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Enzymatic Synthesis of (-)-6-[^{18}F]-Fluoronorepinephrine from 6-[^{18}F]-Fluorodopamine by Dopamine β-Hydroxylase

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ABSTRACT

The visualization and investigation of the sympathetic innervation of the heart with positron emission tomography (PET) has become a new and active area in nuclear cardiology. The radiofluorinated amine (-)-6-[^{18}F]-fluoronorepinephrine ((-)-6-[^{18}F]FNE) has been suggested as a potential radiotracer for imaging the sympathetic nervous system and providing kinetic information regarding the utilization of the native adrenergic neurotransmitter norepinephrine. In this study, it was found that (-)-6-[^{18}F]FNE can be synthesized stereospecifically from 6-[^{18}F]-fluorodopamine (6-[^{18}F]FDA) under *in vitro* conditions through the catalytic action of the enzyme dopamine β-hydroxylase (DβH), which is responsible for the *in vivo* biosynthesis of endogenous norepinephrine from dopamine. The estimated K_m and V_{max} values for the enzymatic activity of DβH on 6-[^{18}F]FDA and on dopamine were 0.87 ± 0.07 mM, 0.011 ± 0.001 μmoles/unit/min, and 1.4 ± 0.3 mM, and 0.079 ± 0.017 μmoles/unit/min

Key Words: Positron Emission Tomography (PET), Dopamine β-hydroxylase, (-)-6-[^{18}F]-Fluoronorepinephrine

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respectively. The differences between the K_m and V_{max} values of the two substrates were both statistically significant at $p<0.05$. Provided that 6-[^{18}F]FDA with a sufficiently high specific activity is available, results from this study suggests that the enzymatic conversion of 6-[^{18}F]FDA into (-)-6-[^{18}F]FNE by DβH may be used to synthesize (-)-6-[^{18}F]FNE suitable for intravenous administration into humans. Furthermore, this study also opens up the possibility of exploiting the low substrate specificity of DβH for the synthesis of other ^{11}C- or ^{18}F-labeled biogenic phenylethanolamines in their natural enantiomeric form for PET studies.

INTRODUCTION

Sympathetic nervous system is one of the two major autonomic nervous systems controlling and regulating various involuntary activities in the body (1). Abnormal sympathetic adrenergic activities have been implicated in the pathogenesis of various diseases, including hypertrophic cardiomyopathy, congestive heart failure, myocardial ischemia, and infarction (2-7). Despite improved pharmacotherapeutic management of these clinical disorders over the past years, acute arrhythmias and sudden death contribute significantly to the mortality of these patients (8-10). Difficulties in the direct *in vivo* assessment of adrenergic activities have impeded attempts to elucidate the role of the sympathoneural system in the pathogenesis of the above clinical conditions. Recent development of several non-catecholaminergic analogues of norepinephrine labeled with positron-emitting radioisotopes, such as 6-[^{18}F]-fluorometaraminol and [^{11}C]-hydroxyephedrine, has allowed visualization of the adrenergic innervation of the heart with the positron emission tomographic (PET) technology (11-12). Unfortunately, they provide little information on the function of the native neurotransmitter (-)-norepinephrine. In comparison, (-)-6-[^{18}F]-fluoronorepinephrine ((-)-6-[^{18}F]FNE) may mimic native norepinephrine, and thus may offer a more truthful method for studying the adrenergic neurotransmission process.

The radiofluorinated (-)-6-[^{18}F]FNE has been shown to accumulate in adrenergic nerve terminals of the baboon heart (13-15). These studies also strongly suggested that (-)-6-[^{18}F]FNE

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closely followed the metabolism of endogenous norepinephrine *in vivo*. Therefore, together with PET, (-)-6-[^{18}F]FNE has the potential for not only visualizing sites of sympathetic innervation, but also assessing the sympathetic tone under various physiological or pathological conditions.

At present, the only known method for synthesizing high specific activity (-)-6-[^{18}F]FNE is the aromatic nucleophilic substitution method developed by Ding et al., 1991 (14). This method is not stereospecific, and therefore, requires chiral separation of the two enantiomers towards the end of the synthesis. Recently, several *in vivo* studies have strongly suggested that the enzyme dopamine β-hydroxylase (DβH) (E.C. 1.14.17.1) converts 6-[^{18}F]FDA stereospecifically to (-)-6-[^{18}F]FNE (13,16-17). This was confirmed by Ding et al., 1991, who demonstrated *in vitro* the enzymatic conversion of unlabelled 6-fluorodopamine into (-)-6-fluoronorepinephrine by DβH (14). However, the extent and the kinetics of this enzymatic reaction have not been previously reported. Therefore, it was not known whether this enzymatic reaction would proceed under *in vitro* conditions at a rate fast enough to be exploited as a synthetic method for producing (-)-6-[^{13}F]FNE for clinical use. The present study compares the kinetics of the enzymatic action of DβH on 6-fluorodopamine and dopamine. More importantly, the possibility of using isolated DβH for the stereospecific synthesis of (-)-6-[^{18}F]FNE for human administration is explored.

