for the same baboon) in Figures 2, 7 and 8. The response functions are model-independent and provide information about the relative amount of tissue uptake in response to a unit input with no input from plasma (after the initial impulse). Since the response functions from different studies are normalized to the same unit input, different studies can be compared directly. In addition, the exponents ($\alpha_i$) can be used to examine the loss of tracer from tissue and to calculate the

**TABLE 1**
Response Function (ml/g/min) for (−) and (+)-6-[$^{18}$F]FNE in Baboon Heart 0.5 to 90 Minutes After Injection

| | | Response function (RF) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Baboon | Study | 0.5 | 1 | 10 | 30 | 60 | 90 | $\alpha_1$ | $\alpha_2$ |
| Leah | (−)FNE | 0.36 | 0.26 | 0.20 | 0.16 | 0.15 | 0.14 | 0.10 | 0.002 |
| | (+)FNE | 0.34 | 0.23 | 0.16 | 0.14 | 0.12 | 0.10 | 0.24 | 0.0054 |
| | Ratio* | 0.95 | 0.88 | 0.80 | 0.88 | 0.80 | 0.71 | 2.4 | 2.7 |
| Peace | (−)FNE | 0.41 | 0.36 | 0.29 | 0.25 | 0.23 | 0.21 | 0.16 | 0.0029 |
| | (+)FNE | 0.39 | 0.28 | 0.19 | 0.15 | 0.12 | 0.10 | 0.16 | 0.0071 |
| | Ratio* | 0.95 | 0.78 | 0.66 | 0.60 | 0.52 | 0.48 | 1.0 | 2.4 |

*Ratio is (+)FNE:(−)FNE.

**TABLE 2**
Response Function (ml/g/min) for 6-[$^{18}$F]FDA in Baboon Heart at Different Times Before and After Pretreatment with Desipramine (DMI) (0.5 mg/kg)

| | | Response function (RF) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Baboon | Condition | 0.5 | 1 | 10 | 30 | 60 | 90 | $\alpha_1$ | $\alpha_2$ |
| Peace | Control | 2.08 | 1.89 | 1.30 | 0.78 | 0.50 | 0.33 | 0.09 | 0.014 |
| | DMI* | 0.86 | 0.56 | 0.32 | 0.20 | 0.15 | 0.12 | 0.11 | 0.008 |
| | Ratio† | 0.41 | 0.30 | 0.25 | 0.25 | 0.30 | 0.36 | 1.2 | 0.57 |
| Carm | Control | 1.55 | 1.52 | 1.12 | 0.75 | 0.53 | | 0.09 | 0.01 |
| | DMI* | 1.40 | 1.09 | 0.74 | 0.52 | 0.43 | | 0.12 | 0.005 |
| | Ratio† | 0.90 | 0.72 | 0.66 | 0.69 | 0.81 | | 1.3 | 0.5 |

*DMI pretreatment at 40 min prior to administration of 6-[$^{18}$F]FDA.
†Ratio is for DMI-to-control.

half-life of tracer in tissue without the continued input from the plasma. Values for the smallest (slowest) two exponents are designated as $\alpha_1$ and $\alpha_2$ and are also reported in the tables. The slowest of these ($\alpha_2$) is the most easily determined (and the most important one over most of the time course of the experiment) and is related to the overall half-life of retention in tissue while the larger of the two ($\alpha_1$) is a measure of more rapid processes.

### PET Studies in Baboons

Comparative PET studies with (−)-6-[$^{18}$F]FNE, (+)-6-[$^{18}$F]FNE and 6-[$^{18}$F]FDA were carried out in the same baboon. There were no significant changes in blood pressure, heart and respiratory rates following the injection of each of the three tracers. This was consistent with the low chemical mass associated with high specific activity 6-[$^{18}$F]FDA and (−) and (+)-6-[$^{18}$F]FNE produced by nucleophilic aromatic substitution reaction with NCA [$^{18}$F]fluoride ion.

There was a longer retention of $^{18}$F activity after injection of (−)-6-[$^{18}$F]FNE than for the (+)-enantiomer (Figs. 2 and 3). For example, 86% of the peak $^{18}$F heart uptake for (−)-6-[$^{18}$F]FNE was retained at 60 min postinjection, in contrast to 62% for (+)-6-[$^{18}$F]FNE (Fig. 2, time-activity curve for baboon Peace). The more rapid clearance of (+)-6-[$^{18}$F]FNE can be seen in the response function values of Table 1 for both Leah and Peace as well as in the time-ln(RF) curves inserted in Figure 2 (baboon Peace). From Table 1, the initial values of the response function are similar (at 0.5 min), but by 90 min, values of (+)-6-[$^{18}$F]FNE are 71% (baboon Leah) and 48% (baboon Peace) of (−)-6-[$^{18}$F]FNE. This more rapid clearance is also reflected in values of the slowest exponent ($\alpha_2$) which for (+)-6-[$^{18}$F]FNE is two to three times greater than for (−)-6-[$^{18}$F]FNE in both studies. There are also individual differences between the two baboons. Peace shows a more rapid washout of (+)-6-[$^{18}$F]FNE than Leah even though $\alpha_1$ is larger for Leah. From the response function itself, the major effect must therefore be in the slower exponent $\alpha_2$ which is greater for Peace.

