Sensitivity of [^{11}C]Phenylephrine Kinetics to Monoamine Oxidase Activity in Normal Human Heart

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Phenylephrine labeled with ^{11}C was developed as a radiotracer for imaging studies of cardiac sympathetic nerves with PET. A structural analog of norepinephrine, (−)-[^{11}C]phenylephrine (PHEN) is transported into cardiac sympathetic nerve varicosities by the neuronal norepinephrine transporter and stored in vesicles. PHEN is also a substrate for monoamine oxidase (MAO). The goal of this study was to assess the importance of neuronal MAO activity on the kinetics of PHEN in the normal human heart. MAO metabolism of PHEN was inhibited at the tracer level by substituting deuterium atoms for the two hydrogen atoms at the α-carbon side chain position to yield the MAO-resistant analog D2-PHEN. **Methods:** Paired PET studies of PHEN and D2-PHEN were performed in six normal volunteers. Hemodynamic and electrocardiographic responses were monitored. Blood levels of intact radiotracer and radiolabeled metabolites were measured in venous samples taken during the 60 min dynamic PET study. Myocardial retention of the tracers was regionally quantified as a retention index. Tracer efflux between 6 and 50 min after tracer injection was fit to a single exponential process to obtain a washout half-time for all left ventricular regions. **Results:** Although initial heart uptake of the two tracers was similar, D2-PHEN cleared from the heart 2.6 times more slowly than PHEN (mean half-time 155 ± 52 versus 55 ± 10 min, respectively; $P < 0.01$). Correspondingly, heart retention of D2-PHEN at 40–60 min after tracer injection was higher than PHEN (mean retention indices 0.086 ± 0.018 versus 0.066 ± 0.011 mL blood/min/mL tissue, respectively; $P < 0.003$). **Conclusion:** Efflux of radioactivity from normal human heart after uptake of PHEN is primarily due to metabolism of the tracer by neuronal MAO. Related mechanistic studies in the isolated rat heart indicate that vesicular storage of PHEN protects the tracer from rapid metabolism by neuronal MAO, suggesting that MAO metabolism of PHEN leaking from storage vesicles leads to the gradual loss of PHEN from the neurons. Thus, although MAO metabolism influences the rate of clearance of PHEN from the neurons, MAO metabolism is not the rate-determining step in the observed efflux rate under normal conditions. Rather, the rate at which PHEN leaks from storage vesicles is likely to be the rate-limiting step in the observed efflux rate.

Received Jun. 9, 1997; revision accepted Apr. 12, 1998.

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Key Words: ^{11}C-phenylephrine; monoamine oxidase; norepinephrine transporter; sympathetic nervous system; PET

J Nucl Med 1999; 40:232–238

In an effort to provide clinicians with a noninvasive method to regionally assess cardiac sympathetic nerve status in the living human, several radiolabeled analogs of norepinephrine have been developed for nuclear medicine studies of cardiac sympathetic innervation (*1–4*). Our laboratory recently produced (−)-[^{11}C]phenylephrine (PHEN) as an agent for investigating the integrity of cardiac sympathetic nerves with PET (*5*). PHEN is transported into sympathetic neurons by the neuronal norepinephrine transporter (uptake-1) and stored in vesicles by the vesicular monoamine transporter (*6*). PHEN is also a substrate for monoamine oxidase (MAO).

Initial clinical evaluation of PHEN demonstrated that it clears from the normal human heart with a mean half-time of 59 ± 5 min (*7*). This finding, along with results of mechanistic studies in the isolated rat heart (*8*), suggests that the rate of clearance of PHEN from the heart is influenced by neuronal MAO activity. To assess the importance of MAO activity on the kinetics of PHEN in the normal human heart, an analog of PHEN was made with deuterium atoms substituted for the two hydrogen atoms at the α-carbon of the side chain of PHEN, [^{11}C]-(−)-α-α-dideutero-phenylephrine (D2-PHEN) (*9*). Substitution of deuterium at the α-carbon of an MAO substrate is known to slow the rate of metabolism of the substrate (*10,11*).

We report here the results of paired PET imaging studies with PHEN and D2-PHEN in normal volunteers. These studies have demonstrated that the kinetics of PHEN are sensitive to neuronal MAO activity. However, other processes involved in the neuronal handling of norepinephrine, such as vesicular storage function and leakage from vesicles, are also important in determining the observed kinetics of PHEN in the human heart.

MATERIALS AND METHODS

Radiotracer Preparation

The chemical structures of norepinephrine, PHEN and D2-PHEN are shown in Figure 1. The radiochemical syntheses for PHEN and D2-PHEN have been reported previously (*5,9*). Specific activities exceeded 500 Ci/mmol with typical radiochemical yields >50% end of synthesis. The product was dissolved in sterile isotonic sodium phosphate saline solution.