MATERIALS

6-[^{18}F]-Fluorodopamine was synthesized as previously described by Chirakal et al., 1996 (18). The specific activity of the 6-[^{18}F]FDA preparation used in this study was approximately 2 to 4 mCi/μmole. Unlabelled 6-fluorodopamine was kindly provided by Dr. K. Kirk (Laboratory of Chemistry, National Institutes of Health, Bethesda, Maryland). Catalase (from bovine liver) (E.C. 1.11.1.6), dopamine β-hydroxylase (from bovine adrenals) (E.C. 1.14.17.1), dopamine HCl, (±)-norepinephrine, (±)-norepinephrine HCl, (-)-norepinephrine HCl, 1-octanesulfonic acid (sodium salt), fumaric acid (disodium salt), and tetrahydrofuran (HPLC grade) were purchased from Sigma Chemical Co. (St. Louis, MO). Trifluoroacetic acid (99%) was purchased from

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Aldrich Chemical Co., Inc. (Milwaukee, WI). L-ascorbic acid (AnalaR grade), anhydrous diethyl ether (Assured grade), disodium EDTA (Assured grade), glacial acetic acid (Assured grade), anhydrous sodium acetate (ACS Assured grade), sodium hydroxide (Assured grade), trichloroacetic acid (ACS Assured grade), and triethylamine (Assured grade) were purchased from BDH Inc. (Toronto, ON). (±)-6-Fluoronorepinephrine HCl was purchased from Research Biochemicals International (Natick, MA). Hydrochloric acid (Certified A.C.S. grade) and o-phosphoric acid (85%) (Certified ACS grade) were purchased from Fisher Scientific Company: Chemical Manufacturing Division (Fair Lawn, NJ). Acetonitrile (HPLC grade) and hexanes were purchased from Caledon Laboratories Ltd. (Georgetown, ON). Methanol (distilled) was purchased from EM Science (Gibbstown, NJ), and sterile water for injection USP was purchased from Baxter Corporation (Toronto, ON).

METHODS

Enzymatic Synthesis of (-)-6-[^{18}F]FNE

6-[^{18}F]FDA was incubated in an open sterile depyrogenized reaction vessel at 37°C with constant stirring in a 2 M sodium acetate buffer (pH 5.5) containing ascorbic acid, 40 mM; sodium fumarate, 40 mM; catalase, 2 mg/mL; and DβH, 1.21 units/mL (1 unit converts 1.0 μmol of tyramine to octopamine per min at pH 5.0 at 37 °C). The final volume of the reaction mixture was 2 mL. For the time profile experiments, 50 μL samples were taken out from the reaction mixture at various times during incubation. Following incubation, 6 N HCl (1 mL) was added to the reaction mixture to precipitate the proteins. Then, 1 N NaOH (4 mL) was added slowly to bring the pH of the solution up to the range of about 4 to 5.5. The reaction mixture was then passed through a series of 25mm diameter 0.45μm and 0.22μm syringe filters into a sterile evacuated vial. Samples were then taken for HPLC analysis.

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Quantitative Assay for 6-[^{18}F]FNE

Fifty-μL samples of the filtered 6-[^{18}F]FDA reaction mixture were loaded onto a high performance liquid chromatography (HPLC) column (Varian Spherisorb® 5μ ODS(2) 80 Å; 250 x 4.6 mm, Harbor City, CA) with a flow rate set at 1.0 mL/min. Sixty 1-mL fractions of the HPLC eluate were collected into separate tubes by an automatic fraction collector. The tubes were then put into an automatic gamma-counter (MINAXI Auto-gamma 5000 Series, Canberra Packard Canada) and each tube was counted for 1 minute. The counts of the fractions which corresponded to the 6-[^{18}F]FDA and 6-[^{18}F]FNE peaks were summed up. All the counts were corrected for the decay time of ^{18}F, the environmental and chromatographic background counts, and the counts of the corresponding peaks of the blank samples. The quantities of 6-[^{18}F]FDA and 6-[^{18}F]FNE in the injected 50-μL sample were then determined by dividing the net peak count by the specific activity of the substrate corrected for the decay time of ^{18}F and the efficiency of the gamma-counter. The rate of production of 6-[^{18}F]FNE was then calculated by dividing the total amount of 6-[^{18}F]FNE produced by the quantity of enzyme and the time of incubation.