An even greater difference in kinetics was observed when (−)-6-[$^{18}$F]FNE and 6-[$^{18}$F]FDA were compared in the same baboon. The uptake curves of (−)-6-[$^{18}$F]FNE and 6-[$^{18}$F]FDA (Fig. 4) show an initially higher and quicker uptake, but faster washout in the heart for 6-[$^{18}$F]FDA than for (−)-6-[$^{18}$F]FNE (e.g., 86% of the peak value for (−)-6-[$^{18}$F]FNE remaining in the heart at 60 min postinjection compared to only 37% for 6-[$^{18}$F]FDA). The fast washout of 6-[$^{18}$F]FDA was also demonstrated in Figure 5 by comparing the PET images of heart at 90 min after injection of (−)-6-[$^{18}$F]FNE and 6-[$^{18}$F]FDA. From Tables 1, 2 and 3, the faster clearance of 6-[$^{18}$F]FDA compared to (−)-6-[$^{18}$F]FNE is also evident in the larger value of $\alpha_2$ which is in the range 0.01–0.014 min$^{-1}$ for 6-[$^{18}$F]FDA and 0.0005–0.003 min$^{-1}$ for (−)-6-[$^{18}$F]FNE. From Figure 4, the uptake in %dose/cc at 60 min is greater for (−)-6-[$^{18}$F]FNE than 6-[$^{18}$F]FDA by a factor of ~2. The twofold larger value for the response function (Tables 1 and 2, baboon Peace) for 6-[$^{18}$F]FDA is because the metabolism of 6-[$^{18}$F]FDA is much greater at earlier times (Fig. 6), as reflected in the ratio of integrated plasma radioactivity (6-[$^{18}$F]FDA/(−)-6-[$^{18}$F]FNE) for baboon Peace which is ~0.2 at 60 min (data not shown). Thus, because the response function is the tissue response normalized to the same unit input, the response function for 6-[$^{18}$F]FDA is greater at 60 min even though the uptake (%dose/cc) is less than (−)-6-[$^{18}$F]FNE.

### Appearance Rate of Labeled Metabolites in Plasma

The fraction of $^{18}$F in plasma present as unchanged parent tracer decreased rapidly for (−) and (+)-6-[$^{18}$F]-

**TABLE 3**
Response Function (ml/g/min) for (−)-6-[$^{18}$F]FNE in Baboon Heart at Times from 0.5 to 90 Minutes Before and After Pretreatment with Desipramine (DMI) (0.5 mg/kg)

| | | Response function (RF) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Baboon | Study | 0.5 | 1 | 10 | 30 | 60 | 90 | $\alpha_1$ | $\alpha_2$§ |
| Carm | Control | 0.42 | 0.35 | 0.32 | 0.29 | 0.28 | 0.27 | 0.06 | 0.0005 |
| | DMI* | 0.26 | 0.079 | 0.032 | 0.027 | 0.026 | 0.027 | 0.23 | 0.0005 |
| | Ratio‡ | 0.62 | 0.23 | 0.10 | 0.09 | 0.09 | 0.10 | 3.8 | 1.0 |
| | DMI† | 0.36 | 0.32 | 0.25 | 0.22 | 0.21 | 0.19 | 0.19 | 0.0023 |
| | Ratio‡ | 0.86 | 0.91 | 0.78 | 0.77 | 0.75 | 0.71 | 3.2 | 4.6 |
| Brie | Control | 0.52 | 0.45 | 0.43 | 0.41 | 0.37 | 0.35 | | 0.0026 |
| | DMI† | 0.33 | 0.32 | 0.25 | 0.24 | 0.23 | 0.22 | 0.24 | 0.0011 |
| | Ratio‡ | 0.63 | 0.71 | 0.58 | 0.59 | 0.62 | 0.63 | | |
| Peace | Control | 0.29 | 0.28 | 0.25 | 0.22 | 0.20 | 0.19 | 0.06 | 0.0023 |
| | DMI† | 0.22 | 0.19 | 0.15 | 0.13 | 0.12 | 0.11 | 0.15 | 0.0035 |
| | Ratio‡ | 0.76 | 0.68 | 0.60 | 0.59 | 0.60 | 0.58 | | |

*Forty minutes after single DMI treatment.
†Twenty-four hours after single DMI treatment.
‡Ratio is for DMI-to-control.
§Changes in values of $\alpha_2$ after pretreatment have relatively low significance due to the very slow clearance of (−)-6-[$^{18}$F]FNE from tissue.