PET Imaging

The study protocol was reviewed and approved by the Institutional Review Board of the University of Michigan Medical Center. Six normal volunteers (four women, two men; mean age 39 ± 13 y; range 25–55 y) were recruited and underwent tandem studies with PHEN and D2-PHEN. On the basis of asymptomatic status and normal resting electrocardiogram, all subjects had low likelihood of hemodynamically important coronary heart disease or prior myocardial infarction. None of the subjects had any evidence of diabetes mellitus or hypertension, and none were taking any antihypertensive or other cardioactive drugs including beta-blockers, angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists, calcium channel blockers, digoxin or antidepressants. For five of the six volunteers, resting myocardial perfusion was measured with a dynamic [^{13}N]ammonia PET study. None of the subjects were smokers. All volunteers gave written informed consent before participation in the studies. Heart rate and blood pressure were monitored continually throughout the study using a blood pressure cuff placed around the subject's upper arm (the same arm used for venous blood sampling) and a Marquette Model 7010 rate-pressure monitor (Marquette Medical Systems, Milwaukee, WI).

A Siemens/CTI ECAT931 15-slice whole-body tomograph (Siemens Medical Systems, Hoffman Estates, IL) was used for all PET imaging studies. A 22-gauge intravenous cannula was placed in an antecubital vein to establish an injection port. A 20-gauge intravenous cannula was placed in the antecubital vein of the other arm to provide a port for venous blood samples. A scout image was acquired after injection of 74 MBq (2 mCi) of [^{13}N]ammonia to aid in positioning the patient's heart within the PET camera's field of view. A 15-min transmission scan was acquired using a retractable ^{68}Ge ring source for attenuation correction of the emission data. Data acquisition was started as approximately 740 MBq (20 mCi) [^{13}N]ammonia were administered as a bolus injection. A 15-min dynamic imaging sequence was used (20 image frames, frame rates: 12 × 10 s, 6 × 30 s, 2 × 300 s). Approximately 45 min after the end of the dynamic [^{13}N]ammonia scan, 740 MBq (20 mCi) PHEN or D2-PHEN were administered intravenously as a bolus injection. The dynamic imaging sequence lasted 1 h (23 image frames, frame rates: 12 × 10 s, 2 × 60 s, 2 × 150 s, 2 × 300 s, 2 × 600 s, 1 × 1200 s). The same imaging protocol was used after injection of approximately 740 MBq (20 mCi) of the other tracer. The order in which the two tracers were injected was chosen randomly. A delay of at least 60 min between the two studies was imposed to allow myocardial levels of ^{11}C to decay and clear to negligible levels. For the PHEN and D2-PHEN studies, blood samples (3 mL each) were drawn at 1, 5, 10, 20, 40 and 60 min after tracer injection for the determination of radiolabeled metabolites and intact radiotracer in blood using a previously reported method (*7*).

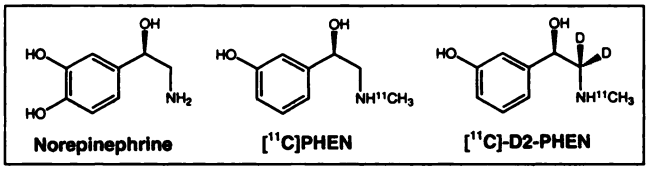

FIGURE 1. Chemical structures of norepinephrine, PHEN and D2-PHEN.

After initially positioning the subject inside the PET scanner for the [^{13}N]ammonia scan the subject was marked using a laser positioning device integral to the camera. Subjects were repositioned if needed before each subsequent scan. If repositioning was needed, a new transmission scan was performed at this time. For subject motion during a given PET scan, the image analysis software allowed correction of in-plane motion when deriving the time-activity curves; however, this was not needed for any of the scans included in this study.

Image Reconstruction

The emission data were attenuation corrected and reconstructed using filtered backprojection. A Hann filter with a cutoff of 0.3 cycles/pixel was used, yielding an in-plane resolution of 12.5 mm in the reconstructed images. The transaxial reconstructed images were transferred to a SUN workstation (SUN Microsystems, Mountain View, CA), formatted into three-dimensional volume data and resliced into 12 short-axis sections, including the entire left ventricle from apex to base (slice thickness ~0.8 cm). Multiple vertical long-axis and horizontal long-axis sections were also produced.

Image Processing

Polar maps of left ventricular radioactivity concentrations were generated as reported previously (*7*). Each polar map was constructed from 8 short-axis slices covering the left ventricle from apex to base. Each left ventricular slice was divided circumferentially into a set of angular sectors. For the [^{13}N]ammonia blood-flow studies, 8 sectors/slice were used for a total of 64 sectors in the polar map. For the PHEN and D2-PHEN scans, 60 sectors/slice were used for a total of 480 sectors. Time-activity curves for each sector were generated and stored for later quantitative analyses. Arterial blood time-activity data were obtained directly from the PET images by placing a 5 × 5 pixel region of interest over the left ventricular chamber in the most basal plane of the 12-plane short-axis dataset. For PHEN and D2-PHEN, the arterial blood time-activity data were corrected for metabolites. This was accomplished by first performing a cubic spline interpolation of the estimated fractions of intact radiotracer in blood onto a 1-s time grid. The metabolite-corrected blood time-activity curve was then obtained by multiplying the total blood PET counts by the value of the estimated fraction of intact tracer at the midtime for each image frame.

