With the solution binding constants in hand, we should be able to determine if the allosteric activity differences observed are solely due to the differences in the interactions observed in the X-ray studies or due to the intrinsic nature (activity) of the compounds to shift the allosteric equilibrium. It is possible that two molecules with very similar or identical binding constants can differ markedly in their allosteric effector capabilities. The concept that a molecule's biological effect might arise not only from its binding affinity but incorporate a potency factor (called intrinsic activity) has been well established in receptor research.[27]

Acknowledgment. We gratefully acknowledge grant support for these studies from the National Institutes of Health NIH HLBI-1 RO1 H1 32793. The authors thank Drs. M. F. Perutz and G. Fermi for helpful discussions and for the coordinates of the urea derivatives in series **3**.

Supplementary Material Available: Orthogonal coordinates of compounds **4a-c** and **5a-c** with reference to human deoxyhemoglobin coordinates after applying the translation 16.61, 13.72, 37.65 to *X*, *Y*, and *Z*, respectively, in accordance with the matrix information in the Brookhaven Protein Data Bank (7 pages). Ordering information is given on any current masthead page.

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Synthesis of High Specific Activity (+)- and (-)-6-[^{18}F]Fluoronorepinephrine via the Nucleophilic Aromatic Substitution Reaction

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The first example of a no-carrier-added ^{18}F-labeled catecholamine, 6-[^{18}F]fluoronorepinephrine (6-[^{18}F]FNE), has been synthesized via nucleophilic aromatic substitution. The racemic mixture was resolved on a chiral HPLC column to obtain pure samples of (-)-6-[^{18}F]FNE and (+)6-[^{18}F]FNE. Radiochemical yields of 20% at the end of bombardment (EOB) for the racemic mixture (synthesis time 93 min), 6% for each enantiomer (synthesis time 128 min) with a specific activity of 2-5 Ci/μmol at EOB were obtained. Chiral HPLC peak assignment for the resolved enantiomers was achieved by using two independent methods: polarimetric determination and reaction with dopamine β-hydroxylase. Positron emission tomography (PET) studies with racemic 6-[^{18}F]FNE show high uptake and retention in the baboon heart. This work demonstrates that nucleophilic aromatic substitution by [^{18}F]fluoride ion is applicable to systems having electron-rich aromatic rings, leading to high specific activity radiopharmaceuticals. Furthermore, the suitably protected dihydroxynitrobenzaldehyde 1 may serve as a useful synthetic precursor for the radiosynthesis of other complex ^{18}F-labeled radiotracers.

Positron emission tomography (PET) is an in vivo imaging modality which measures the spatial and temporal distribution of positron emitter labeled compounds and their labeled metabolites in a volume element of living tissue.[1] Although the heart has been extensively studied with PET, it has been examined mainly from the perspective of assessing perfusion and substrate metabolism.[2] However, the neuronal integrity of the heart is also important in assessing cardiac physiology and pathophysiology[3,4] and there has been an interest in probing this function in vivo by using external imaging beginning with the synthesis of [^{11}C]norepinephrine in the early 1970's.[5,6] Recently, interest has intensified with the development of [^{18}F]fluorometaraminol[7] and [^{11}C]*m*-hydroxyephedrine.[8] Both compounds are metabolically stable false neurotransmitters for norepinephrine. These tracers share the same uptake and storage mechanisms as norepinephrine and provide excellent images of the neuronal distribution in dog heart,[9,10] and human heart.[11] Ring-fluorinated catecholamines such as 6-fluoronorepinephrine[12,13] and

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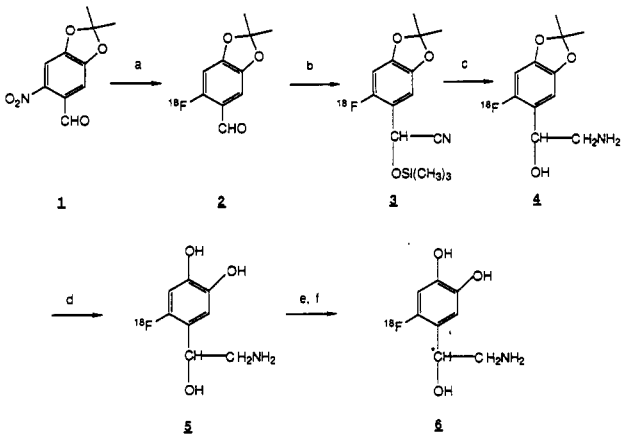

Figure 1. Synthesis and resolution of 6-[^{18}F]FNE. Steps are as follows: (a) K ^{18}F/kryptofix 222; (b) $(CH_3)_3SiCN$, ZnI_2; (c) $LiAlH_4$; (d) HCOOH; (e) semipreparative HPLC; (f) chiral HPLC.