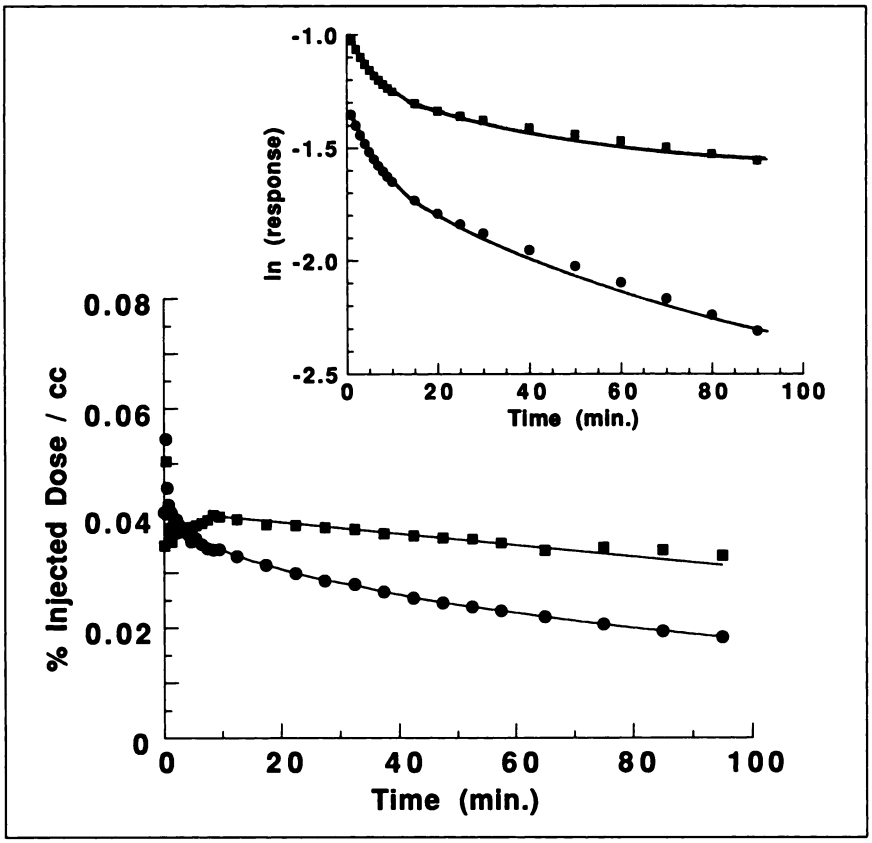


**FIGURE 2.** Uptake and clearance of $^{18}$F after the injection of (−)-6-[$^{18}$F]FNE (squares) and (+)-6-[$^{18}$F]FNE (circles) in baboon heart. The time versus natural log of the response function (ln(Response)) is shown in the insert.

FNE and for 6-[$^{18}$F]FDA. In each case, most of the radioactivity was in noncatechol methylated metabolites. Metabolites appeared more rapidly in plasma for 6-[$^{18}$F]FDA than for (−) and (+)-6-[$^{18}$F]FNE. For example, only 1%–2% of the $^{18}$F remained as parent tracer at 10 min after injection of 6-[$^{18}$F]FDA, in contrast to 28% and 17% remaining after injection of (−) and (+)-6-[$^{18}$F]FNE, respectively (Fig. 6). Since previous studies with tritium-labeled tracers demonstrated that 6-fluoronorepinephrine was barely detectable in plasma at any time point after injection of tritiated 6-fluorodopamine (*30*), it was assumed that the trace amount of 6-[$^{18}$F]FNE in plasma arising from 6-[$^{18}$F]FDA after intravenous injection of 6-[$^{18}$F]FDA would not interfere with the detection of


**FIGURE 3.** Images of the baboon heart (2 levels) at 40 min injection of (−) (top) and (+)-6-[$^{18}$F]FNE (bottom).

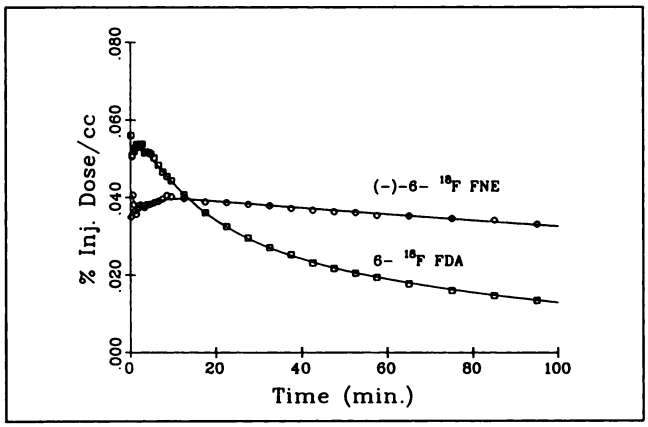


**FIGURE 4.** Uptake and clearance of $^{18}$F after injection of 6-[$^{18}$F]FDA (circles) and (−)-6-[$^{18}$F]FNE (squares) in the baboon heart.

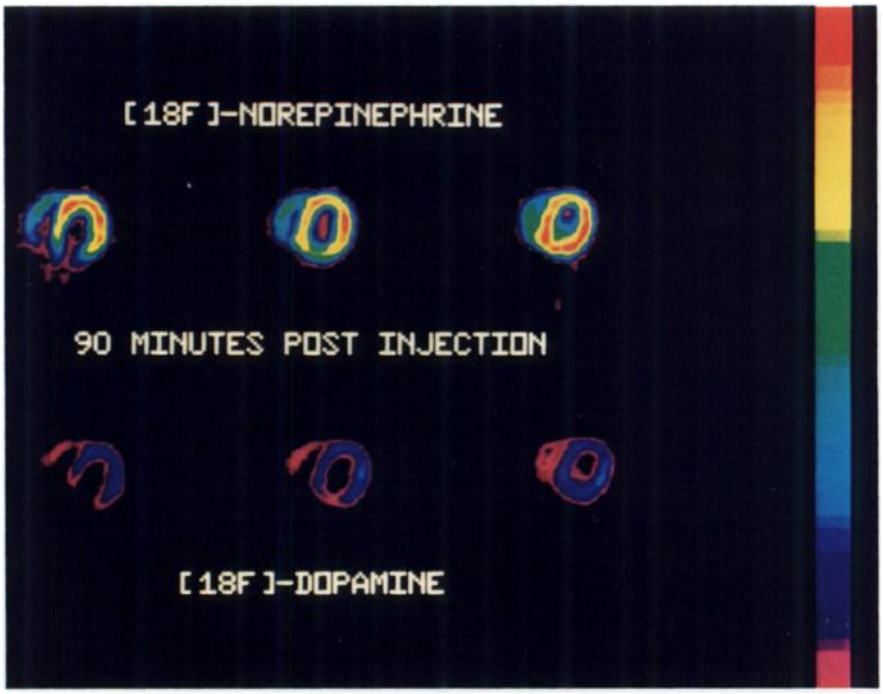


**FIGURE 5.** Images of the baboon heart (3 levels) at 90 min after injection of (−)-6-[$^{18}$F]FNE (top) and 6-[$^{18}$F]FDA (bottom).