Kinetics

Different concentrations of the substrates, dopamine (0.5 to 8 mM) or 6-fluorodopamine (0.25 to 4 mM), were incubated in open plastic tubes at 37 °C with constant agitation for 15 to 30 minutes in a 2 M sodium acetate buffer (pH 5.5) containing ascorbic acid, 4 mM; sodium fumarate, 10 mM; catalase, 500 μg/mL; and DβH 0.302 unit/mL. The final volume of the reaction mixture was 1 mL. The enzyme reaction was stopped by the adding 3 M trichloroacetic acid (100 μL). The reaction tube was then capped, centrifuged at 3000 rpm for 10 minutes to precipitate the proteins. The supernatant was filtered through a 0.22 μm filter. A substrate blank sample and an enzyme blank sample were prepared in each experiment by excluding the substrate and the enzyme from the incubation mixture respectively.

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Quantitative Assay for Norepinephrine and 6-Fluoronorepinephrine

Fifty-μL samples of the filtered reaction mixtures were injected into the ODS HPLC system. The mobile phase was adapted from Chiueh et al., 1983 (11), with slight modifications. It contained per liter of aqueous solution: 1-octanesulfonic acid 1.3 g, disodium EDTA 0.1 g, triethylamine 6 mL, 85% o-phosphoric acid 6 mL, and acetonitrile 40 mL. The flow rate was set at 1.5 mL/min. The UV absorbance of the effluent at 280 nm was monitored (Waters Associates Model 440 Absorbance Detector, Mississauga, ON), and recorded with a stripchart recorder (Brinkmann/Sybron Model BR-100). The capacity factor (k') and area were calculated for each peak. The quantity of norepinephrine or 6-fluoronorepinephrine present in each of the 50 μl samples could then be read from an external standardization curve prepared beforehand by loading known amounts of norepinephrine or 6-fluoronorepinephrine onto the HPLC column. The total amount of norepinephrine or 6-fluoronorepinephrine produced was then calculated by correcting for the volume ratio between the total sample volume and the injection volume. Dividing the total amount of norepinephrine or 6-fluoronorepinephrine produced by the quantity of enzyme and the time of incubation would then give the rate of norepinephrine or 6-fluoronorepinephrine production in μmoles/unit/min.

Determination of K_m and V_{max} values

The K_m and V_{max} values of the conversion of the two substrates by DβH to their respective products were determined for each separate experiment by applying Eadie-Hofstee linear transformation to the Michaelis-Menten equation. Least-square linear regression analysis was applied to obtain the best fit line for the transformation. The average and standard deviation of the K_m and V_{max} values derived from separate experiments were then calculated for each substrate. Statistical significance of the differences between the estimated K_m and V_{max} values for the two substrates was determined by applying the Student's t test for unpaired data. A p value of <0.05 was considered statistically significant.

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Chiral HPLC

Chiral HPLC was used to check the optical purity and enantiomeric identity of the 6-[^{18}F]FNE synthesized from 6-[^{18}F]FDA through the enzymatic action of DβH. In order to separate the optical isomers of 6-[^{18}F]FNE, a mobile phase which was hydrophobic in nature was required. Therefore, various aqueous authentic and test samples of 6-fluoronorepinephrine had to be first extracted into organic solvents before they could be loaded onto the chiral HPLC column. Extration was done by first titrating the pH of the aqueous solutions of the catecholamines with 1 N NaOH or 100 mg/mL $NaHCO_3$ to within the range of 7 to 7.5. The catecholamines were then extracted into diethyl ether. The ether layer of the extract was evaporated to dryness under a stream of nitrogen gas. The residue was then redissolved in the mobile-phase solution which was to be used for chiral HPLC analysis. It contained per liter: tetrahydrofuran, 250 mL; methanol, 75 mL; trifluoroacetic acid, 2.5 mL; and hexanes, 672.5 mL. Aqueous solutions of authentic (±)-norepinephrine HCl and (-)-norepinephrine HCl were also extracted by using the same technique into the mobile-phase solution just described. These extracts were then chromatographed on a chiral HPLC column (Phenomenex Chirex® column; model 3017; (S)-proline and (S)-1-(α-naphthyl)ethylamine packing; 150 x 4.6 mm; Torrance, CA) at a flow rate of 1.0 mL/min. UV absorbance at 280 nm of the eluate was monitored and recorded. The distance travelled by the absorbance peaks, their heights, and the capacity factors (k') were then measured and calculated.

