# Influence of Vesicular Storage and Monoamine Oxidase Activity on [$^{11}$C]Phenylephrine Kinetics: Studies in Isolated Rat Heart

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[$^{11}$C]Phenylephrine (PHEN) is a radiolabeled analogue of norepinephrine that is transported into cardiac sympathetic nerve varicosities by the neuronal norepinephrine transporter and taken up into storage vesicles localized within the nerve varicosities by the vesicular monoamine transporter. PHEN is structurally related to two previously developed sympathetic nerve markers: [$^{11}$C]-*meta*-hydroxyephedrine and [$^{11}$C]epinephrine. To better characterize the neuronal handling of PHEN, particularly its sensitivity to neuronal monoamine oxidase (MAO) activity, kinetic studies in an isolated working rat heart system were performed. **Methods:** Radiotracer was administered to the isolated working heart as a 10-min constant infusion followed by a 110-min washout period. Two distinctly different approaches were used to assess the sensitivity of the kinetics of PHEN to MAO activity. In the first approach, oxidation of PHEN by MAO was inhibited at the enzymatic level with the MAO inhibitor pargyline. In the second approach, the two hydrogen atoms on the α-carbon of the side chain of PHEN were replaced with deuterium atoms ([$^{11}$C](−)-α-α-dideutero-phenylephrine [D2-PHEN]) to inhibit MAO activity at the tracer level. The importance of vesicular uptake on the kinetics of PHEN and D2-PHEN was assessed by inhibiting vesicular monoamine transporter–mediated storage into vesicles with reserpine. **Results:** Under control conditions, PHEN initially accumulated into the heart at a rate of 0.72 ± 0.15 mL/min/g wet. Inhibition of MAO activity with either pargyline or di-deuterium substitution did not significantly alter this rate. However, MAO inhibition did significantly slow the clearance of radioactivity from the heart during the washout phase of the study. Blocking vesicular uptake with reserpine reduced the initial uptake rates of PHEN and D2-PHEN, as well as greatly accelerated the clearance of radioactivity from the heart during washout. **Conclusion:** These studies indicate that PHEN kinetics are sensitive to neuronal MAO activity. Under normal conditions, efficient vesicular storage of PHEN serves to protect the tracer from rapid metabolism by neuronal MAO. However, it is likely that leakage of PHEN from the storage vesicles and subsequent metabolism by MAO lead to an appreciable clearance of radioactivity from the heart.

**Key Words:** [$^{11}$C]phenylephrine; norepinephrine transporter; sympathetic nervous system; PET; [$^{11}$C]hydroxyephredrine; [$^{11}$C]epinephrine

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Carbon-11-phenylephrine (PHEN) was developed in our laboratory as a radiotracer for PET studies of the sympathetic innervation of the heart (*1*). PHEN is structurally related to two previously developed sympathetic nerve markers: [$^{11}$C]-*meta*-hydroxyephedrine (HED) (*2*) and [$^{11}$C]epinephrine (EPI) (*3*). As analogues of the endogenous neurotransmitter norepinephrine, these tracers are transported into cardiac sympathetic nerves by the neuronal norepinephrine transporter (uptake$_1$) and stored in vesicles by the vesicular monoamine transporter (*4*). PHEN and EPI are substrates for monoamine oxidase (MAO), whereas HED is not. Clinical PET studies of these tracers in humans have shown that although there is very little clearance of HED and EPI from normal human heart, PHEN clears from the heart with a mean half-time of 59 ± 5 min (*5*). To investigate the importance of neuronal MAO activity and vesicular storage on PHEN retention and kinetics, studies were performed in the isolated working rat heart model. The influence of MAO activity on PHEN kinetics was assessed using two distinctly different approaches. First, MAO activity was inhibited pharmacologically with pargyline. Second, deuterium substitution at the α-carbon of the side chain of PHEN to form the analogue [$^{11}$C](−)-α-α-dideutero-phenylephrine (D2-PHEN) was used to slow the rate of MAO metabolism at the tracer level (*6*). In addition, the importance of vesicular uptake on the kinetics of PHEN and D2-PHEN was studied using hearts isolated from rats injected with the vesicular uptake blocker reserpine. The results of these studies show that vesicular uptake of PHEN protects the tracer from rapid metabolism by neuronal MAO. Furthermore, the rate at which PHEN clears from the isolated rat heart is significantly influenced by neuronal MAO activity.

## MATERIALS AND METHODS

### Radiotracer Preparation

The radiochemical syntheses for PHEN and D2-PHEN have been reported previously (*1,6*). Specific activities exceeded 500 Ci/mmol with typical radiochemical yields of >50% end of synthesis. The product was dissolved in sterile 20 mmol/L sodium phosphate monobasic solution.

### Chemicals

All chemicals used for preparation of the Krebs-Henseleit (K-H) perfusion buffer were American Chemical Society grade reagents obtained through the University of Michigan stores from several different chemical firms. Reserpine was obtained from Sigma Chemical Co. (St. Louis, MO). Pargyline was obtained from Aldrich Chemical Co. (Milwaukee, WI).