Flow Estimation and Retention Index Calculation

Regional estimates of resting myocardial blood flow were estimated from the [^{13}N]ammonia time-activity data using the method of Hutchins et al. (*12*). Myocardial retention of PHEN and

retention of D2-PHEN were quantitatively assessed using a retention index (RI, mL blood/min/mL tissue) as described previously (*7*). A retention index represents the tissue concentration of tracer (C_{tissue}) in a given image frame (with start time T_1 and stop time T_2) normalized to the integral of the blood concentration of intact tracer (C_{blood}) from the start of the study out to time T_2.

$$RI = \frac{C_{tissue}(T_1:T_2)}{\int_0^{T_2} C_{blood}}$$

The RI for the final frame of the dynamic imaging sequence (40–60 min) was calculated for each left ventricular region of interest (i.e., each sector in the polar map).

Washout Kinetics

The time-activity data between image frame midtimes of 6 and 50 min were fit to a single exponential as a measure of the washout of PHEN and D2-PHEN from the heart during the study. The estimated washout rate constants were averaged over all sectors in the polar map to estimate a global mean washout rate, expressed as a mean washout half-time.

Statistical Analysis

Data are expressed as the mean ± 1 SD. Statistical comparisons between PHEN and D2-PHEN were made using the paired 2-tailed *t* test. For other significance tests, the unpaired 2-tailed *t* test was used. In both cases, $P < 0.05$ was considered significant.

RESULTS

Physiological Effects of Tracer Injection

Injection of PHEN or D2-PHEN caused no significant changes in heart rate or blood pressure recorded during the study. No untoward symptoms were reported by any volunteer.

Blood Levels of PHEN and D2-PHEN

The mean rate of D2-PHEN degradation in blood was 25% slower than that of PHEN (Table 1; $P < 0.05$). This may be due to a slower rate of radiotracer metabolism systemically in MAO-containing organs such as the liver, heart and lung (*13,14*).

Tracer Quantification

For the five subjects who underwent dynamic [^{13}N]ammonia imaging, estimated resting myocardial blood flows (0.72 ± 0.15 mL/min/g) were within reported normal ranges (*12,15*). The mean RI values for all six normal volunteers and the mean washout half-times of PHEN and D2-PHEN are given in Table 2. Individual global mean RI values for each normal volunteer are plotted in Figure 2. Individual global mean washout half-times are shown in Figure 3. Representative time-activity curves of PHEN and D2-PHEN in heart and blood are presented in Figure 4.

TABLE 1
Parameters for Intact PHEN and D2-PHEN in Blood

	% Intact radiotracer = $A_1 \exp(-\lambda t) + C$		
	A_1 (%)	λ (min^{-1})	C (%)
PHEN	94.3 ± 6.5	0.060 ± 0.016	6.4 ± 5.4
D2-PHEN	89.4 ± 11.6	0.045 ± 0.017*	11.2 ± 10.2

Values are mean ± SD (n = 6).
*$P < 0.05$ relative to PHEN.

TABLE 2
Retention Indices and Washout Half-times for PHEN and D2-PHEN

	Retention index (mL blood/min/mL tissue)	Washout half-time (min)
PHEN	0.066 ± 0.011	59 ± 10
D2-PHEN	0.086 ± 0.018*	155 ± 52†

Values are mean ± SD (n = 6).
*$P < 0.003$ relative to PHEN.
†$P < 0.01$ relative to PHEN.

DISCUSSION

PHEN is structurally related to two previously developed radiotracers for PET studies of cardiac sympathetic innervation, [^{11}C]-*meta*-hydroxyephedrine (HED) and [^{11}C]epinephrine (EPI). The PHEN molecule is derived by either removing the α-methyl group of HED or the para-hydroxyl group of EPI. The α-methyl group of HED prevents its oxidation by MAO. PHEN and EPI lack this α-methyl group and are thus vulnerable to oxidative deamination by MAO.

When PHEN is cleaved at the α-carbon by MAO, the radiolabeled metabolite formed is [^{11}C]methylamine. Methylamine is a small molecule that readily diffuses across lipid bilayer membranes (*16*). Thus the rate constant for leakage of [^{11}C]methylamine from the neuronal axoplasm into the interstitium is much higher than that of PHEN itself. Because methylamine is not a substrate for neuronal transport, once it diffuses from the neuron it is not transported

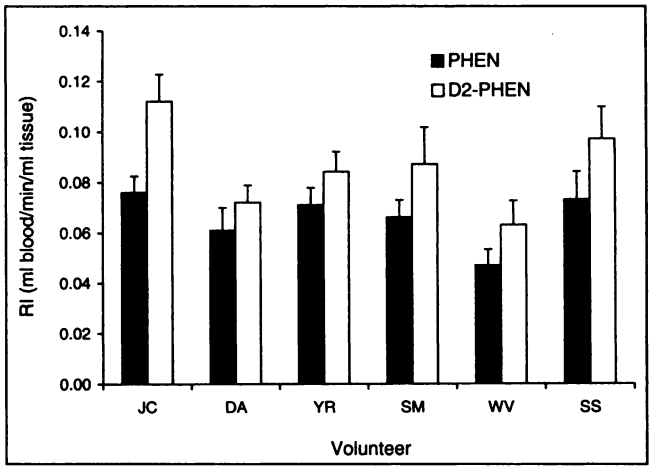

FIGURE 2. Mean tracer retention index (RI) values of PHEN and D2-PHEN. Mean ± SD of all 480 sectors of left ventricle are shown for each normal volunteer.