RESULTS

6-[^{18}F]FDA as a substrate of DβH

When a sample of the incubation mixture was injected into the ODS HPLC system, a distinct UV absorbance peak was observed. This peak was absent in the chromatograms of the blank samples in which either the enzyme or the substrate was excluded. The capacity factor (k') of this peak was identical to that of authentic 6-fluoronorepinephrine within experimental error.

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Moreover, a single enhanced peak was observed when a mixture of the reaction mixture and the authentic 6-fluoronorepinephrine sample was injected. These two peaks, in fact, were superimposable upon each other (Table 1). This showed that 6-fluoronorepinephrine was present in the incubation mixture post-incubation. DβH catalyzed the conversion of 6-[^{18}F]FDA to produce 6-[^{18}F]FNE *in vitro* (Figure 1).

Time Profile

Figure 2 shows that 1 μmole of 6-[^{18}F]FDA was totally converted to 6-[^{18}F]FNE over a period of 2 hours. The [DβH]/[6-[^{18}F]FDA] ratio was 2 units/μmole. At 1 hour into the reaction, more than 95% of 6-[^{18}F]FDA had been converted into 6-[^{18}F]FNE. Because of this, an one-hour incubation time could be used for all subsequent enzymatic synthesis of 6-[^{18}F]FNE from 6-[^{18}F]FDA.

Table 1. Identification of the presence of 6-[^{18}F]FNE in the reaction mixture containing DβH and 6-[^{18}F]FDA. Samples of authentic (±)-6-fluoronorepinephrine and reaction mixture containing 6-[^{18}F]FDA and DβH were injected into the ODS HPLC system, alone and in combination. (mobile phase flow rate: 1.5 mL/min, UV wavelength: 280 nm, recording scale: 0.5 AUFS, recording paper advancing speed: 30 cm/hr). The distance travelled, height, and base width of the UV absorbance peak which was either known or suspected to represent 6-fluoronorepinephrine were measured. The capacity factor and the peak area were then calculated.

HPLC Injections	Capacity Factor (k')	Peak Area (mm²)
a) 0.5μg of authentic (±)-6-fluoronorepinephrine	3.70 ± 0.14	130 ± 10
b) 10μl of reaction mixture containing 6-fluorodopamine and DβH after incubation	3.62 ± 0.14	192 ± 15
c) A combination of a) and b)	3.65 ± 0.14	329 ± 25

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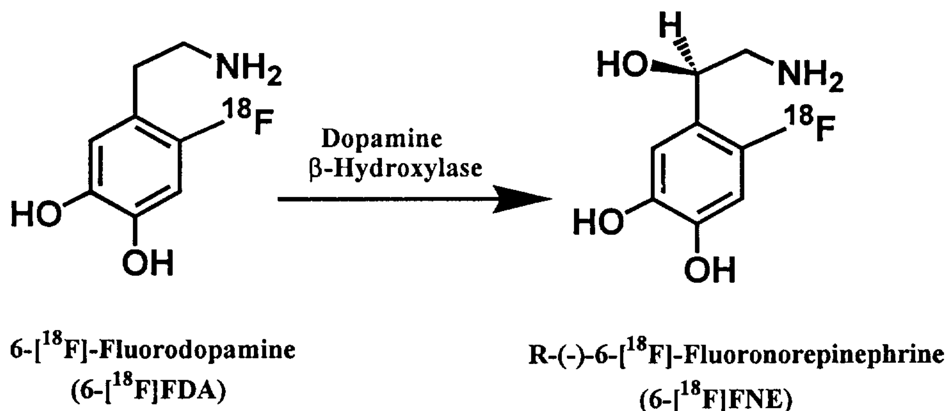

Figure 1. Enzymatic conversion of 6-[^{18}F]-fluorodopamine to (-)-6-[^{18}F]-fluoronorepinephrine by dopamine β-hydroxylase.