### Working Heart Perfusion

Hearts from male Sprague-Dawley rats (weight range 160–540 g) were perfused at moderate workload conditions (7.3 mm Hg preload and 73 mm Hg postload) using a working heart preparation based on the system developed by Taegtmeyer et al. (*7*). Two separate perfusion circuits were used in parallel and were connected to the left atrial cannula with a three-way connector to allow rapid switching from one perfusion circuit to the other. The perfusion medium was K-H bicarbonate buffer (118 nmol/L NaCl, 4.7 mmol/L KCl, 2.55 mmol/L $CaCl_2$, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L $K_2H_2PO_4$ and 25 mmol/L $NaHCO_3$) containing 5 mmol/L glucose and oxygenated with a 95% $O_2$/5% $CO_2$ gas mixture.

Hearts were excised rapidly from ether-anesthetized rats and were placed in ice-chilled K-H buffer. The heart was immediately attached to the perfusion apparatus by cannulation of the aorta, and retrograde (Langendorff) perfusion of K-H buffer was begun. A timer started at this point served as the master clock of the experiment. A small opening was cut into the pulmonary artery at the point where it crosses over the base of the aorta to facilitate expulsion of perfusate from the right heart during right ventricular contraction. The heart was then reoriented on the aortic cannula to align the left atrium with the left atrial cannula. After removing excess pulmonary vein tissue, the left atrium was cannulated and the heart was switched from retrograde perfusion to working heart perfusion. The period of retrograde perfusion ranged from 2 to 4 min. Hearts were not paced and were allowed to determine their own coronary and aortic flows.

### External Detection System

Radioactivity in the heart was externally measured using a pair of bismuth germanate ($Bi_4Ge_3O_{12}$) detectors. The front faces of the two $Bi_4Ge_3O_{12}$ detector crystals (2.5-cm diameter × 2.5-cm height) were positioned 3.7 cm apart, directly opposing each other with the heart centered between them. Each detector was enclosed in a cylindrical lead collimator (3.5-cm inner diameter, 6.3-cm wall thickness and 25-cm long) to minimize detected counts originating from radioactive sources outside the heart. Two coincident detection circuits were established between the detectors using Nuclear Instrument Modules standard electronic modules. One circuit measured total coincident events between the two detectors (true + random coincident events) and the second measured only random coincident events.

### Data Acquisition

A data acquisition system composed of a counter/timer board (PC-TIO-10; National Instruments, Austin, TX) interfaced to a personal computer (model 4DX-66V; Gateway 2000, North Sioux City, SD) was used to acquire count data from the external detection system. Four channels of data were acquired simultaneously: the counting rates from each detector individually, the total coincidence event rate and the random coincidence event rate. The true coincidence rate was estimated by subtracting the random coincidence measurement from the total coincidence measurement for each time point. Data were acquired at four samples per second and were later compressed to one sample per second for analysis.

### Experimental Protocols

Hearts were perfused in working mode for a 30-min stabilization period using K-H buffer in the first of the two perfusion circuits. During this time, the $^{11}C$-labeled tracer being studied was added to perfusate in the second perfusion circuit and was allowed to mix for several minutes. Before the start of tracer infusion, three 1.0-mL aliquots of the perfusate were drawn and placed in separate polypropylene sample tubes for counting in a gamma counter (Packard model MINAXI, Meriden, CT) to determine the radioactivity concentration in the perfusate ($C_{perf}$). After the 30-min stabilization period, data acquisition from the external detection system was started. After 2 min of data acquisition to assess background counting rates, the heart was rapidly switched to the second perfusion circuit to begin a constant infusion of the $^{11}C$-labeled neuronal tracer. After a 10-min constant infusion period, the heart was switched back to the first perfusion circuit to effect tracer washout. The washout period was at least 110 min. Longer washout periods were not feasible because the 20-min half-life of $^{11}C$ leads to very low counting rates beyond 110 min. Radioactivity concentrations of 0.7–1.0 μCi/mL were used. These concentrations were found to provide good counting statistics, with peak coincidence counting rates of several thousand counts per second. With the high specific activities of these $^{11}C$-labeled neuronal tracers (>400 Ci/mmol), the radioactivity concentrations used correspond to molar concentrations of at most 2.5 nmol/L, with typical concentrations of <1 nmol/L. The 50% inhibitory concentration value for inhibition of neuronal uptake of norepinephrine by (−)-phenylephrine has been estimated to be 5.6 μmol/L (*8*); therefore, these can be considered to be tracer-level concentrations.

Experiments characterizing the uptake and washout kinetics of PHEN were performed using hearts obtained from rats covering a wide range of ages. An age effect was looked for in the kinetics of PHEN because it has been well documented that cardiac MAO levels dramatically increase with age in rats (*9–11*). The rats used in this study had ages ranging from 7 to 20 wk (160–540 g whole-body wet).