6-[$^{18}$F]FDA. Therefore, the same solid extraction technique was applied to determine the percentages of unchanged catecholamines in plasma after injection of 6-[$^{18}$F]FNE and 6-[$^{18}$F]FDA into baboons. The rapid metabolism of 6-FDA was previously reported in rats, where the amount of circulating 6-[$^{3}$H]FDA decreased to less than 2%–3% by 5 min after injection of 6-[$^{3}$H]FDA (*30*).

### Desipramine Pretreatment

Flow measurements in the heart using [$^{13}$N]ammonia before and after intravenous injection of desipramine indicated that there was no significant change in regional myocardial blood flow as a result of this drug intervention (data not shown).

A significant blocking effect was observed with (−)-6-

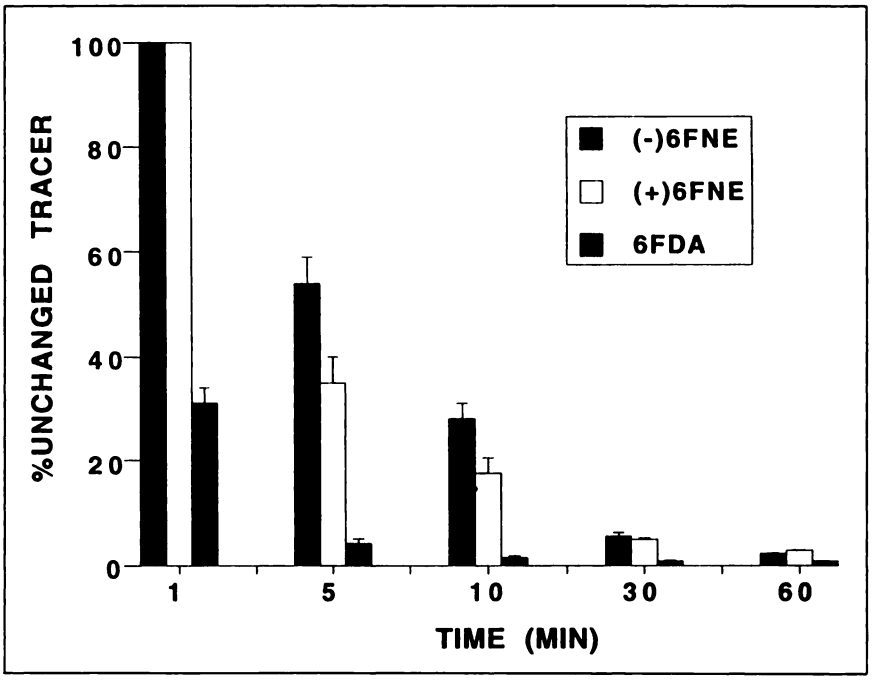


**FIGURE 6.** Percentage of unchanged tracer in plasma for 6-[$^{18}$F]FDA (n = 6), (+)-6[$^{18}$F]FNE (n = 2) and (−)-6-[$^{18}$F]FNE (n = 9) at 1, 5, 10, 30 and 60 min postinjection.

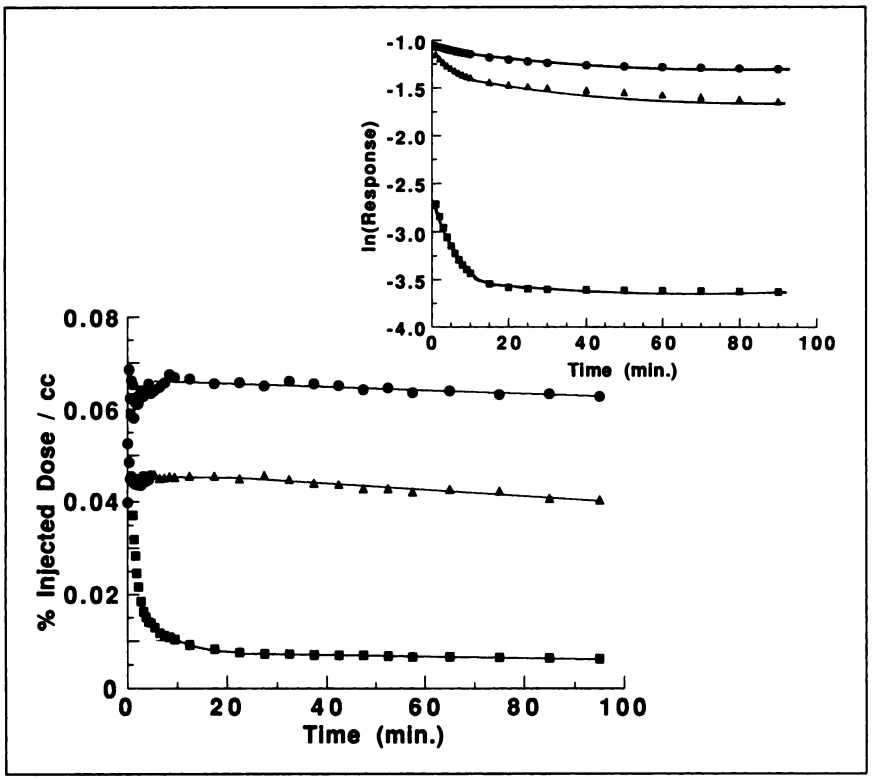


**FIGURE 7.** Uptake and clearance of $^{18}F$ after the injection of (−)-6-[$^{18}F$]FNE in the baboon heart after a single dose of desipramine (0.5 mg/kg); control (circles), pretreatment with desipramine 40 min prior to injection of the radiotracer (squares) and 24 hr after desipramine pretreatment (triangles). The time versus natural log of the response function (ln (Response)) is shown in the insert.