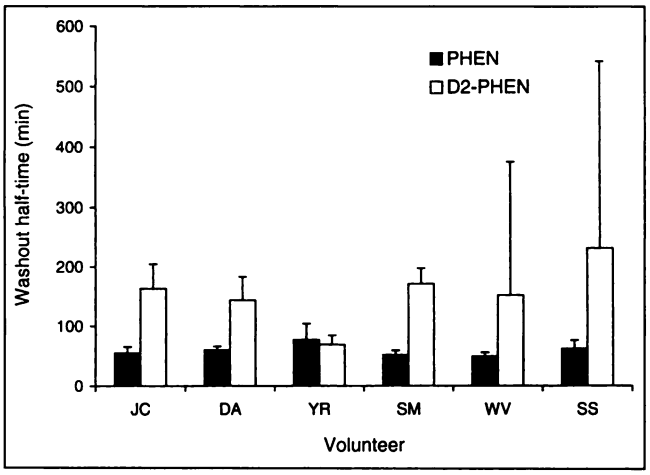

FIGURE 3. Mean washout half-time of PHEN and D2-PHEN. Mean ± SD of all 480 sectors of left ventricle are shown for each normal volunteer.

back into the neuron and would most likely enter into the coronary circulation. These considerations led to the hypothesis that the radioactivity clearing from the heart after neuronal uptake of PHEN should primarily be in the form of [^{11}C]methylamine, and as such, the rate at which radioactivity cleared from the heart would be a measure of neuronal MAO activity.

Initial clinical characterization of PHEN was performed in tandem PET studies with HED in normal volunteers (*7*). Examination of the kinetics of the two tracers showed that heart uptake of PHEN was comparable to HED soon after tracer injection, but PHEN cleared from the heart at an

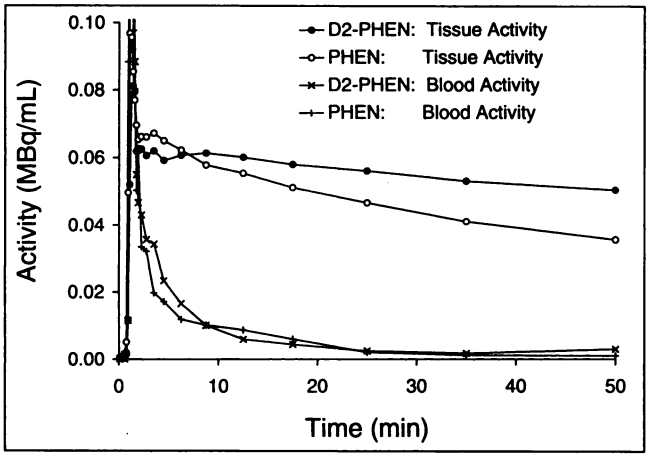

FIGURE 4. Representative time-activity curves for PHEN and D2-PHEN in normal volunteer. Time-activity curves have been converted from PET counts to MBq/mL using appropriate PET scanner calibration factor, determined weekly by imaging 20-cm cylindrical flood phantom filled with aqueous solution of ^{18}F at known concentration. Blood data are metabolite corrected and represent time course of intact tracer in blood. Peaks of blood curves are off scale; PHEN injection in this subject peaked at 0.14 MBq/mL, whereas peak of D2-PHEN activity in blood was 0.17 MBq/mL. Note faster efflux of PHEN relative to D2-PHEN, despite very similar blood curves.

appreciable rate during the 60 min imaging study, while heart levels of HED did not change. PET studies in normal volunteers after oral administration of desipramine, a potent inhibitor of the neuronal norepinephrine transporter, demonstrated that most cardiac PHEN and HED uptake is neuronal (*7*). Thus PHEN and HED selectively localize in cardiac sympathetic neurons, but PHEN clears from the normal human heart after initial uptake, while HED levels remain constant.