The effect of MAO activity on the kinetics of PHEN was examined in two ways. The first approach was to inhibit MAO activity at the enzyme level by adding pargyline (100 μmol/L) to the perfusate. The heart was infused with pargyline-laden perfusate for 30 min before the switch to the second perfusion circuit containing PHEN as well as 100 μmol/L pargyline. After the 10-min constant infusion period, the heart was switched back to the first perfusion circuit to effect tracer washout as described previously. The second approach to inhibiting MAO activity was to use the deuterium isotope effect to inhibit MAO activity at the tracer level. The kinetics of D2-PHEN were characterized under control conditions as described previously for PHEN.

To assess the importance of vesicular uptake, kinetic studies were performed with PHEN, PHEN during pargyline block and D2-PHEN using hearts isolated from rats that had been treated with the vesicular uptake blocker reserpine (1 mg/kg intraperitoneally 3 h before being killed). With vesicular uptake blocked, tracer that would normally be sequestered inside vesicles is left vulnerable to neuronal MAO activity. Washout of PHEN was sufficiently rapid in these hearts to allow a second infusion and washout of tracer to be performed in the same heart.

### Data Analysis

Two quantitative analyses were performed on the data from each experiment. First, the initial uptake rate ($K_{up}$; in mL/min/g wet) was

assessed by fitting the time-activity data between t = 1 min and t = 4 min of the 10-min constant infusion phase of the experiment to a line. The fitted slope ($m_{up}$; in cps/min/heart, in which cps = counts/s) was converted to $K_{up}$ using the following equation:

$$K_{up} = \frac{m_{up}}{C_{perf} Z_{calib} M_{wet}},$$

where $C_{perf}$ is the concentration of radioactivity in the perfusate (in μCi/mL), $Z_{calib}$ is the external detection system calibration factor (in cps/μCi) and $M_{wet}$ is the wet mass of the heart (in g wet/heart). Second, the coincidence count data during the washout phase of the experiment were fit to multiple exponential decay processes. The exponential washout rate constants ($\lambda_i$) were used to calculate corresponding washout half-times ($t_{1/2} = \ln 2/\lambda_i$). Although the original counting rate data were used for the fitting process, the amplitudes of the exponentials (in cps/heart) were converted to an apparent distribution volume (ADV) with the following units: mL perfusate/g wet. ADV is defined as the ratio of the amount of radioactivity in the heart at a given time divided by the amount of radioactivity in 1 mL perfusate during the constant infusion ($C_{perf}$). The count data for the entire experiment can be expressed as ADV, which normalizes the data from different studies such that they can be plotted on the same scale and compared. Just as the slope of the initial uptake ($m_{up}$) was converted to the uptake rate constant $K_{up}$, the observed coincidence count data Q(t) (in cps/heart) are converted to ADV(t) by the following equation:

$$ADV(t) = \frac{Q(t)}{C_{perf} Z_{calib} M_{wet}}.$$

The amount of PHEN or D2-PHEN in the tissue at the end of the 10-min infusion period (denoted $ADV_{tot}$) was estimated as the sum of the exponential amplitudes associated with tracer in tissue. The implementation of the Marquardt-Levenberg nonlinear least-squares fitting algorithm devised by Bevington (*12*) was used for all data fitting. The analysis of variance method was used to determine the minimum number of exponential decay terms required to adequately fit the washout data.

### Measurement of $Z_{calib}$ and $M_{wet}$

Determination of the external detection system calibration factor ($Z_{calib}$) was performed for each study. This was performed with a second infusion of radiotracer, using either the remaining radioactivity from the radiosynthesis of the tracer used in the first infusion (at low specific activity) or, if available, a small amount of HED that had been prepared for clinical PET studies (at high specific activity). In either case, the second infusion was performed to ensure that there was sufficient radioactivity in the heart tissue to allow for an accurate calibration of the detection system. At the end of the washout phase of the first infusion-washout experiment, available radiotracer was added to perfusate in the second perfusion circuit. A second data acquisition study was started and the isolated heart was switched from the first perfusion circuit to the second to begin a second infusion of tracer. After 5–10 min of infusion, the heart was switched back to the first circuit to begin tracer washout. After 2 min of washout to clear radioactivity from the cannulae and vascular spaces, the perfusion lines into and out of the heart were clamped off and the amount of radioactivity trapped in the heart was measured for a 2-min period. The detectors were then moved back and the heart was removed from the perfusion apparatus, placed in a polypropylene sample tube and counted in a gamma counter (Packard model MINAXI) to determine the tissue concentration of $^{11}C$. The mean coincident counting rate observed in the final plateau of the calibration study (in cps/heart) was divided by the amount of radioactivity in the heart (in μCi/heart) to estimate the calibration factor (in cps/μCi). Uncertainties in the measurements were propagated in quadrature to obtain the uncertainty in the system calibration factor.