[$^{18}F$]FNE. Fluorine-18 uptake in the heart was almost completely blocked at a dose of 0.5 mg/kg of desipramine (Fig. 7, time-activity curve for baboon Carm). This can also be seen in the decrease in the value of the response function for this study [Table 3 and Fig. 7 (insert)]. The response function ratio (desipramine to control) was 0.1 at 10 min. The decrease in the response function ratio with time corresponds to accelerated loss of tracer relative to control ($\alpha_1$ is approximately four times greater than control).

The measurement of (−)-6-[$^{18}F$]FNE uptake 24 hr after pretreatment with desipramine revealed a 60%–70% recovery of $^{18}F$ uptake in the heart (Fig. 7, baboon Carm). The response functions (Table 3, n = 3) were 60%–80% of control. The initial washout still was accelerated.

Desipramine pretreatment also decreased the uptake and response function for 6-[$^{18}F$]FDA (Fig. 8 and Table 2). From the data in Table 2, one can see that the effect was greater in baboon Peace (maximum reduction in response function was 0.25 at 10 min compared to 0.66 for baboon Carm), even though the administered dose of desipramine was the same (0.5 mg/kg) for both animals. The decrease in the response function ratio at the early time points indicates an initial accelerated washout of 6-[$^{18}F$]FDA, while the increase in the ratio at later times indicates that desipramine slows the overall loss from tissue (smaller $\alpha_2$) so that the clearance half-life for the slow phase was increased by a factor of ~2.

## DISCUSSION

Circulating (−)-norepinephrine undergoes three major presynaptic processes in the cardiac sympathetic neuron: (1) uptake 1, in which it interacts with the norepinephrine reuptake site and is transported into the neuron; (2) storage, in which intraneuronal (−)-norepinephrine is transported into the intrasynaptic vesicle and stored; and (3) release, in which stored (−)-norepinephrine is released into the synapse by exocytosis or leaked from the vesicle into the axoplasm where it is metabolized (*31*). Although (−)-[$^{11}C$]norepinephrine would be the ideal PET tracer for the endogenous substrate, it has only been synthesized in racemic form (*32*). Since neuronal uptake is not stereospecific (*33*), the uptake of (±)-[$^{11}C$]norepinephrine would track that of (−)-norepinephrine. However, the stereoselectivity of (−)-norepinephrine for the vesicular transporter (*34*) would limit the use of the racemic mixture for labeling vesicular storage sites and complicate the interpretation of kinetic data. Additionally the 20.4 min half-life of $^{11}C$ would limit the examination of clearance kinetics. On the other hand, it has been shown that although fluorine substitution in the sixth position of norepinephrine changes its postsynaptic activity (*7*), it does not significantly alter the behavior of norepinephrine at the presynaptic terminal (*5*). Reports that 6-FNE appears to be a valid tracer for the turnover of endogenous NE support the development of a synthetic route to high

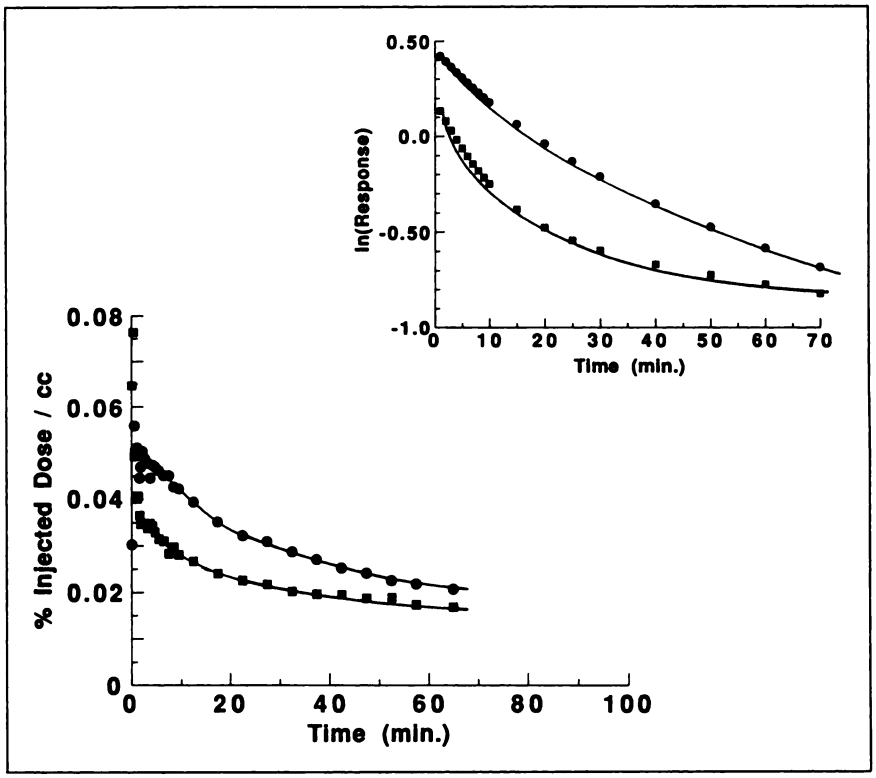


**FIGURE 8.** Uptake and clearance of $^{18}$F after the injection of 6-[$^{18}$F]FDA; control (circles) and pretreatment with desipramine (0.5 mg/kg) at 40 min prior to injection of the radiotracer (squares). The time versus natural log of the response function (ln(Response)) is shown in the insert.