6-fluorodopamine[14,15] have also been synthesized and shown to share the same presynaptic mechanisms for the uptake, storage, and synthesis as the parent molecules. Recently 6-[^{18}F]fluorodopamine has been synthesized via the electrophilic fluorination route and used in PET studies of the canine heart.[16]

In spite of the fact that both [^{18}F]fluorometaraminol and [^{18}F]dopamine display binding properties appropriate to their use in PET studies of myocardial innervation, their vasoactivity which result in hemodynamic effects when these tracers are administered in vivo combine to limit their application in humans.[16,7] Clearly, a route to high specific activity ^{18}F-labeled catecholamines is needed so that the full potential of this class of compounds as neuronal imaging tracers can be objectively assessed. For these reasons, we have examined the feasibility of preparing ^{18}F-labeled catecholamines in high specific activity using [^{18}F]fluoride and nucleophilic aromatic substitution.[17] We report here the synthesis of no-carrier-added (NCA) 6-[^{18}F]FNE and its resolution in practical yields for PET studies. We also report PET studies in the baboon with racemic 6-[^{18}F]FNE.

Chemistry

The synthesis of 6-[F^{18}]FNE is shown in Figure 1. A suitably protected dihydroxy aromatic substrate, 3,4-*O*-isopropylidene-6-nitrobenzaldehyde (**1**), was chosen as the precursor of 6-[^{18}F]FNE in the expectation of achieving an acceptable radiochemical yield for the substitution and easy removal of the protecting group under mild conditions. Table I shows the time taken and relative yield (corrected for decay) for each step as the synthesis progressed. Nucleophilic aromatic substitution of compound **1** by kryptofix/K_2CO_3 activated [^{18}F]fluoride ion in DMSO at 120 °C for 10 min gave a radiochemical yield of 40–45%. The isolated crude product **2** was converted to the corresponding cyanohydrin trimethylsilyl ether **3**, followed by in situ lithium aluminum hydride reduction to give the protected dihydroxy amino alcohol **4**.[12] Quantitative yields

Table I. Time Elapsed and Relative Yield at Various Stages in the Radiosynthesis and Resolution of 6-[^{18}F]FNE

step completed	relative yield, mCi (EOB)	total time, min
[^{18}F]fluoride dried at 120 °C azeotropically with CH_3CN	100	0
fluorination at 120 °C for 10 min, crude product extracted with CH_2Cl_2	40	18
cyanohydrin formation at room temperature for 10 min, solvent evaporated under vacuum	32	40
reduction at 50 °C for 10 min, crude product extracted with CH_2Cl_2	29	62
hydrolysis at 100 °C for 5 min, 6-[^{18}F]FNE collected from semipreparative HPLC	20	93
resolution on chiral HPLC, (+)- and (−)-6-[^{18}F]FNE collected	6 × 2	128

were obtained for both steps. The product was hydrolyzed with 88% formic acid in a closed vessel (fitted with a 0.2-mm tube to release pressure) at 120 °C for 7 min. 6-Nitropiperonal was also examined as a precursor in the synthesis of 6-FNE and although it gave a higher radiochemical yield for the substitution step (51% vs 42%), harsh conditions (heating with strong acid for longer time) were required for the hydrolysis, resulting in decomposition and byproduct formation.