After counting the heart in the gamma counter, it was removed from the sample tube, cut open and blotted dry on a paper towel. The heart was then weighed to determine its wet mass ($M_{wet}$).

### Statistical Analysis

Data are expressed as means ± 1 SD. Statistical comparisons between groups were made using the unpaired 2-tailed *t* test. A probability value of $P < 0.05$ was considered significant.

## RESULTS

### Initial Uptake Rate

Representative time-activity curves for PHEN and $D_2$-PHEN in control hearts and in hearts in which vesicular uptake was blocked with reserpine are shown in Figure 1. Values for the initial uptake of PHEN and D2-PHEN under the experimental conditions studied are given in Table 1. For PHEN and D2-PHEN in control hearts, there was no correlation between the rate of uptake and age (data not shown). Under control conditions, PHEN accumulates into the perfused rat heart with a rate constant of 0.72 ± 0.15 mL/min/g wet. MAO blockade did not significantly alter this rate. The uptake rate for the di-deuterated PHEN analogue (D2-PHEN) was higher, 0.82 ± 0.10 mL/min/g wet, but was not significantly different from PHEN controls. Likewise, when MAO activity was pharmacologically inhibited with 100 μmol/L pargyline the uptake rate was not significantly different from PHEN controls. Blocking vesicular uptake with reserpine pretreatment led to a 54% reduction in the estimated uptake rate for PHEN ($P < 7.9 \times 10^{-7}$). Reserpine pretreatment reduced the uptake rate of D2-PHEN by 45%

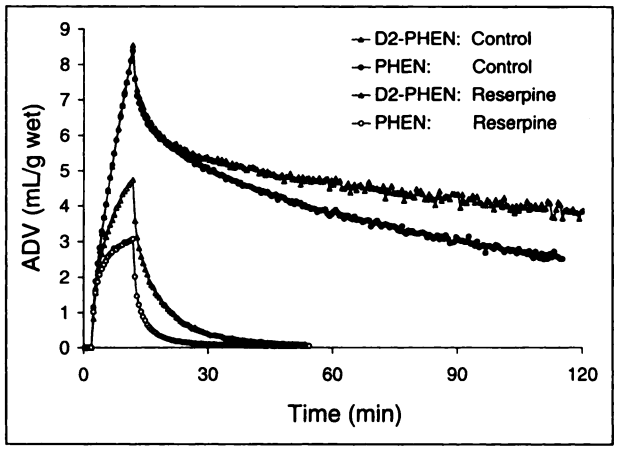


**FIGURE 1.** Representative studies of PHEN and $D_2$-PHEN under control conditions and in hearts in which vesicular uptake was blocked by injecting rat with 1 mg/kg reserpine 3 h before heart isolation. After 10-min constant infusion of tracer, isolated heart was switched to normal perfusate to effect tracer washout. ADV = apparent distribution volume.

**TABLE 1**
Initial Uptake Rates of PHEN and D2-PHEN

| Tracer + inhibitors | n | $K_{up}$ (mL/min/g wet) |
|---|---|---|
| PHEN | 11 | 0.72 ± 0.15 |
| D2-PHEN | 6 | 0.82 ± 0.10 |
| PHEN + pargyline | 4 | 0.74 ± 0.07 |
| PHEN + reserpine | 7* | 0.33 ± 0.05 |
| D2-PHEN + reserpine | 6† | 0.45 ± 0.16 |
| PHEN + pargyline + reserpine | 2‡ | 0.51 ± 0.07 |

*Seven infusions in four hearts (two infusions each in three hearts and a single infusion in the fourth).
†Six infusions in three hearts.
‡Two infusions in one heart.
PHEN = [$^{11}$C]phenylephrine; $D_2$ = PHEN = [$^{11}$C](−)-α-α-dideutero-phenylephrine; $K_{up}$ = initial uptake rate.

($P < 0.0007$). The combination of reserpine block of vesicular uptake with pargyline block of MAO activity led to a 31% reduction in the initial uptake rate of PHEN when compared with the uptake rate observed during pargyline block alone ($P < 0.02$). The reductions of the initial uptake rates after inhibition of vesicular uptake, together with the observation that inhibition of MAO activity did not lead to a significantly higher initial uptake rate relative to control hearts, suggest a tight coupling between neuronal transport and vesicular transport of PHEN under normal conditions.

### Washout Kinetics

For all experimental conditions studied, analysis of variance showed that the sum of four exponential terms best described the kinetics during the washout phase of the experiment. The fastest of the four exponential terms represents radiotracer leaving the perfusion cannulae, capillary bed and heart chambers after the rapid switch back to radioactivity-free perfusate. This exponential term, which was not normalized to the wet mass of the heart, had a mean amplitude of 1.05 ± 0.26 mL for all experiments (n = 36) with a mean half-time of 4.9 ± 3.5 s. The amplitudes (normalized to heart wet mass) and washout half-times for the other three exponential terms are presented in Table 2.