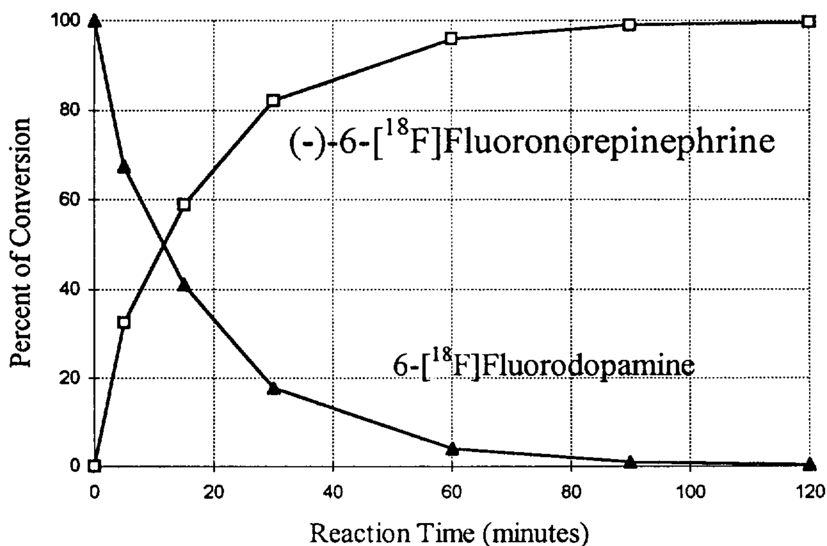

Figure 2. Enzymatic production of 6-[^{18}F]-fluoronorepinephrine. 1 μmole of 6-[^{18}F]FDA was incubated in the reaction mixture at 37 °C with a [DβH]/[6-[^{18}F]FDA] ratio of 2 units/μmole. Samples of the reaction mixture were taken at various times for up to 120 minutes. The 6-[^{18}F]FDA and 6-[^{18}F]FNE contents in these samples were analyzed by HPLC. The percentages of each of 6-[^{18}F]FDA and 6-[^{18}F]FNE relative to the total amount of the two compounds in the reaction mixture samples were plotted as a function of time.

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Kinetics

The estimated K_m and V_{max} values for the enzymatic action of DβH on dopamine and 6-[^{18}F]FDA are summarized on Table 2. The differences between the K_m and V_{max} values for the two substrates were both statistically significant at $p < 0.05$. Figures 3 and 4 show a typical Eadie-Hofstee plot for each of the substrates. Between 3% to 38% of dopamine and 2% to 11% of 6-fluorodopamine were converted to their respective products by DβH in these kinetic experiments.

Radiochemical Purity

Samples of the 6-[^{18}F]FNE preparation were injected into the HPLC column. The flow rate of the mobile phase was set at 1.0 mL/min. Radioactivity counting of successive 1-mL HPLC eluate fractions revealed a single prominent peak of radioactivity followed by a much smaller, but observable peak (Figure 5). The prominent peak represented the desired product 6-[^{18}F]FNE. (This was confirmed by co-injecting the 6-[^{18}F]FNE sample with an excessive amount of non-radioactive authentic 6-fluoronorepinephrine, and monitoring the UV absorbance peak.) The main radiochemical contaminant in the preparation was represented by the small second peak, which

Table 2. Estimated K_m and V_{max} values for the enzymatic action of DβH on dopamine and 6-[^{18}F]FDA. For each substrate, the K_m and V_{max} values listed represent the average ± standard deviation from three separate determinations.

Substrate	K_m (mM)	V_{max} (µmole/unit/min)	$k = V_{max}/K_m$ (ml/unit/min)
6-[^{18}F]FDA	0.87 ± 0.07	0.011 ± 0.001	0.013
Dopamine	1.4 ± 0.3	0.079 ± 0.017	0.056

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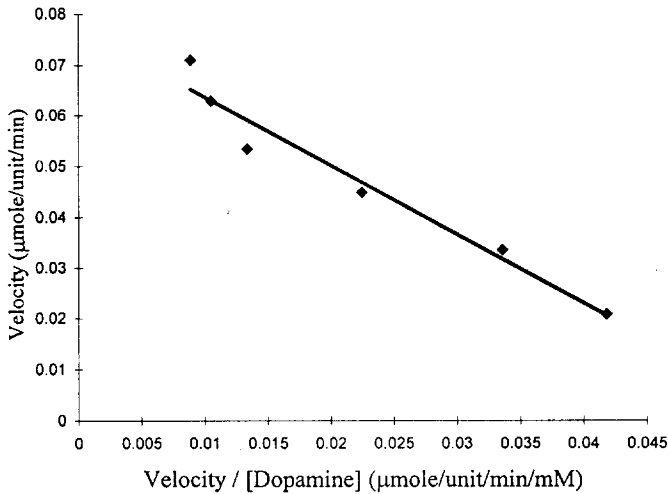

Figure 3. Eadie-Hofstee plot of enzymatic conversion of dopamine to norepinephrine. The reaction mixture consisted of a 2 M sodium acetate buffer (pH 5.5) containing 4 mM ascorbic acid, 10 mM sodium fumarate, 500 μg/mL catalase, and 0.302 unit/mL of DβH at 37 °C.