Resolution of Racemic 6-FNE. In principle, enantiomerically pure 6-FNE could be derived from optically active cyanohydrins, prepared through the use of chiral catalysts,[18] enzyme-catalyzed ester cleavage[19] or esterification,[20] or enzyme-catalyzed enantioselective addition of hydrogen cyanide to aldehydes.[21,22] However, these approaches were not suitable for the radiosynthesis of 6-[^{18}F]FNE as, in addition to the poor optical yields and the long reaction times, the rapid, sequential reduction, hydrolysis, and purification steps required for ^{18}F-labeled radiosynthesis would result in racemized product. For these reasons resolution was carried out at the last stage of the purification with use of a chiral HPLC column. After injecting the racemic mixture onto the chiral HPLC column, two fractions with identical amounts of radioactivity and a 5-min difference in retention times were collected. No derivatization was necessary; the separation was efficient (separation factor $\alpha = 1.33$), and the enantiomers were afforded in practical yields with a specific activity of 2–5 Ci/μmol (EOB). For example, on injection of 20 mCi of racemic 6-[^{18}F]FNE, 6 mCi of each enantiomer was obtained. It is important to note that the individual enantiomers of 6-FNE racemize very easily and thus care must be taken to avoid racemization. For example, evaporation of HPLC fractions containing individual enantiomers causes extensive racemization and therefore direct neutralization of the chiral HPLC fraction

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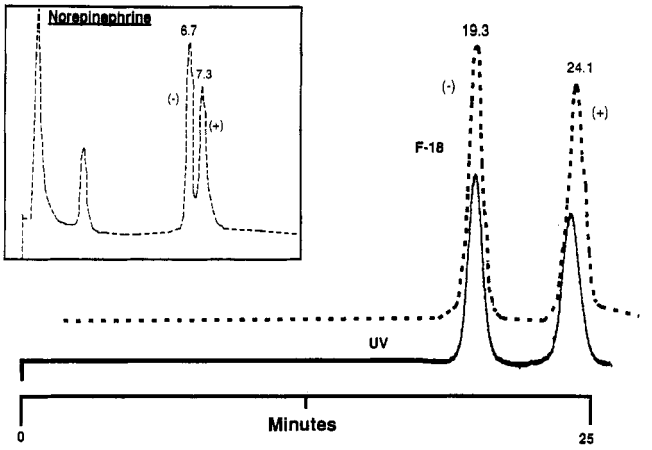

Figure 2. HPLC chromatogram of the resolved enantiomers of 6-FNE obtained by coinjecting the final racemic 6-[^{18}F]FNE reaction mixture (dotted line) with an authentic sample of racemic 6-FNE (solid line). An HPLC chromatogram of the resolved enantiomers of NE is inserted for comparison.

must be used to prepare the tracer for PET studies.

By coinjecting the final racemic 6-[^{18}F]FNE reaction mixture (dotted line in Figure 2) with an authentic sample of racemic 6-FNE (solid line in Figure 2), an HPLC chromatogram of the resolved enantiomers was obtained. It is noteworthy that the separation factor for the enantiomers of 6-fluoronorepinephrine was significantly greater than that for the enantiomers of norepinephrine itself (1.33 vs 1.13; the chromatogram of the resolved enantiomers of NE is inserted in Figure 2 for comparison). The assignment of the two peaks from racemic NE was achieved by coinjecting with an authentic sample of (-)-NE. The first peak (retention time 6.7 min) corresponded to (-)-NE, and therefore the second peak (retention time 7.3 min) was (+)-NE.

Peak Assignment of Resolved 6-FNE Enantiomers. Chiral HPLC peak assignment for the resolved enantiomers of racemic 6-FNE was performed by using two independent methods: polarimetric determination and reaction with dopamine β-hydroxylase. The resolved enantiomers obtained from the chiral HPLC column (max 0.1 mg per injection to obtain baseline resolution) were neutralized and carefully concentrated to a small volume. A sample of the enantiomer having the shorter retention time was submitted for optical rotation analysis (Rudolph Instruments, Inc.). Although the concentration of the sample was too low to give a quantitative value for the optical rotation which could be used to calculate an absolute rotation with the polarimeter system used, the results consistently indicated the substance to be levorotatory (25 trials). Thus, while this system has proven ideal for the rapid efficient resolution of NCA 6-[^{18}F]FNE in practical yields for PET studies, the small capacity of the chiral column and the ease of racemization precluded preparation of large quantities of resolved 6-FNE.