For PHEN and D2-PHEN under control conditions, there was no correlation between either the amplitudes or washout rates and animal age (data not shown). Included in Table 2 is the sum of the amplitudes of the three exponentials, which represent an estimate of the amount of tracer distributed into the heart tissue at the time washout was started. Although it is not possible to associate each exponential with a particular tissue compartment, general trends in the way the exponential terms changed as a result of each experimental condition provide some insight into the neuronal handling of PHEN. Under control conditions, the largest (and slowest) exponential component had a half-time of 98 ± 14 min. The half-times for this term during inhibition of MAO activity were significantly longer. When MAO was inhibited pharmacologically with pargyline, the corresponding half-time was 140 ± 28 min ($P < 0.001$). For di-deuterium substitution, the half-time was 163 ± 39 min ($P < 0.0001$). The amplitude of this exponential term was significantly higher for D2-PHEN relative to PHEN controls ($P < 0.04$) and to PHEN during pargyline block ($P < 0.004$). The half-times for the other two exponential terms were not significantly different from control values; therefore, only the slowest term appears to be sensitive to MAO blockade. For both PHEN and D2-PHEN, reserpine block of vesicular uptake greatly decreased the amplitude ($P < 1.1 \times 10^{-8}$ and $P < 0.0001$, respectively) and increased the washout rate ($P < 1.7 \times 10^{-9}$ and $P < 3.1 \times 10^{-6}$, respectively) of the slowest exponential term relative to controls, indicating that vesicular storage of the tracer is an important process in determining the size and rate of this term. Thus, the largest and slowest term is primarily related to neuronal uptake and storage of PHEN. In reserpine-treated hearts, the kinetics of D2-PHEN were similar to those of PHEN when MAO was inhibited with pargyline (Fig. 2 and Table 2). Together these results indicate that although MAO activity does significantly influence the efflux rate of PHEN from the heart, vesicular storage of PHEN is an important process that prevents rapid degradation of the tracer by neuronal MAO. Under control conditions, vesicular storage of PHEN leads to increased neuronal accumulation of the tracer and a much slower efflux of radioactivity from the heart.

**TABLE 2**
Amplitudes and Washout Half-Times from Fits of Time-Activity Curves During Washout Period

| Tracer and inhibitors | n | $ADV_2$ (mL/g wet) | $ADV_3$ (mL/g wet) | $ADV_4$ (mL/g wet) | $ADV_{tot}$ (mL/g wet) | $T_{1/2,2}$ (min) | $T_{1/2,3}$ (min) | $T_{1/2,4}$ (min) |
|---|---|---|---|---|---|---|---|---|
| PHEN | 11 | 0.94 ± 0.47 | 1.15 ± 0.42 | 4.22 ± 1.11 | 6.31 ± 1.33 | 1.8 ± 0.6 | 12.9 ± 7.2 | 98.2 ± 13.7 |
| D2-PHEN | 6 | 0.79 ± 0.53 | 1.28 ± 0.68 | 5.30 ± 0.47 | 7.37 ± 0.77 | 2.3 ± 1.3 | 8.0 ± 2.5 | 163.2 ± 39.0 |
| PHEN + P | 4 | 0.86 ± 0.40 | 1.73 ± 0.53 | 4.00 ± 0.56 | 6.60 ± 0.74 | 2.8 ± 1.4 | 11.5 ± 4.8 | 140.4 ± 27.8 |
| PHEN + R | 7 | 1.05 ± 0.18 | 1.10 ± 0.45 | 0.20 ± 0.12 | 2.35 ± 0.41 | 1.1 ± 0.3 | 4.1 ± 0.8 | 31.2 ± 6.0 |
| D2-PHEN + R | 6 | 0.72 ± 0.20 | 1.85 ± 0.80 | 0.91 ± 0.47 | 3.48 ± 1.05 | 1.2 ± 0.8 | 4.9 ± 0.9 | 14.6 ± 3.4 |
| PHEN + R + P | 2 | 0.26 ± 0.37 | 2.90 ± 0.22 | 0.59 ± 0.14 | 3.74 ± 0.28 | 2.0 ± 0.1 | 5.5 ± 0.3 | 14.9 ± 1.3 |

ADV = apparent distribution volume; $ADV_{tot}$ = sum of the amplitudes for the three exponential terms; T = time; PHEN = [$^{11}$C]phenylephrine; D2-PHEN = [$^{11}$C](−)-α-α-dideutero-phenylephrine; P = pargyline; R = reserpine.
Amplitudes are expressed as apparent distribution volumes. Values of n are the same as shown in Table 1.