Substitution of deuterium atoms for hydrogen atoms as a means of slowing chemical and enzymatic reactions is a technique often used in kinetic studies (*10*). Because the bond energy of a carbon-deuterium bond is higher than that of a carbon-hydrogen bond, reactions are slowed by substituting deuterium for hydrogen in the bond broken in the rate-determining step of the reaction. For example, the deuterium substitution technique has been used for in vitro kinetic studies of MAO activity on dopamine (*11*). Deuterium substitution has also been used to assess the influence of MAO activity on PET radiotracers developed for in vivo investigations of MAO activity in brain (*17,18*) and cardiac sympathetic neurons (*19,20*). The merit of this approach is that MAO activity is inhibited at the tracer level while not significantly altering other molecular properties of the tracer. Thus, the influence of MAO activity alone on the tracer kinetics can be assessed in the living human subject. An advantage of this technique over pharmacological inhibition of MAO is that there are no complicating drug-induced physiological changes that might influence the observed tracer kinetics, such as changes in myocardial blood flow or norepinephrine levels. With the previous successful applications of this method in mind, deuterium substitution of the two hydrogen atoms on the α-carbon side chain position of PHEN was used to assess the influence of neuronal MAO activity on the kinetics of PHEN in the normal human heart. To our knowledge, this is the first time the deuterium substitution approach has been used in tracer kinetic studies in the human heart.

In our first PET studies with D2-PHEN, we performed tandem imaging studies of PHEN and D2-PHEN in dogs. However, we found no significant differences in the kinetics of these two agents in the three dogs imaged. Although this result was initially surprising, existing evidence suggests that MAO levels in the canine heart are very low (*21,22*). It is well documented that there is considerable species variability in cardiac MAO, both in the amount of MAO activity and the relative ratios of the MAO-A and MAO-B isoforms (*13,21,23*). It is also possible that a considerable amount of PHEN and D2-PHEN uptake in the canine heart occurs extraneuronally. Because the dog proved to be a problematic animal model for studying the MAO sensitivity of PHEN, paired PET studies of PHEN and D2-PHEN in normal human volunteers were undertaken.

The washout rate of PHEN measured in this study (59 ± 10 min; n = 6) agrees with the previously determined value of 59 ± 5 min (n = 14) (*7*). Di-deuterium substitution

led to a mean washout rate of 155 ± 52 min, approximately 2.6 times slower than that of PHEN (Table 2). The slower washout rate of D2-PHEN led to higher tissue concentrations of D2-PHEN relative to PHEN at the end of the dynamic PET imaging study (40–60 min after tracer injection). When these final tissue concentrations were normalized to integrated blood radioactivity associated with intact radiotracer (i.e., the RI calculation), neuronal retention of D2-PHEN was significantly higher than that of PHEN. These findings are consistent with the hypothesis that neuronal MAO activity converts significant amounts of PHEN to [^{11}C]methylamine in the normal human heart, leading to a faster efflux of radioactivity for PHEN relative to D2-PHEN.

For two of the volunteers, washout of D2-PHEN was quite slow from a few areas of the heart, leading to highly skewed distributions of washout half-times. The sectors with much slower washout were responsible for the high calculated SD of the mean washout for these patients (Fig. 3). The washout rates in most areas of the left ventricle in these two subjects were within normal ranges. However, it should be noted that these two volunteers were the oldest in the study (55-y-old woman and 54-y-old man). Determining whether this is an age-related effect would require assessment of a larger population. Also, the washout rates of D2-PHEN and PHEN were approximately the same for one volunteer. Integrated blood activity (the denominator of the RI calculation) was low for both the PHEN and D2-PHEN studies in this subject. However, final tissue values were also lower, so that calculated RI values were comparable to those of the other volunteers. This volunteer was a 30-y-old Native American woman. Whether the similarity of washout rates of PHEN and D2-PHEN in this patient was related to the subject's race is unknown and would require further study.

PET studies in baboon heart with (−)-6-[^{18}F]fluorodopamine and (−)-6-[^{18}F]fluorodopamine-α, α-D_2 by Ding et al. (*20*) demonstrated that di-deuterium substitution significantly slowed tracer efflux, leading to increases in washout half-times by factors of 1.52, 2.34 and 3.04 in the three individual baboons studied. These values are comparable to the mean value of 2.6 found for D2-PHEN in the normal human heart.

Although the washout of D2-PHEN was on average slower than that of PHEN, it did occur at a measurable rate. This is notably different from the kinetic behavior of the structurally related tracers HED and EPI. Neither HED nor EPI exhibits any appreciable washout from the normal human heart (*24,25*). Mechanistic studies of all four tracers in the isolated working rat heart have allowed construction of a working model of the differences in the neuronal handling of these agents (*8,26,27*). Several factors dictate these differences, including (a) the rate of neuronal transport; (b) the rate of vesicular transport; (c) vulnerability to MAO; and (d) lipophilicity, which largely determines the rate of diffusion from vesicles (vesicular leakage rate) and across neuronal membranes (neuronal leakage rate) (*28*). The most lipophilic tracer is HED, which leaks rapidly from vesicles and across the neuronal membrane. This sets up a dynamic recycling of HED by the neuron, because most HED molecules leaking from the neuron are transported back into the neuron by the neuronal transporter. EPI is the least lipophilic of the tracers and is very efficiently stored in vesicles. PHEN has an intermediate lipophilicity and thus probably leaks more quickly from vesicles than EPI, but not as quickly as HED.