Dopamine β-hydroxylase is known to enantiospecifically convert dopamine to (-)-norepinephrine.[23,24] Two enzyme incubations, with dopamine and 6-fluorodopamine, respectively, were performed simultaneously. In less than 1 h, a peak corresponding to (-)-NE (retention time 6.7 min) appeared when the enzyme mixture which contained dopamine as substrate was injected onto a chiral HPLC column; (+)-NE was not observed (Figure 3). An analogous result was obtained when 6-fluorodopamine was used as a substrate; a new HPLC peak at 18 min appeared (Figure 4). This UV peak coeluted with the first radioactive peak collected from 6-[^{18}F]FNE. Thus the levorotatory isomers of both NE and 6-FNE elute before their respective dextrorotatory enantiomers. This order of

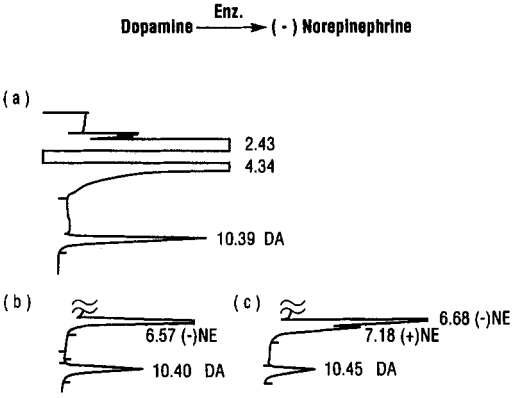

Figure 3. HPLC traces of a reaction mixture resulting from the incubation of dopamine with dopamine β-hydroxylase: (a) reaction at 1 min; (b) reaction at 1 h; (c) coinjection of b with racemic NE.

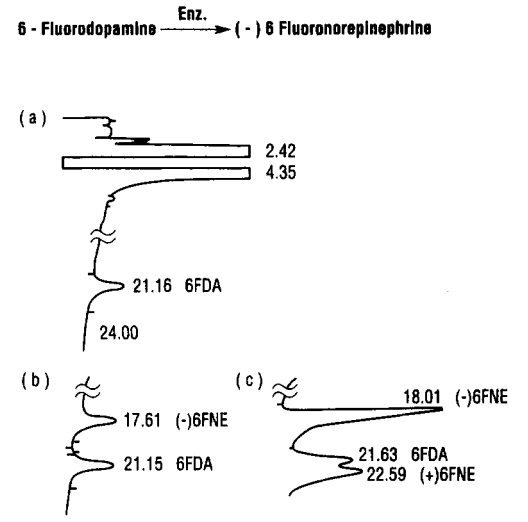

Figure 4. HPLC traces of a reaction mxiture resulting from the incubation of 6-fluorodopamine with dopamine β-hydroxylase: (a) reaction at 1 min; (b) reaction at 1 h; (c) coinjection of b with racemic 6-FNE.

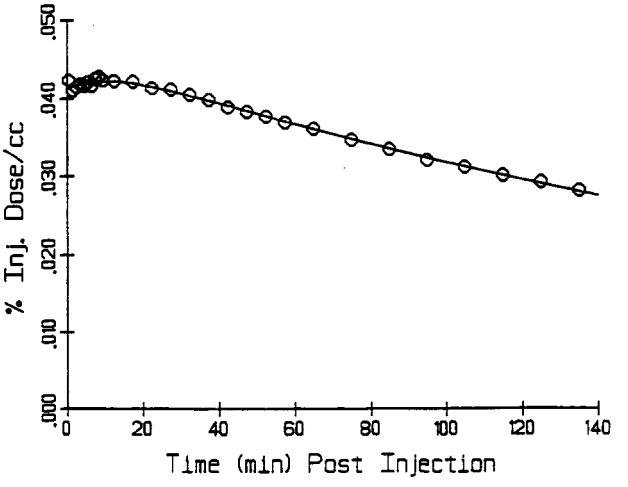

Figure 5. Time-activity curve for F-18 in the baboon myocardium (left ventricular wall and septum) after injection of 5 mCi of racemic 6-[^{18}F]FNE.