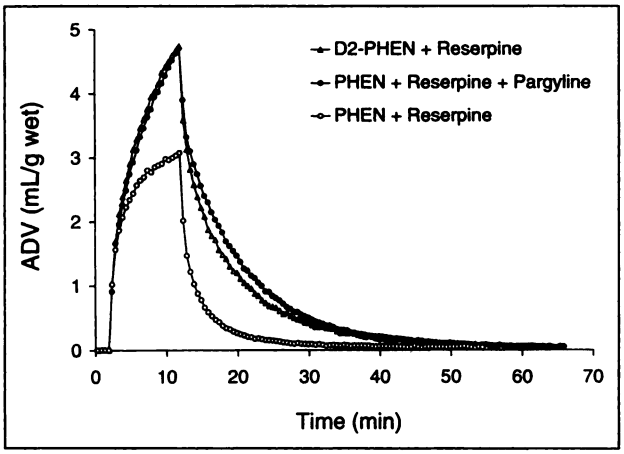


**FIGURE 2.** PHEN and D2-PHEN in hearts isolated from reserpine-treated rats. Note similarity of kinetics of PHEN during pharmacological inhibition of MAO with pargyline to those of D2-PHEN. ADV = apparent distribution volume.

## DISCUSSION

Rawlow et al. (*13*) performed studies of the uptake and metabolism of [$^3$H](−)-phenylephrine in isolated rat hearts perfused using the Langendorff technique. They showed that phenylephrine is transported by both the neuronal and extraneuronal norepinephrine uptake systems in the rat heart (uptake$_1$ and uptake$_2$). They also found that MAO metabolism of phenylephrine occurred neuronally and extraneuronally and that deamination of phenylephrine preferentially produced the glycol metabolite hydroxyphenylglycol rather than the acid metabolite hydroxymandelic acid. Thus, phenylephrine is a substrate for both uptake systems in the rat heart and is readily metabolized by MAO.

PHEN represents a structural hybrid of two previously developed markers of cardiac sympathetic neurons, HED and EPI. Removal of either the α-methyl group of HED or the parahydroxyl group of EPI yields PHEN. Because of its α-methyl group, HED does not undergo oxidative deamination by MAO. PHEN and EPI lack this α-methyl group, leaving both tracers vulnerable to catabolism by MAO. Because the $^{11}$C label is on the N-methyl group of PHEN and EPI, oxidative deamination of these two tracers by MAO generates [$^{11}$C]$CH_3NH_2$ or [$^{11}$C]methylamine. Methylamine possesses a high permeability for cellular membranes and should rapidly diffuse from the neuron (*14*). It was hypothesized that the kinetics of PHEN could provide information on neuronal MAO activity because the efflux of radioactivity would primarily reflect egress of [$^{11}$C]methylamine from the heart. Initial clinical studies comparing the kinetics of PHEN with HED in normal volunteers have shown that PHEN is initially taken up by the human heart at levels comparable to HED but has a significantly faster efflux rate (*5*). This finding is consistent with the hypothesis that the efflux of radioactivity is related to metabolism of PHEN within the neuron. However, more proof is needed to substantiate this interpretation of the clinical observations. The studies presented here were undertaken to investigate the importance of MAO activity and vesicular storage on the kinetics of PHEN in the isolated perfused rat heart.

Under control conditions, the initial uptake rate of PHEN in the isolated rat heart (0.72 mL/min/g wet) is considerably lower than the value of 2.7 mL/min/g wet measured for HED (*15*) but is comparable to the value of 0.60 mL/min/g wet measured for EPI (*16*). After the 10-min constant infusion of PHEN, washout of the tracer from the heart was best described by four exponential clearance terms. The most rapid of these clearance terms ($t_{1/2}$ ~ 5 s; amplitude ~1 mL) represented tracer being cleared from the perfusion cannulae, capillary bed and heart chambers. The three remaining terms represented clearance of tracer from myocardial tissue (extraneuronal uptake) and neuronal compartments. The largest of these three components (67% of the total heart uptake of 6.3 mL/g wet) was also the slowest ($t_{1/2}$ ~98 min). The two remaining terms were nearly equal in size (15% and 18% of the total) and had half-times around 1.8 and 13 min, respectively.

MAO blocking studies with PHEN during pargyline block or with D2-PHEN alone led to significantly longer clearance times for the largest of the three clearance processes. The initial uptake rates of D2-PHEN and PHEN during pargyline block were not significantly different from control values. Thus, the most significant change in the kinetics of PHEN during MAO block was a longer half-time for the major clearance component. For pargyline block, the washout half-time was 1.4 times longer than the control value; for D2-PHEN, it was 1.7 times longer. These results clearly indicate that MAO activity does influence the clearance kinetics of PHEN in the isolated rat heart.

Although both methods of MAO blockade slowed the efflux of radioactivity from the heart, blocking vesicular uptake with reserpine caused a dramatic acceleration in the washout kinetics. For PHEN, reserpine caused large decreases in both the size and half-time of the major clearance term. The amplitude of this component was decreased to only 5% of its control value, with the half-time decreased from 98 to 31 min. The remaining uptake in this component was probably caused by some residual vesicular uptake because it is unlikely that 100% blockade of vesicular monoamine transporters was achieved with the reserpine pretreatment. The initial uptake rate of PHEN was reduced to 46% of the control uptake rate after reserpine block. Also, it is apparent that without vesicular uptake, the kinetics of PHEN begin to approach a steady-state equilibrium during the 10-min constant infusion (Fig. 1). These findings indicate that the largest, slowest clearance component of PHEN under control conditions is associated with neuronal uptake of PHEN. Vesicular storage of PHEN is a critical process for achieving high neuronal levels of tracer under normal conditions and acts to significantly slow the efflux of radioactivity from the heart.