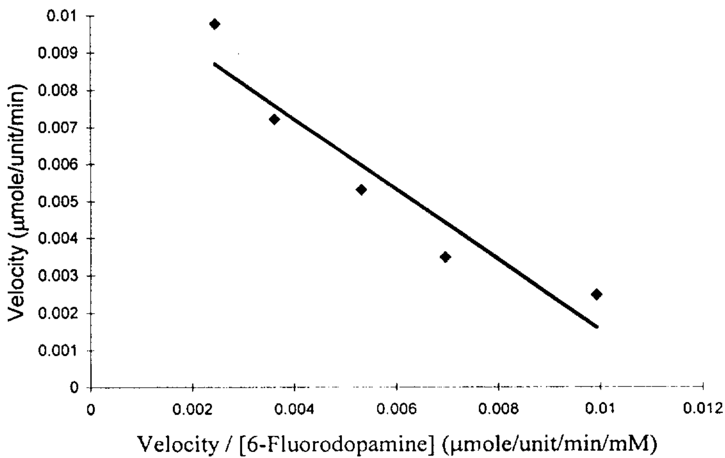

Figure 4. Eadie-Hofstee plot of enzymatic conversion of 6-[^{18}F]FDA to 6-[^{18}F]FNE. The reaction mixture consisted of a 2 M sodium acetate buffer (pH 5.5) containing 4 mM ascorbic acid, 10 mM sodium fumarate, 500 μg/mL catalase, and 0.302 unit/mL of DβH at 37 °C.

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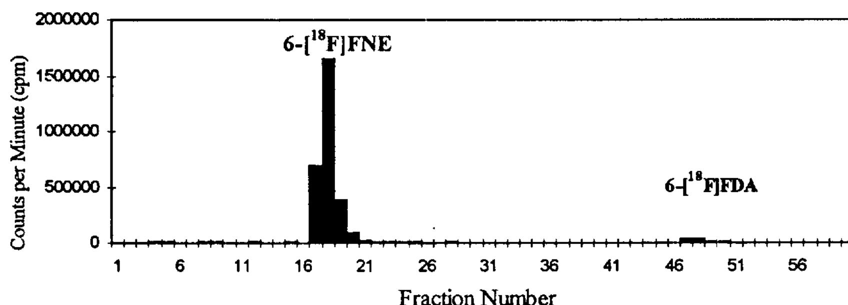

Figure 5. Radiochemical Purity of 6-[^{18}F]FNE Preparation. A sample of the 6-[^{18}F]FNE preparation was loaded onto the ODS HPLC column with the mobile phase flow rate at 1.0 mL/min. Sixty 1-mL fractions were collected into separate tubes by an automatic fraction collector. The collected fractions were then counted for radioactivity.

was identified to be 6-[^{18}F]FDA. (This was confirmed by spiking the radioactivity peak with an excessive amount of authentic 6-[^{18}F]FDA.) The radiochemical purity of the 6-[^{18}F]FNE preparation was above 95%.

Optical Purity and Enantiomeric Identity

Injection of an extracted sample of enzymatically synthesized 6-[^{18}F]FNE onto the chiral HPLC column resulted in a single UV absorbance peak. This was in contrast to the pair of partially resolved peaks which was obtained from an injection of authentic (±)-6-fluoronorepinephrine. The single peak of the 6-fluoronorepinephrine test sample was superimposable upon the earlier peak of the twin peaks from the authentic sample of racemic 6-fluoronorepinephrine (Table 3). This showed that 6-[^{18}F]FNE produced from the enzymatic reaction was of a single enantiomeric form. No enantiomeric impurities were detected. Taking into account of the minimal detectable quantity of the separate enantiomers, which was estimated to be approximately 100 nmol, the enzymatically produced 6-[^{18}F]FNE preparation had an optical purity of at least 90%.

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Table 3. Optical purity of 6-[^{18}F]FNE synthesized from 6-[^{18}F]FDA by DβH. Extracted samples of authentic (±)-6-fluoronorepinephrine and the 6-[^{18}F]FNE isolated from the DβH enzymatic reaction mixture were loaded onto the chiral HPLC column, alone and in combination. (Mobile phase flow rate: 1.0 mL/min, UV wavelength: 280 nm, recording scale: 0.05 AUFS, recording paper advancing speed: 30 cm/hr). The height and the distance travelled by the UV absorbance peaks were measured. The capacity factor of the peaks were then calculated.