Considering the differences in the way these tracers are retained in cardiac sympathetic neurons, one might expect the in vivo kinetics of D2-PHEN to be similar to those of HED. The fact that D2-PHEN clears from the normal human heart with a measurable half-time, while heart levels of HED remain constant during an imaging study, is probably due to the lower transport rate of D2-PHEN at the neuronal norepinephrine transporter. Isolated rat heart studies have demonstrated that PHEN, D2-PHEN and EPI are transported more slowly than HED (*8,26,27*). This is not surprising, because structure-activity studies have demonstrated that methylation of the α-carbon on the side chain of phenolamines results in a considerable increase in affinity for the neuronal norepinephrine transporter (*29*). Thus, whereas PHEN and D2-PHEN probably undergo some degree of neuronal recycling, their lower affinity for the neuronal norepinephrine transporter relative to HED may lead to a higher probability that a PHEN or D2-PHEN molecule that leaves the neuron (either through passive diffusion or vesicular exocytosis) will escape from the interstitium into the coronary vasculature rather than being taken back up into the neuron. Alternatively, it is possible that the di-deuterium substitution does not completely block MAO activity but merely slows the rate of MAO metabolism. However, studies in the isolated rat heart found that the kinetics of D2-PHEN were very similar to those of PHEN after inhibition of MAO activity with high concentrations of pargyline, suggesting that the inhibitory effect of di-deuterium substitution is substantial (*8*).

How the kinetics of PHEN change as the result of cardiac diseases that impact sympathetic nerve populations is a complex issue, but some hypotheses can be put forth based on our understanding of its neuronal retention. Initial uptake of the tracer is highly dependent on intact sympathetic nerve varicosities with functional neuronal norepinephrine transporters. Areas of the heart that are denervated or have nerves with reduced neuronal norepinephrine transporter function will have low initial uptake of PHEN. However, in areas in which neuronal uptake does occur, vesicular storage is the critical process in prolonging neuronal retention of PHEN, because it protects the tracer from rapid metabolism by mitochondrial-bound MAO in the neuronal axoplasm. If a particular cardiac disease were to impair vesicular storage function, an increase in the efflux rate of PHEN from the heart would be expected. Thus the kinetics of PHEN are not only sensitive to neuronal MAO activity but are highly dependent on vesicular storage function.

The effects of heart diseases on MAO activity within cardiac sympathetic neurons have not been studied extensively. Studies by Bajusz and Jasmin (*30,31*) in the early 1960s showed that as little as 5 min of coronary artery ligation in the rat heart caused significant reduction of MAO concentrations in the heart, as demonstrated by histological staining. This would suggest that MAO activity is sensitive to damage under ischemic conditions. However, attempts to reproduce these findings showed the loss of MAO in both ischemic and nonischemic areas to be quite variable (*32*) or absent (*33*). The fact that the majority of MAO in the rat heart is localized extraneuronally further complicates interpretation of these studies (*34,35*). Acute ischemia is known to cause a rapid release of norepinephrine from vesicles, which in combination with an elevated axoplasmic sodium concentration drives the neuronal norepinephrine transporter into outwardly transporting norepinephrine from the neuronal axoplasm into interstitium (*36*). It is possible that acutely high levels of norepinephrine during ischemic episodes bind to MAO throughout the rat heart and prevent the histochemical stain from properly marking tissue levels of MAO. A study by Lamontagne et al. (*37*) in the isolated rat heart has demonstrated that part of the tissue norepinephrine stores that are lost during acute ischemia occurs because of metabolism by MAO. Studies of MAO in rat liver demonstrated that 60 min of ischemia caused no alterations of MAO activity, suggesting that MAO may in fact be insensitive to damage by ischemic conditions (*38*). It seems unlikely then that cardiac diseases would lead to a selective decrease of MAO activity in surviving cardiac sympathetic neurons. Because vesicular storage function is highly energy dependent, it may be the neuronal process most sensitive to perturbation by pathological conditions. For example, studies of cardiac sympathetic innervation in heart failure have found evidence suggesting that vesicular storage is impaired (*39,40*). Thus, in neurons at risk of cellular death, it may be that neuronal transport and MAO are largely intact but vesicular storage function is compromised. Such conditions would elevate the importance of MAO metabolism in determining the observed efflux rate of PHEN from the heart. In the extreme case of no vesicular uptake, very little radioactivity would be expected to accumulate in the heart, since most of the PHEN entering the neuron would be rapidly metabolized by MAO to [^{11}C]methylamine. The kinetics of PHEN in this case would be ambiguous because they would be indistinguishable from the kinetics expected in an area of localized denervation.

To summarize, although breakage of an α-carbon C-H bond is the rate-limiting step in MAO oxidation of substrates such as PHEN, MAO oxidation is not the sole neuronal process governing the observed rate of clearance of PHEN from the heart under normal conditions. The rate of egress of PHEN-derived radioactivity from the heart is determined by a complex interplay between leakage of PHEN from storage vesicles, vesicular reuptake and MAO metabolism of PHEN into [^{11}C]methylamine. Thus the potential diagnostic/prognostic value of PHEN heart clearance rates does not stem from PHEN's oxidation by neuronal MAO per se, but rather from its ability to reflect impairment of vesicular storage function. The sensitivity of PHEN heart clearance to deficits of vesicular storage—deficits that may occur in the early stages of heart disease—will likely determine PHEN's clinical utility.