When reserpine block of vesicular uptake is combined with conditions in which neuronal MAO activity is inhibited, either with pargyline or D2-PHEN, further insights into the neuronal handling of PHEN are obtained. The amplitude and washout half-times of the slowest exponential were significantly reduced for D2-PHEN during reserpine block. The amplitude of this component was decreased to 17% of control with the half-time reduced from 163 to 15 min. The initial uptake rate of D2-PHEN was reduced to 55% of the control uptake rate, similar to the findings with PHEN. Thus, vesicular uptake is important for neuronal retention of D2-PHEN as well as for PHEN. The kinetics of PHEN during simultaneous inhibition of MAO with pargyline and vesicular uptake with reserpine were nearly identical to those of D2-PHEN during vesicular block (Fig. 2). This suggests that di-deuterium substitution is a very effective method of inhibiting degradation of the tracer by MAO.

The total amount of radioactivity in the heart after the 10-min constant infusion period was significantly higher during simultaneous blockade of MAO and vesicular uptake than for blockade of vesicular uptake alone ($P < 0.002$). This indicates that when vesicular uptake is blocked, significant amounts of PHEN are metabolized into [$^{11}$C]methylamine by MAO, leading to reduced accumulation of radioactivity in the heart. This indirectly validates our prediction that [$^{11}$C]methylamine would readily diffuse out of the neuron and rapidly clear from the heart tissue. If this were not the case and [$^{11}$C]methylamine accumulated in the neuronal spaces during PHEN infusion, there would not be any difference in the accumulation of total radioactivity in the heart observed with and without MAO block during reserpine block.

The observation that inhibition of MAO activity with either pargyline or di-deuterium substitution did not lead to a significantly higher initial uptake rate and the marked reductions of the initial uptake rate after inhibition of vesicular uptake suggest that under control conditions, there is a tight coupling between neuronal transport and vesicular transport of PHEN. If significant amounts of PHEN entering the neuronal axoplasm after transport by the neuronal norepinephrine transporter were immediately metabolized into [$^{11}$C]methylamine, then MAO blockade would be expected to cause a more rapid initial uptake rate. Because no significant change in the uptake rate occurred during MAO blockade, this suggests that most of the PHEN molecules initially entering the neuron are rapidly stored in vesicles. Similar results have been reported for the initial uptake of [$^{3}$H]norepinephrine in the rat heart (*17*) and rabbit heart (*18*).

Control studies with PHEN were performed in animals over a wide range of ages to see if the dramatic increases in cardiac MAO levels with age influenced the kinetics of PHEN (*9–11*). We found no age-related effects on the kinetics of PHEN, despite the fact that the hearts of the older rats had levels of cardiac MAO that were several hundred percent higher than the younger rats (*10*). This is probably because nearly all cardiac MAO is localized extraneuronally in myocytes in the rat heart (*19*). Because there are no storage vesicles within the myocytes to protect PHEN from MAO catabolism, it is likely that even in the youngest rats, PHEN is rapidly converted into [$^{11}$C]methylamine after entering the myocytes via extraneuronal uptake. Because the [$^{11}$C]methylamine produced should quickly diffuse out of the myocytes, the distribution volume for PHEN into the extraneuronal compartment is likely to be small. Thus, the age-related increases of extraneuronal MAO do not impact on the kinetics of PHEN, which are dominated by the neuronal uptake and storage of the tracer. However, it is important to point out that because PHEN is concentrated into neurons by neuronal uptake and vesicular storage, the relatively small pool of neuronal MAO in the rat heart does influence the kinetics of PHEN.

When we compared our results with PHEN to previous studies of HED and EPI in the isolated rat heart, differences in the neuronal handling of these three PET tracers become apparent (Fig. 3). As reflected in the initial uptake rates, HED is more avidly transported by the neuronal norepinephrine transporter than PHEN or EPI. Studies in which the vesicular monoamine transporter has been pharmacologically blocked have shown that all three tracers are transported into vesicles. However, there are significant differences in the ability of the vesicles to retain the tracers. It is likely that a major factor involved in the differences in vesicular storage is the lipophilicity of each tracer (*20*), as shown in Table 3. HED is the most lipophilic tracer, which allows it to quickly diffuse across the vesicular and neuronal membranes, setting up a rapid recycling of HED by cardiac sympathetic neurons (*15*). Because HED is not an MAO substrate, it can be recycled by the neuron indefinitely. EPI is the most hydrophilic of the tracers by virtue of its catechol structure, which leads to very efficient vesicular retention of EPI. Although EPI is vulnerable to neuronal MAO, efficient