	(+)-6FNE Peak		(-)-6FNE Peak	
HPLC Injections	Capacity Factor (k')	Peak Height (mm)	Capacity Factor (k')	Peak Height (mm)
a) 0.3mL of extracted 6-[^{18}F]FNE from reaction mixture	---	0	9.5	9.0
b) 10μl of extracted authentic (±)-6FNE	10.3	17.0	9.6	18.9
c) Combination of a) & b)	10.4	16.5	9.6	26.0

In order to find out the enantiomeric identity of the enzymatically synthesized 6-[^{18}F]FNE, it would be ideal to have an authentic sample of optically pure (S)-(+)- or (R)-(-)-6-fluoronorepinephrine. Unfortunately, isolated enantiomers of 6-fluoronorepinephrine were not commercially available. What was commercially available, however, was an optically pure sample of (R)-(-)-norepinephrine. Injection of a sample of extracted (R)-(-)-norepinephrine into the chiral HPLC column produced a single UV absorbance peak. Parallel to the observation for 6-fluoronorepinephrine, a HPLC injection of an authentic sample of (±)-norepinephrine also

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produced a pair of incompletely separated peaks. The earlier one of the twin peaks was superimposable upon the single peak obtained from optically pure (R)-(-)-norepinephrine (Table 4). This established that the (R)-(-)-enantiomer of norepinephrine was eluted from the chiral HPLC column before the (S)-(+)- enantiomer. By analogy, the same should also be true for 6-fluoronorepinephrine because the extra fluorine atom on the aromatic ring of 6-fluoronorepinephrine does not alter the chemical nature of the functional groups at the chiral center. Therefore, the first eluted peak from racemic 6-fluoronorepinephrine should represent the

Table 4. Enantiomeric identity of the chiral HPLC chromatographic peaks of (±)-norepinephrine. Extracted samples of authentic (±)-norepinephrine and (-)-norepinephrine were loaded onto the chiral HPLC column, alone and in combination. (Mobile phase flow rate: 1.0 mL/min, UV wavelength: 280 nm, recording scale: 0.05 AUFS, recording paper advancing speed: 30 cm/hr). The height and the distance travelled by the UV absorbance peaks were measured. The capacity factor of the peaks were then calculated.

	(+)-Norepinephrine ((+)-NE) Peak		(-)-Norepinephrine ((-)-NE) Peak	
HPLC Injections	Capacity Factor (k')	Peak Height (mm)	Capacity Factor (k')	Peak Height (mm)
a) 20μl of 1:10 dilution of extracted authentic (-)-NE	---	0	10.4	22.7
b) 15μg of authentic (±)-NE	11.2	24.9	10.3	27.1
c) Combination of a) & b)	11.1	23.3	10.3	57.1

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(R)-(-)-enantiomer, while the later peak would represent the (S)-(+)-enantiomer. Since the enzymatically synthesized 6-[^{18}F]FNE sample produced a peak which was superimposable upon the first eluted peak of racemic 6-fluoronorepinephrine, it could be inferred that the 6-[^{18}F]FNE manufactured using DβH was of the (R)-(-)-enantiomeric form.

The present study confirms previous results by Ding et al., 1991, who demonstrated qualitatively by using optical rotation analysis that a sample of unlabelled 6-fluoronorepinephrine produced enzymatically through the catalytic action of DβH was optically active and had a tendency towards the levorotatory orientation (14). In both studies, the low concentration of the enzymatically produced sample precludes a more quantitative measurement of the optical rotation.

Radiochemical Yield and Specific Activity

Given that 6-[^{18}F]FDA was available, the time required for the synthesis of 6-[^{18}F]FNE was about 70 minutes. The radiochemical yield of the enzymatic conversion step, which was defined as the ratio of the radioactivity of the product at the end of the synthesis process to the radioactivity of the substrate at the beginning of the enzymatic reaction, was 42.9 ± 1.6 %. The specific activity of the 6-[^{18}F]FNE preparation at the end of synthesis (EOS) was 1.7 ± 0.3 mCi/μmole.