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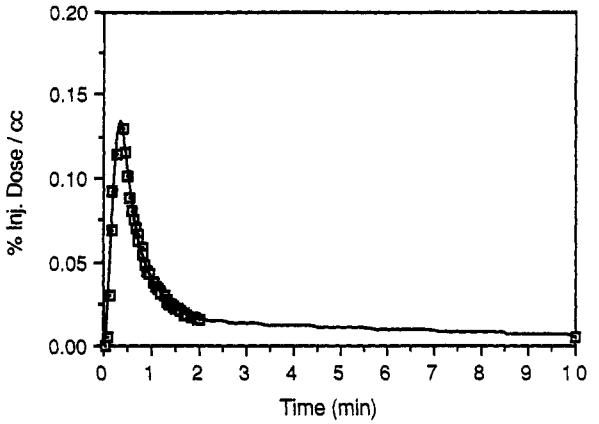

Figure 6. Plasma clearance of F-18 in the baboon after injection of racemic 6-[^{18}F]FNE.

elution was also recently reported for [^{11}C]octopamine enantiomers when a Crownpak CR(+) HPLC column was used.[22]

PET Studies of 6-[^{18}F]FNE in Baboons. PET studies in female baboons (*Papio anubis*) showed high uptake and retention of F-18 in the myocardium (Figure 5) and rapid clearance from blood (Figure 6) after the injection of racemic 6-[^{18}F]FNE. A peak concentration of 0.042% of the injected dose/cc was observed at 10 min post injection. Radioactivity remained at 0.032% of the peak activity by 120 min post injection. The radio of myocardium to plasma was 7 at 5 min and 11.2 at 135 min.

Immediately following completion of the dynamic acquisition, a rectilinear scan (qualitative) was performed beginning at the head and continuing throughout the rostral-caudal extent of the trunk. Radioactivity was distributed in the parotid glands, the heart, liver, and bladder (data not shown). There was no change in vital signs at any time during the study.

Discussion

Fluorine-18, because of its readily availability and relatively long half life and low positron energy, is a very attractive nuclide for PET studies. However, because of the inherent difficulties in the formation of carbon–fluorine bonds, its incorporation into organic molecules and labeling with fluorine-18 remains a challenge.[25] For example, the only methods so far described for introducing F-18 into an aromatic ring bearing the catecholamine moiety require the use of low specific activity electrophilic fluorination reagents derived from F-18 elemental fluorine. The use of [^{18}F]fluoride ion results in an increase in specific activity of a factor of 1000 or greater. Consequently, for equal amounts of radioactivity, the chemical mass of an [^{18}F]F_2 derived radiotracer exceeds than of an [^{18}F]fluoride derived radiotracer by a factor of about 1000. Thus the application of electrophilic fluorination reagents in the synthesis of ^{18}F-labeled compounds for tracer studies in human subjects is limited.

Since the nucleophilic aromatic substitution reaction is currently the only practical route to NCA aryl [^{18}F]-fluorides, we recently examined the feasibility of carrying out this reaction on aromatic systems bearing electron-donating substituents.[17] The synthesis reported here applies this strategy to an aromatic ring bearing a suitably protected catechol and yields 6-FNE in high specific activity and in practical yields for PET studies. Moreover the resolution of the racemic mixture with chiral HPLC proceeds efficiently and in high yield providing for the first time the opportunity to examine the biological behavior of the individual enantiomers of 6-FNE in vivo.

Initial PET studies with racemic 6-[^{18}F]FNE show a high uptake and a slow clearance of radioactivity from baboon heart. While PET measures the concentration of radioactivity and its uptake and clearance in a volume element of tissue, it does not provide information on the chemical form of the radioactivity or the cellular or subcellular substrate to which it is bound. This information must be obtained by direct tissue assay or by measuring the effect of specific pharmacological intervention on the behavior of the tracer in vivo. However, on the basis of these PET studies showing a high uptake and slow clearance of 6-[^{18}F]FNE from baboon heart and previous studies showing that racemic 6-fluoronorepinephrine is taken up and stored by adrenergic nerve terminals,[13] it is likely that the image represents neuronal, and perhaps vesicular 6-[^{18}F]FNE. Although, the individual enantiomers of 6-fluoronorepinephrine have not been studied previously, it is known that the neuronal uptake of norepinephrine itself is not stereoselective whereas only the levorotatory enantiomer serves as a substrate for the vesicular transporter.[26] If 6-fluoronorepinephrine exhibits the same stereoselectivity for vesicular storage as norepinephrine itself, PET studies of the individual labeled enantiomers should provide the potential for assessing vesicular storage. Comparative studies are underway along with serial studies involving pharmacological intervention and the development of methods for rapidly assessing the unchanged tracer in the plasma for future quantitation. The application of nucleophilic aromatic substitution to the synthesis of other ^{18}F-labeled catecholamines such as 6-[^{18}F]fluorodopamine is also being pursued for comparative PET studies in baboons.