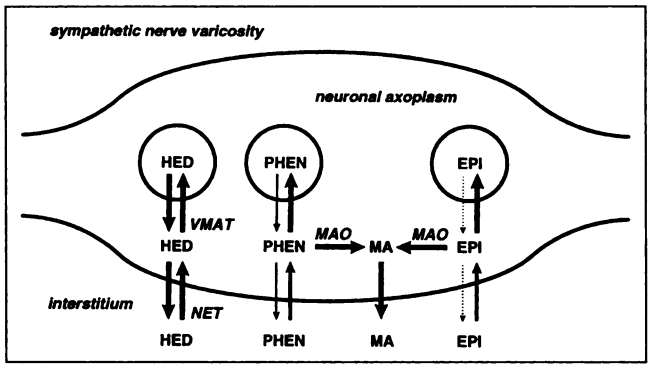


**FIGURE 3.** Neuronal handling of HED, PHEN and EPI. Circles represent storage vesicles. HED is more avidly transported by norepinephrine transporter than PHEN or EPI. Differences in lipophilicity lead to variable rates of tracer leakage from vesicles into neuronal axoplasm as well as across neuronal membranes into interstitium. NET = norepinephrine transporter; VMAT = vesicular monoamine transporter; MAO = monoamine oxidase; MA = [$^{11}$C]methylamine.

**TABLE 3**
Log *P* Values of Sympathetic Nerve Radiotracers and Norepinephrine

| Molecule | Log *P* | |
|---|---|---|
| [$^{11}$C]-*Meta*-hydroxyephedrine | +0.31 | ↑ Increasing lipophilicity |
| [$^{11}$C]-Phenylephrine | −0.30 | |
| [$^{11}$C]-Epinephrine | −1.34 | |
| Norepinephrine | −1.74 | |

Values obtained or calculated from data in Mack and Bönisch (*20*). Measured at pH 7.4.

vesicular storage causes extremely slow clearance of the tracer from the perfused rat heart under control conditions (*16*). The intermediate lipophilicity of PHEN likely leads to a slower diffusion rate across membranes relative to HED but more rapid than is observed with EPI. Evidence supporting this interpretation is the fact that MAO-resistant D2-PHEN washes out more slowly from the perfused heart than HED but more quickly than EPI. Because coronary flows in the working rat heart model at moderate workload are approximately five times higher than physiological flows, the probability that a tracer molecule that diffuses out of the neurons into the interstitium escapes into the coronary circulation is much higher than would be observed in vivo. If, for example, D2-PHEN were leaking from the neurons at the same rate as HED, one would expect a washout rate for D2-PHEN that was at least as high as that observed with HED. This is particularly true in light of the fact that D2-PHEN is not as avidly transported by the neuronal norepinephrine transporter as HED, which would make the probability of D2-PHEN escape from interstitium to coronary circulation higher than for HED. The fact that HED washes out of the perfused rat heart more quickly than D2-PHEN eliminates this possibility. Likewise, if D2-PHEN was stored as efficiently in vesicles as EPI, there would not be any washout of D2-PHEN from the isolated rat heart. Thus, the intermediate washout rate of D2-PHEN strongly suggests an intermediate level of diffusion across vesicular and neuronal membranes for PHEN and D2-PHEN. This would imply that neuronal recycling of PHEN occurs, albeit at a slower rate than HED. This recycling pool of PHEN would be expected to be slowly depleted by neuronal MAO activity, which would account for the observed slowing of the washout rate of PHEN during MAO block conditions. Because neuronal uptake and vesicular uptake appear to be tightly coupled, it seems likely that MAO degradation of PHEN occurs primarily after leakage of tracer from vesicles into the neuronal axoplasm. However, it remains to be determined whether or not any of the PHEN molecules leaking from vesicles escape across neuronal membranes into interstitium. Is neuronal MAO activity so high that no PHEN molecules diffuse from the neuronal axoplasm into the interstitium? Or is there appreciable reuptake of interstitial PHEN after leakage from the neurons? These questions will be addressed in future studies in the isolated working rat heart model.

## CONCLUSION

Studies of PHEN and the MAO-resistant analogue D2-PHEN in isolated working rat hearts showed that the efflux rate of PHEN in this model is sensitive to MAO activity. The critical importance of vesicular uptake in increasing neuronal tracer retention and greatly slowing tracer efflux was shown in studies where vesicular uptake was blocked with reserpine. Thus, although PHEN is highly susceptible to rapid metabolism by MAO, under normal conditions, vesicular storage protects the tracer from MAO. As PHEN leaks from vesicles, it is metabolized by MAO into [$^{11}$C]methylamine, which quickly diffuses from the neuron. The combination of the inherent vulnerability of PHEN to rapid MAO metabolism and the importance of vesicular storage in protecting PHEN from MAO implies that PHEN kinetics are sensitive not only to neuronal MAO activity but also to alterations in vesicular storage function.

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