CONCLUSION

Paired PET studies with PHEN and D2-PHEN have directly demonstrated that the in vivo kinetics of PHEN are sensitive to neuronal MAO activity in the normal human heart. However, MAO activity is not the rate-limiting step of the observed efflux rate under normal conditions. Because vesicular storage of PHEN is necessary to protect the tracer from rapid metabolism by neuronal MAO, the kinetics of PHEN are also highly dependent on vesicular storage function.

ACKNOWLEDGMENTS

We thank University of Michigan PET technologists Jill Rothley, Edward McKenna and Andrew Weeden for their technical assistance; the Cyclotron/PET Radiochemistry staff for their production of the radiotracers; and Linder Markham for her assistance in preparing the manuscript. This work was supported by grants R01-HL47543 and R01-HL27555 from the National Institutes of Health, Bethesda, MD.

REFERENCES

1. Wieland DM, Brown LE, Rogers WL, et al. Myocardial imaging with a radioiodinated norepinephrine storage analog. *J Nucl Med.* 1981;22:22–31.
2. Rosenspire KC, Haka MS, Van Dort ME, et al. Synthesis and preliminary evaluation of carbon-11-*meta*-hydroxyephedrine: a false transmitter agent for heart neuronal imaging. *J Nucl Med.* 1990;31:1328–1334.
3. Ding Y-S, Fowler JS, Gatley S, Dewey S, Wolf A, Schlyer D. Synthesis of high specific activity (+) and (−) 6-[^{18}F]fluorodopamine for PET studies of sympathetic nervous tissue. *J Med Chem.* 1991;34:861–863.
4. Chakraborty PK, Gildersleeve DL, Jewett DM, et al. High yield synthesis of high specific activity *R*-(−)-[^{11}C]epinephrine for routine PET studies in humans. *Nucl Med Biol.* 1993;20:939–944.
5. del Rosario RB, Jung Y-W, Chakraborty PK, Sherman PS, Wieland DM. Synthesis and preliminary evaluation of [^{11}C] phenylephrine for mapping heart neuronal function. *Nucl Med Biol.* 1996;23:611–616.
6. Raffel DM, Corbett JR, Schwaiger M, Wieland DM. Mechanism-based strategies for mapping heart sympathetic nerve function. *Nucl Med Biol.* 1995;22:1019–1026.
7. Raffel DM, Corbett JR, del Rosario RB, et al. Clinical evaluation of carbon-11-phenylephrine: MAO sensitive marker of cardiac sympathetic neurons. *J Nucl Med.* 1996;37:1923–1931.
8. Raffel DM, Wieland DM. Influence of vesicular storage and monoamine oxidase activity on [^{11}C]phenylephrine kinetics: studies in isolated rat heart. *J Nucl Med.* 1999;40:323–330.
9. del Rosario RB, Wieland DM. Synthesis of [^{11}C](−)-α-α-dideutero-phenylephrine for in vivo kinetic isotope studies. *J Labeled Compds Radiopharm.* 1995;36:625–630.
10. March J. *Advanced Organic Chemistry.* 4th ed. New York, NY: John Wiley & Sons; 1992.
11. Yu PH, Bailey BA, Durden DA, Boulton AA. Stereospecific deuterium substitution at the α-carbon position of dopamine and its effects on oxidative deamination catalyzed by MAO-A and MAO-B from different tissues. *Biochem Pharmacol.* 1986;35:1027–1036.
12. Hutchins GD, Schwaiger M, Rosenspire KC, Krivokapich J, Schelbert H, Kuhl DE. Noninvasive quantification of regional blood flow in the human heart using N-13 ammonia and dynamic positron emission tomographic imaging. *J Am Coll Cardiol.* 1990;15:1032–1042.