specific activity (−)-6-[$^{18}$F]FNE (*19*). The PET studies described here represent the first biological examination of the individual enantiomer of 6-fluoronorepinephrine and extend previous studies with unlabeled racemic 6-fluoronorepinephrine. Additionally, the ability to compare each enantiomer of 6-fluoronorepinephrine with 6-fluorodopamine in the same baboon provides an important perspective on the behavior of these $^{18}$F-labeled catecholamines.

### Specific Activity

By preparing 6-[$^{18}$F]FNE and 6-[$^{18}$F]FDA using the methods described in this manuscript, a specific activity of 0.7–1.9 Ci/$\mu$mol for 6-[$^{18}$F]FNE and 1–2.5 Ci/$\mu$mol for 6-[$^{18}$F]FDA at EOS was achieved. The chemical mass at EOS using a molecular weight of 187 for 6-[$^{18}$F]FNE and 171 for 6-[$^{18}$F]FDA was calculated to be 0.27–0.1 $\mu$g/mCi (avg. 0.18 $\mu$g/mCi) for 6-[$^{18}$F]FNE and 0.17–0.07 $\mu$g/mCi for 6-[$^{18}$F]FDA (avg. 0.1 $\mu$g/mCi) which was $10^4$ times less than that of 6-[$^{18}$F]FDA prepared by electrophilic fluorination (*10*). The vasopressor potency of 6-FNE is about the same as that of (−)-NE, thus, the mass of 6-FNE injected could be considered to add to the resting mass of norepinephrine in the plasma (0.25 ng/ml, (*4*)). It is known that 2–4 $\mu$g/min of norepinephrine will elicit a pressor response in humans (*35*). Thus, if 5 mCi (1 $\mu$g) were injected as a bolus in 1 min, no pressor effect would be expected.

Even though the present studies were carried out with this high specific activity radiotracer and no hemodynamic effects were detected, the chemical mass introduced is of the same order as the amount of norepinephrine in the coronary arteries. For example, the injection of 5 mCi (1 $\mu$g) of 6-[$^{18}$F]FNE into a 70-kg human subject would give a *transient* peak concentration reaching the heart of 1 ng/ml (assuming that the bolus is diluted to about 1 liter by the time it reaches the heart). Since the myocardial extraction of 6-[$^{18}$F]FNE is very high, it is unlikely that the chemical mass of 6-[$^{18}$F]FNE affected the extraction. Moreover, the present PET measurements of peak heart uptake permit the calculation of the concentration of 6-[$^{18}$F]FNE in nerve terminals (assuming heart uptake represents 6-[$^{18}$F]FNE in nerve terminals) to be about 400 pg/cc for an injected dose of 1 $\mu$g and an uptake of 0.04% dose/cc). This is far below the known concentration of norepinephrine in the human heart (1.04 $\mu$g/g, (*36*)) and thus 6-[$^{18}$F]FNE would be present in tracer concentration.

### Neuronal Uptake

Neuronal uptake is the most important mechanism for terminating the action of (−)-norepinephrine. Thus, the development and validation of tracers for assessing the function of the neuronal reuptake system is of potentially great importance. The ability of (−)-6-[$^{18}$F]FNE to serve as a substrate for neuronal uptake was examined by pretreatment with desipramine, a tricyclic antidepressant drug which is an inhibitor of the norepinephrine reuptake site (*21*). It has been shown in rat tissues (such as the left ventricle, submaxillary gland and spleen) that received

tritiated dopamine alone, desipramine (4 mg/kg i.v.) reduced the accumulation of tritiated dopamine by 60%–90% and tritiated norepinephrine by 73%–94% (*9*). Additionally, recent studies in humans with (−)-[$^3$H]-norepinephrine showed that the human heart is exceptionally dependent on neuronal uptake for in vivo removal of circulating norepinephrine and that uptake is significantly reduced in desipramine treated patients (*3*). In the present studies, desipramine pretreatment reduced myocardial uptake for (−)-6-[$^{18}$F]FNE and 6-[$^{18}$F]FDA. These data are in reasonable agreement with previous PET studies in dogs using carrier-added 6-[$^{18}$F]FDA and pretreatment with a dose of 2 mg/kg of desipramine (*10*). Interestingly, with the relatively low dose of desipramine (0.5 mg/kg) used in the present study, an almost complete blockade of $^{18}$F uptake was observed after the injection of (−)-6-[$^{18}$F]FNE. Studies 24 hr after desipramine treatment also showed considerable blocking of (−)-6-[$^{18}$F]FNE uptake. The greater blocking effect on (−)-6-[$^{18}$F]FNE in comparison to 6-[$^{18}$F]FDA may be due to uptake of 6-[$^{18}$F]FDA by other mechanisms in addition to neuronal uptake and/or a higher selectivity of desipramine for blocking the neuronal uptake of (−)-6-[$^{18}$F]FNE versus 6-[$^{18}$F]FDA. These blocking studies with desipramine and previous studies indicate that both 6-[$^{18}$F]FDA and (−)-6-[$^{18}$F]FNE would be useful tracers for the functional activity of the norepinephrine transporter, although the almost total blockade of (−)-6-[$^{18}$F]FNE uptake in contrast to 6-[$^{18}$F]FDA indicates that the uptake of (−)-6-[$^{18}$F]FNE into the neuron is more selective.