DISCUSSION

The present study demonstrates that DβH converts the un-natural substrate 6-[^{18}F]FDA stereospecifically into (-)-6-[^{18}F]FNE. This result is not surprisig because DβH does not show a high degree of substrate specificity *in vitro* (19-20). The kinetic constants for 6-[^{18}F]FDA are, however, quite different from those of the native substrate dopamine. The presence of fluorine at the 6-position of the aromatic ring of 6-[^{18}F]FDA has apparently increased the affinity (lower K_m) of DβH for this substrate. Conversely, the capacity (V_{max}) of DβH for 6-[^{18}F]FDA was found to be lower than that for dopamine. The well known ability of fluorine to form strong hydrogen bonds may be the reason for the observed differences in both kinetic constants, K_m and V_{max}, for

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the enzymatic action of DβH on the two substrates. If one assumes that the fluorine substitution is responsible for the increase in the affinity of the fluorinated substrate, 6-[^{18}F]FDA, for the enzyme, it then follows, that the enzyme-product complex ((-)-6-[^{18}F]FNE-DβH complex) cannot dissociate as readily. This, in turn, leads to a reduction in the capacity of DβH to work on 6-[^{18}F]FDA when compared to dopamine.

The decay-corrected yield was 67% for the 70-minute synthesis process. During that time, DβH converts more than 95% of 6-[^{18}F]FDA into (-)-6-[^{18}F]FNE in a remarkably clean reaction. The losses in the practical yield apparently occur during the isolation of (-)-6-[^{18}F]FNE from the reaction mixture. The precipitation of DβH traps some (-)-6-[^{18}F]FNE, and at the same time eliminates traces of protein in the final solution.

The enzymatic method for synthesizing (-)-6-[^{18}F]FNE has a potential advantage over the method described by Ding et al., 1991 (14), which is the only method currently available for producing high specific activity (-)-6-[^{18}F]FNE. Their method is not stereospecific, and requires the use of chiral HPLC to isolate (-)-6-[^{18}F]FNE from the chemically produced racemic mixture of (±)-6-[^{18}F]FNE. Our stereospecific method eliminates the need for the chiral HPLC separation step.

The specific activity of (-)-6-[^{18}F]FNE preparation made in this study (1.7 mCi/μmol) is rather low, and is not suitable for human use. For human administration, it is important to minimize the vasopressor pharmacological effect of (-)-6-[^{18}F]FNE. The hemodynamic effects of (-)-6-[^{18}F]FNE administered intravenously depends on both the total chemical mass of the injection as well as the rate of administration. Although there is no direct pharmacological data on the vasopressor effect of (-)-6-[^{18}F]FNE in humans, several previous animal studies have shown that (-)-6-fluoronorepinephrine has a vasopressor potency approximately equal to that of (-)-norepinephrine (21-23). It is also known that an intravenous infusion of (-)-norepinephrine at a rate of less than 0.012 μmole per minute does not normally trigger a pressor response in humans (15,24). Therefore, it can be reasonably inferred that the vasopressor effect of (-)-6-[^{18}F]FNE can be minimized by administering a maximum of 0.012 μmole of the radiotracer over a period of

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at least one minute. Assuming that 5 mCi of the radiotracer will be injected into the patient, (-)-6-[^{18}F]FNE synthesized with a specific activity of approximately 420 mCi/µmole or higher should prove to be useful for human studies.

In order to apply the enzymatic method for synthesizing high specific activity (-)-6-[^{18}F]FNE, a source of high specific activity starting substrate 6-[^{18}F]FDA is necessary. At present, the nucleophilic aromatic substitution method described by Ding et al., 1991, is the only one capable of producing 6-[^{18}F]FDA with a specific activity as high as 1000 to 2500 mCi/µmole (25). Starting with a commercially available substrate, 6-nitropiperonal, and nucleophilically reactive ^{18}F-fluoride, this synthesis requires 105 minutes and has a radiochemical yield of 20%. In comparison, up to now, electrophilic radiofluorination is only capable of producing low or medium specific activity 6-[^{18}F]FDA (13,18,26). A new approach to overcome this handicap and to produce electrophilically high specific activity ^{18}F-labeled compounds has recently been demonstrated by Solin and Bergman, who synthesized and subsequently used high specific activity [^{18}F]F_2 gas from aqueous ^{18}F-fluoride (27). By applying their method to the synthesis of 6-[^{18}F]FDA, one can expect a specific activity comparable to that achieved through the nucleophilic substitution process developed by Ding et al., 1991 (25). The conversion of high specific activity 6-[^{18}F]FDA by DβH would then afford an optically pure (-)-6-[^{18}F]FNE preparation with a specific activity fit to be used safely in humans (>420 mCi/µmol).

Our study has provided one example of exploiting the low substrate specificity of DβH (19-20) for small scale, stereospecific radiopharmaceutical synthesis. A large number of ^{11}C- or ^{18}F-labeled phenylethylamines can be expected to be β-hydroxylated to their corresponding (-)-phenylethanolamines. Direct enzymatic β-hydroxylation enlarges the armament of radiopharmaceutical synthesis techniques to create the natural enantiomeric form of labeled biogenic amines.

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