13. Lewinsohn R, Glover V, Sandler M. Development of benzylamine oxidase and monoamine oxidase A and B in man. *Biochem Pharmacol.* 1980;29:1221–1230.
14. Saura J, Nadal E, van den Berg B, Vila M, Bombi JA, Mahy N. Localization of monoamine oxidases in human peripheral tissues. *Life Sci.* 1996;59:1341–1349.
15. Hutchins GD, Schwaiger M. Quantifying myocardial blood flow with PET. In: Zaret BL, Beller GA, eds. *Nuclear Cardiology: State of the Art and Future Directions.* St. Louis, MO: Mosby; 1993:305–313.
16. Walter A, Gutknecht J. Permeability of small nonelectrolytes through lipid bilayer membranes. *J Membrane Biol.* 1986;90:207–217.
17. Inoue O, Tominaga T, Yamasaki T, Kinemuchi H. Radioactive *N,N*-dimethylphenylethylamine: a selective radiotracer for in vivo measurement of monoamine oxidase-B activity in the brain. *J Neurochem.* 1985;44:210–216.
18. Fowler JS, Wang G-J, Logan J, et al. Selective reduction of radiotracer trapping by deuterium substitution: comparison of carbon-11-L-deprenyl and carbon-11-deprenyl-D2 for MAO B mapping. *J Nucl Med.* 1995;36:1255–1262.
19. Tominaga T, Inoue O, Suzuki K, Yamasaki T, Hirobe M. [N-13]-β-Phenethylamine ([N-13]PEA). A prototype tracer for measurement of MAO-B activity in heart. *Biochem Pharmacol.* 1987;36:3671–3675.
20. Ding Y-S, Fowler JS, Gatley SJ, Logan J, Volkow ND, Shea C. Mechanistic positron emission tomography studies of 6[^{18}F]fluorodopamine in living baboon heart: selective imaging and control of radiotracer metabolism using the deuterium isotope effect. *J Neurochem.* 1995;65:682–690.
21. White HL, Stine DK. Species differences in monoamine oxidase-A and -B as revealed by sensitivity to trypsin. *Life Sci.* 1984;35:827–833.
22. Takeichi O, Nagao T, Mizunuma Y, et al. Histochemical studies of monoamine oxidase in experimental myocardial infarctions. *Jap Circ J.* 1975;40:29–34.
23. Strolin Bendetti M, Thomassin J, Tocchetti P, Dostert P, Kettler R, Da Prada M. Species differences in changes of heart monoamine oxidase activities with age. *J Neural Transm.* 1994;41(suppl):83–87.
24. Schwaiger M, Hutchins G, Kalff V, et al. Evidence of regional catecholamine uptake and storage sites in the transplanted human heart by positron emission tomography. *J Clin Invest.* 1991;87:1681–1690.
25. Schwaiger M, Wieland D, Muzik O, et al. Comparison of C-11 epinephrine and C-11 HED for evaluation of sympathetic neurons of the heart [abstract]. *J Nucl Med.* 1993;34:13P.
26. DeGrado TR, Hutchins GD, Toorongian SA, Wieland DM, Schwaiger M. Myocardial kinetics of carbon-11-*meta*-hydroxyephedrine: retention mechanisms and effects of norepinephrine. *J Nucl Med.* 1993;34:1287–1293.
27. Nguyen NTB, DeGrado TR, Chakraborty P, Wieland DM, Schwaiger M. Myocardial kinetics of C-11 epinephrine in the isolated working rat heart. *J Nucl Med.* 1997;38:780–785.
28. Mack F, Bönisch H. Dissociation constants and lipophilicity of catecholamines and related compounds. *Naunyn Schmiedeberg's Arch Pharmacol.* 1979;310:1–9.
29. Iversen LL. Uptake processes of biogenic amines. In: Iversen L, Iversen S, Snyder S, eds. *Handbook of Psychopharmacology.* Vol 3. New York, NY: Plenum Press; 1975:381–442.
30. Bajusz E, Jasmin G. Histochemical studies on the myocardium following experimental interference with coronary circulation in the rat. I. Occlusion of coronary artery. *Acta Histochem.* 1964;18:222–237.
31. Bajusz E, Jasmin G. Observations on histochemical differential diagnosis between primary and secondary cardiomyopathies: behavior of monoamine oxidase, phosphorylase, and glycogen in heart muscle. *Am Heart J.* 1965;69:83–92.
32. Fine G, Morales A, Scerpella JR. Experimental myocardial infarction: a histochemical study. *Arch Pathol.* 1966;82:4–8.
33. Hecht A. Enzyme histochemistry of heart muscle in normal and pathological conditions. In: Bajusz E, Jasmin G, eds. *Meth Achievm Exp Path.* Vol 5. Basel, Switzerland: Karger; 1971:384–435.
34. Horita A. Cardiac monoamine oxidase in rat. *Nature.* 1967;215:411–412.
35. Lowe MC, Horita A. Stability of cardiac monoamine oxidase activity after chemical sympathectomy with 6-hydroxydopamine. *Nature.* 1970;228:175–176.
36. Schömig A, Fischer S, Kurz T, Richardt G, Schömig E. Nonexocytotic release of endogenous noradrenaline in the ischemic and anoxic rat heart: mechanism and metabolic requirements. *Circ Res.* 1987;60:194–205.
37. Lamontagne D, Yamaguchi N, Ribuot C, de Champlain J, Nadeau R. Reduction of tissue noradrenaline content in the isolated perfused rat heart during ischemia: importance of monoamine oxidation. *Can J Physiol Pharmacol.* 1991;69:1190–1195.
38. Fredericks WM, Marx F. Changes in cytoplasmic and mitochondrial enzymes in rat liver after ischemia followed by reperfusion. *Exp Mol Pathol.* 1987;47:291–299.
39. Sole M, Helke C, Jacobowitz D. Increased dopamine in the failing hamster heart: Transvesicular transport of dopamine limits the rate of norepinephrine synthesis. *Am J Cardiol.* 1982;49:1682–1690.
40. Rose CP, Burgess JH, Cousineau D. Tracer norepinephrine kinetics in coronary circulation of patients with heart failure secondary to chronic pressure and volume overload. *J Clin Invest.* 1985;76:1740–1747.