### Stereoselectivity

PET studies of (−)-6-[$^{18}$F]FNE with (+)-6-[$^{18}$F]FNE allow an examination of the compounds' behavior within the presynaptic terminal and a comparison with previous studies of the enantiomers of norepinephrine itself. Previous studies with (−)- and (+)-[$^{14}$C] and [$^3$H]norepinephrine have shown that whereas the neuronal uptake of norepinephrine itself is not stereoselective (*33*), there is a preferential accumulation of (−)-norepinephrine in storage vesicles (*34*). Once (−)-norepinephrine is sequestered within the vesicle, it is protected from metabolic degradation and released from the presynaptic terminal. In contrast, (+)-norepinephrine is not a substrate for the vesicular transporter and is exposed to degradative enzymes like monoamine oxidase within the cytosol. In vivo studies examining the accumulation of (+)- and (−)-[$^{14}$C]norepinephrine in the mouse heart showed that the concentration of the (+)-enantiomer declined more rapidly than the (−)-enantiomer (*37*), although the initial accumulation for the two enantiomers was the same. Pretreatment with reserpine, which inhibits the vesicular transporter (*38*), abolished the stereoselective retention. An examination of mouse heart tissue for $^{14}$C-labeled metabolites revealed that there are more O-methylated metabolites formed from (+)-norepinephrine than from (−)-norepinephrine which is consistent with vesicular storage of the (−)-enantiomer protecting it from metabolism (*37*). Similar results have been reported for the isolated perfused rat heart (*39*) and in tissue slices (*40*).

The present PET studies showing high uptake and concentration in myocardial tissue for both (+) and (−)-6-[$^{18}$F]FNE are consistent with the lack of stereoselectivity of norepinephrine for uptake 1. In addition, the faster washout of $^{18}$F radioactivity for (+)-6-[$^{18}$F]FNE than (−)-6-[$^{18}$F]FNE is consistent with stereoselective vesicular compartmentation as was observed with (−)-norepinephrine (*37*). Analysis of plasma showed more O-methylated metabolites with (+)-6-[$^{18}$F]FNE than with (−)-6-[$^{18}$F]FNE, which is similar to the results from previous measurements of metabolites in mouse heart in vivo using [$^{14}$C]norepinephrine (*37*).

### Comparative Kinetics: (−)- and (+)-6-[$^{18}$F]FNE and 6-[$^{18}$F]FDA

The most striking result from these studies is the significantly more rapid clearance of 6-[$^{18}$F]FDA relative to (−)- and (+)-6-[$^{18}$F]FNE (Figs. 2 and 4). The slow clearance of (−)-6-[$^{18}$F]FNE from the heart is similar to the long retention time of the endogenous neurotransmitter (−)-norepinephrine in intact animals (*37*). The half-lives for the (−)- and (+)-enantiomers in mouse heart in vivo were reported to be 7.6 and 2.5 hr, respectively (*37*). Based on response function analysis, the clearance of (+)-6-[$^{18}$F]FNE corresponds to a half-life of 1.5–2 hr. Because the loss of (−)-6-[$^{18}$F]FNE is so slow, the half-life is less accurately determined, but appears to be greater than 4 hr. Of all the compounds, 6-[$^{18}$F]FDA has the shortest tissue half-life (~1 hr). For 6-[$^{18}$F]FDA, the half-life for the rapid phase ($\alpha_1$) is ~8 min. Half-lives for 6-[$^{18}$F]FDA of 22 and 144 min for the rapid and slow phase (*10*) and 13 and 117 min for the rapid and slow phase (*20*) have been reported in canine and baboon hearts, respectively. The half-lives calculated (based on the response function) are shorter than those calculated from time-activity curves since, in the latter case, the tissue has a continued supply of tracer.

The large difference between the kinetics of (−)-6-[$^{18}$F]FNE and 6-[$^{18}$F]FDA is of relevance in the use of these $^{18}$F-labeled catecholamines as tracers for endogenous norepinephrine, especially with respect to the measurement of norepinephrine turnover in vivo. The relationship of 6-[$^{18}$F]FDA kinetics to discrete biochemical processes is complex because it is a multistep process which requires transport of 6-[$^{18}$F]FDA into the neuron via uptake 1, transport of 6-[$^{18}$F]FDA into the vesicle and enzymatic conversion (via dopamine $\beta$-hydroxylase) of 6-[$^{18}$F]FDA to (−)-6-[$^{18}$F]FNE within the vesicle. Although all of these steps have been shown to take place (*9,30*), the present comparative PET study of 6-[$^{18}$F]FDA and (−)-6-[$^{18}$F]FNE in vivo shows a significantly more rapid clearance of 6-[$^{18}$F]FDA relative to (−)-6-[$^{18}$F]FNE

over 2 hr, indicating that the conversion of 6-[$^{18}$F]FDA to (−)-6-[$^{18}$F]FNE is not rapid. Previous studies also support this. For example, after injection of 6-[$^{3}$H]FDA into rats, the percentage of all the radioactivity in the heart as 6-[$^{3}$H]FNE was assayed to be 8%, 21% and 40% at 5, 20 and 60 min, respectively (*30*). Thus, the PET image after 6-[$^{18}$F]FDA probably represents increasing amounts of (−)-6-[$^{18}$F]FNE over the time course of the study as 6-[$^{18}$F]FDA is converted to (−)-6-[$^{18}$F]FNE and as labeled metabolites clear from tissue. This is also supported by recent mechanistic studies of low specific activity 6-[$^{18}$F]FDA, showing that reserpine did not appreciably change the clearance of $^{18}$F early after the injection of 6-[$^{18}$F]FDA but did affect clearance after 1 hr (*10*).