Experimental Section

NMR spectra were recorded with a Bruker 300-MHz spectrometer with $CDCl_3$ as solvent and TMS as internal reference. 6-Fluoronorepinephrine was purchased from RBI (Research Biochemicals Inc.). Dopamine and dopamine β-hydroxylase was obtained from the Sigma Chemical Co. HPLC analyses were carried out with a Perkin-Elmer liquid chromatograph equipped with a radioactivity monitor and UV detector. Optical rotation analysis was performed by Rudolph Instruments, Inc. (NJ).

Preparation of 3,4-*O*-Isopropylidene-6-nitrobenzaldehyde (1). Compound 1 was prepared as described previously.[17]

Synthesis of NCA 3,4-*O*-Isopropylidene-6-[^{18}F]fluorobenzaldehyde (2). K^{18}F/kryptofix 222 was prepared as described previously.[17] A solution of compound 1 (10 mg) in 0.3 mL of dry DMSO was added to the dried K^{18}F/kryptofix 222. The mixture was stirred at 120 °C for 10 min, quenched by addition of water (3 mL), and then extracted with CH_2Cl_2 (2 × 3 mL). The CH_2Cl_2 extracts were dried by passing them through a K_2CO_3 column, concentrated, and used directly in the synthesis of 4. The crude product 2 was analyzed by thin-layer chromatography (TLC) (silica, 7:3 hexane/ethyl acetate, R_f = 0.65).

Synthesis of NCA 3,4-*O*-Isopropylidene-6-[^{18}F]fluorophenethanolamine (4). To the residue containing 2 were added 0.3 mL of trimethylsilyl cyanide and approximately 5 mg of zinc iodide. The mixture was stirred at room temperature for 10 min. The excess trimethylsilyl cyanide was then removed in vacuo. The residue was cooled in an ice bath, followed by sequential addition of 0.3 mL of anhydrous ether and 0.5 mL of 1 M lithium aluminum hydride. After refluxing at 50 °C for 10 min, the reaction mixture was cooled in ice and the excess hydride was decomposed by the sequential addition of 0.1 mL of 20% NaOH and 2 mL of H_2O. The resulting mixture was stirred for 1 min and extracted with

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CH_2Cl_2 (2 × 3 mL). Cyanohydrin trimethylsilyl ether **3** and phenethanolamine **4** had R_f values of 0.73 and 0.04, respectively, on TLC (silica, 7:3 hexane/ethyl acetate). The HPLC analysis of compound **4** was performed on a Phenomenex ODS 2 column (CH_3CN/H_2O = 90:10, 0.8 mL/min, 254 nm, retention time = 3.7 min), or on a Phenomenex ODS 1 column (CH_3CN/0.01 M KH_2PO_4 = 65:35, 0.8 mL/min, 254 nm, retention time = 9 min).

Synthesis of NCA (±)-6-[^{18}F]FNE (5). The CH_2Cl_2 extracts containing **4** were evaporated under a stream of nitrogen. Formic acid (88%, 1 mL) was added to the residue, and the mixture was heated in a closed vessel (fitted with a 0.2-mm tube to release pressure) at 100 °C for 5 min. The solvent was evaporated and the residue was dissolved in 1.5 mL of 2.5% CH_3COOH. The labeled racemic 6-[^{18}F]FNE was purified by a semipreparative HPLC (Phenomenex ODS1 Column, 25 × 1.0 cm) eluting with 2.5% CH_3COOH (2.7 mL/min, 254 nm). The fraction eluting at 8 min was collected and concentrated by rotary evaporation. The product was identified on a Phenomenex ODS1 or ODS2 column (25 × 0.46 cm) under the following conditions: 2.5% CH_3COOH, 0.8 mL/min or CH_3CN/0.01 M KH_2PO_4 = 65:35, pH 3.14, 0.8 mL/min. The radioactive peak corresponding to 6-[^{18}F]FNE had the same retention time as an authentic unlabeled sample which had been added as carrier. Radiochemical purity was also assayed by TLC (silica; $BuOH/H_2O/HOAc/EtOAc$ = 1:1:1:1) in the presence of unlabeled 6-FNE as carrier. 6-FNE was visualized with ninhydrin and radioactivity corresponded to the carrier spot (R_f = 0.69). Radiochemical purity was 98%.