The more rapid clearance of radioactivity after injection of 6-[$^{18}$F]FDA relative to (−)-6-[$^{18}$F]FNE may be due to a number of factors including inefficient β-hydroxylation of 6-[$^{18}$F]FDA in comparison to dopamine (*9*) (leading to an efflux of 6-[$^{18}$F]FDA from storage vesicles), different reactivities of the two tracers toward the metabolizing enzymes such as MAO and COMT or that 6-[$^{18}$F]FDA may be a poorer substrate for the vesicular transporter, leaving it vulnerable to metabolism by cytosolic MAO. Thus, a major issue in the use of $^{18}$F clearance after the injection of 6-[$^{18}$F]FDA is to distinguish turnover from the loss of 6-[$^{18}$F]FDA which has not been converted to 6-[$^{18}$F]FNE or from the loss of MAO metabolites of 6-[$^{18}$F]FDA. From the present work and from the previous studies, an initial clearance time of at least 60 min may be required to observe clearance which at least partially represents the behavior of (−)-6-[$^{18}$F]FNE. This may vary with different subjects. Moreover, even at 60 min the rate of clearance after 6-[$^{18}$F]FDA is more rapid than that of (−)-6-[$^{18}$F]FNE, indicating that other processes are also taking place. It is important to note that by 1 hr after the injection of 6-[$^{18}$F]FDA, the concentration of radioactivity has declined by a factor of 4 relative to peak uptake of 6-[$^{18}$F]FDA. In contrast, (−)-6-[$^{18}$F]FNE reaches a peak uptake at 10–15 min and shows a very slow decrease over a 2 hr period. At 60 min, the $^{18}$F concentration in the heart is double that of 6-[$^{18}$F]FDA in the same baboon (Fig. 4). Thus, a larger proportion of injected radioactivity appears to be contributing to the vesicular storage pool with (−)-6-[$^{18}$F]FNE.

## SUMMARY

The PET studies reported here for (−)-6-[$^{18}$F]FNE and (+)-6-[$^{18}$F]FNE support and extend previous studies with the racemic mixture (*5*) and suggest a close parallel between the behavior of (−)- and (+)-6-[$^{18}$F]FNE and (−)- and (+)-norepinephrine. However, the kinetic behavior of (−)-6-[$^{18}$F]FNE contrasts with that of 6-[$^{18}$F]FDA which clears rapidly from the heart. The rapid efflux of 6-[$^{18}$F]FDA from the heart early postinjection suggests that the conversion of 6-[$^{18}$F]FDA to (−)-6-[$^{18}$F]FNE is not rapid and that the cardiac radioactivity at early times represents predominantly 6-[$^{18}$F]FDA and/or its metabolites and not vesicular (−)-6-[$^{18}$F]FNE. Although the clearance of $^{18}$F after the injection of 6-[$^{18}$F]FDA contains information relevant to the functional activity of the sympathetic neuron, interpretation of clearance patterns is complicated by the occurrence of multiple processes.

The fact that desipramine, a drug which inhibits norepinephrine reuptake, almost totally blocked the uptake of (−)-6-[$^{18}$F]FNE suggests that its uptake into the myocardium is predominately due to its interaction with the norepinephrine transporter. The uptake of 6-[$^{18}$F]FDA into the myocardium is significantly but only partially blocked by desipramine, suggesting a higher specificity for neuronal uptake for (−)-6-[$^{18}$F]FNE and/or to other mechanisms for accumulation in the heart in addition to neuronal uptake for 6-[$^{18}$F]FDA. These studies also illustrate how even a single dose of desipramine almost completely blocks the reuptake of norepinephrine into the myocardium. Even at 24 hr after a single dose of desipramine, (−)-6-[$^{18}$F]FNE uptake has only returned to 60% of baseline values. The inhibition of norepinephrine reuptake may be one of the mechanisms by which desipramine induces cardiac arrhythmias.

Although the present studies suggest that (−)-6-[$^{18}$F]FNE may be a useful tracer for the parent neurotransmitter, the relationship of the kinetics to physiological processes such as vesicular leakage and exocytosis must be examined in vivo with appropriate pharmacological and physiological interventions and tracer kinetic analysis to establish its utility as a tracer for the turnover of endogenous norepinephrine. Mechanistic studies are underway to characterize the factors which contribute to the kinetic patterns observed for the $^{18}$F-labeled catecholamines. These studies are being carried out with a view toward using these tracers for the assessment of the neuronal activity of the normal and diseased heart as well as understanding the mechanism of action of therapeutic drugs. The fact that there were no hemodynamic effects with a large injected dose (9 mCi) further demonstrates that nucleophilic aromatic substitution is, at present, the only practical route to NCA $^{18}$F-labeled catecholamines in sufficiently high specific activity to avoid measurable hemodynamic effects and the risk of carrier-mediated effects on tracer behavior.

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