Resolution of NCA (±)-6-[^{18}F]FNE. The residue obtained upon evaporation of solvent was dissolved in 0.5 mL of 0.02 M $HClO_4$. The (+) and (-) forms of the NCA 6-[^{18}F]FNE were separated on an analytical scale chiral column (Daicel Crownpak CR(+), 15 × 0.46 cm). The following conditions were used: flow, 0.7 mL/min; solvent, 0.02 M $HClO_4$; UV detector, 254 nm. Under these conditions the retention times for (-)- and (+)-6-[^{18}F]FNE were 19.3 and 24.1 min, respectively. The total volume for each fraction was about 2 mL. Each fraction was neutralized with sterile 4.2% $NaHCO_3$ to pH 4, followed by passing through a sterile 0.22-μm filter into a sterile, pyrogen-free injection vial. Specific activity, determined by HPLC analysis (comparing the UV response of a known amount of radioactivity to a standard curve), was about 2–5 Ci/μmol (EOB).

Dopamine β-Hydroxylase Reaction. (a) Dopamine Hydroxylation. A mixture of 0.5 mg of dopamine, potassium phosphate buffer (0.3 mL, pH 5.7), 0.5 M ascorbic acid (0.02 mL, pH 5.7), 1 M sodium formate (0.05 mL), and dopamine β-hydroxylase solution (0.05 mL) was stirred in a closed vial at 30 °C. The cap was removed and the vial was shaken periodically. An aliquot (ca. 1 μL) was withdrawn and subjected to chiral HPLC analysis as the reaction progressed. HPLC conditions were as follows: Crownpak CR(+) column, 15 × 0.46 cm, 254 nm, 0.02 M $HClO_4$, 0.7 mL/min. The retention times for dopamine, (-)-NE, and (+)-NE were 10.4, 6.6, and 7.3 min, respectively.

(b) 6-Fluorodopamine Hydroxylation. The same procedure was used as described as for a except 0.4 mg of 6-fluorodopamine was used as substrate. The retention times for 6-fluorodopamine, (-)-6FNE, and (+)-6FNE were 21.1, 18.0, and 22.6 min, respectively, under the same HPLC conditions.

PET Studies in Baboons. Adult female baboons were anesthetized and prepared for PET studies as described previously.[27] A solution of racemic 6-[^{18}F]FNE (5 mCi, 0.4 μg) in 3 mL of saline was injected intravenously. Scanning was performed for 140 min in a Computer Technology Imaging (CTI) positron tomograph (model 931-08/12; 15 slice, 6.5-mm slice thickness, full width at half maximum (FWHM)) with an in-plane resolution of 6.0 × 6.0 mm (FWHM). The following scanning protocol was used: ten 30-s scans, followed by five 60-s scans, followed by ten 5-min scans, followed by eight 10-min scans. In every case, an initial transmission scan was performed 2.0 min prior to radiotracer injection in order to determine the proper position of the animal in the gantry. Upon completion of this scan, adjustments in position were made as necessary and a regular transmission scan (10 min) was performed in order to correct for the attenuation of the annihilation photons. The tomograph was cross calibrated with the well-counter used for the measurement of radioactivity in the plasma samples. A rectilinear scan was performed by placing the baboon's head in the gantry and moving through the rostral caudal extent of the animal (10-mm increments) to include the bladder at the most caudal level. Scanning was performed for 1.5 minutes at each position. Vital signs including heart and respiratory rate were monitored and recorded throughout the length of the study.

The time–activity curve for F-18 after the injection of 6-[^{18}F]FNE was generated from a region of interest over the left ventricular wall and the septum.

Acknowledgment. This research was carried out at Brookhaven National Laboratories under contract DE-AC02-76CH00016 with the U.S. Department of Energy and support by its Office of Health and Environmental Research and also supported by the National Institutes of Health, Grant No. NS-15380. The authors thank Dr. Kenneth Kirk for a sample of 6-fluorodopamine. They are also grateful for the advice and assistance of David Schlyer, Bernard Bendriem, Robert MacGregor, Payton King, Karin Karlstrom, Elizabeth Jellett, Colleen Shea, Robert Carciello, and Babe Barrett.

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