Myocardial Kinetics of Carbon-11-Epinephrine in the Isolated Working Rat Heart

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The kinetics of EPI were studied in the isolated rat heart model to evaluate ^{11}C-epinephrine (EPI) as a radiotracer for the assessment of sympathetic neuronal function in the heart. **Methods:** Isolated rat hearts were perfused in a working mode. Carbon-11-EPI was added to the perfusate during wash-in period of 20 min, followed by a washout period of 40 min. Radioactivity in the heart was externally monitored and time-activity curves were recorded as a function of time. Effluent samples were collected throughout each study to determine the fraction of ^{11}C radioactivity as intact tracer. **Results:** Time-activity curves of control hearts showed that ^{11}C-EPI is taken up and retained by the myocardium. Desipramine inhibition (DMI) of uptake-1 resulted in a significant decrease in myocardial uptake and retention of ^{11}C-EPI by 91% compared to controls. Addition of DMI to the perfusion medium during washout did not affect kinetics of ^{11}C-EPI compared to control hearts. Reserpine pretreated rat hearts also showed significant decrease in tracer retention of 95% compared to controls. The metabolic data showed that, in control conditions, about 61% of ^{11}C-EPI taken up by the rat heart is rapidly metabolized and released. **Conclusion:** Carbon-11-EPI traces sympathetic nerve terminals in the isolated rat heart. Uptake blockade by DMI and reserpine suggest that uptake and storage of ^{11}C- EPI appear to be similar to that of norepinephrine. However, the prominent metabolic pathway warrants further consideration. These results suggest that ^{11}C-EPI may be a suitable radiolabeled tracer for the evaluation of sympathetic vesicular function of the heart by PET.

Key Words: carbon-11-epinephrine; cardiac sympathetic innervation; PET

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Functional integrity of the sympathetic neurons in the heart has been shown to be influenced by conditions including congestive heart failure, dysrhythmia, ischemia and diabetes (*1–5*). PET has been demonstrated to be a useful tool for noninvasive assessment of sympathetic innervation and function of the heart (*6–11*). Carbon-11- and ^{18}F-labeled catecholamines and functional analogs have been developed for the visualization of neuronal uptake, storage and metabolism of norepinephrine in the heart by PET (*12–20*). One of the catecholamine analogs, ^{11}C-hydroxyephedrine (HED), is a tracer providing important information concerning adrenergic innervation in the heart (*6,7,19–21*). However, HED is a false neurotransmitter and it is not susceptible to degradation by monoamine oxidase (MAO) or catechol-O-methyltransferase (COMT), the enzymes responsible for the metabolism of norepinephrine in the heart. Thus, its fate in the sympathetic nerve endings differs from that of norepinephrine.

To develop a tracer that more closely mimics neuronal uptake, metabolism and storage of norepinephrine, ^{11}C-labeled epinephrine, a true neurotransmitter, was recently synthesized (*16*). Previous studies with ^{3}H-epinephrine in isolated rat hearts showed that epinephrine is taken up by the heart in a similar fashion as norepinephrine, and that epinephrine and norepinephrine share a common transport mechanism into the tissue (*22–25*). Furthermore, epinephrine is also a substrate for the same enzymes MAO, COMT and phenolsulfotransferase as norepinephrine (*26*).

The purpose of this study was to characterize myocardial kinetics involved in the uptake and retention of ^{11}C-EPI in the isolated working rat heart. The isolated rat heart model has been well-established as a useful experimental model that allows control of experimental variables (*21,27*).

MATERIALS AND METHODS

Synthesis of Carbon-11-Epinephrine

Carbon-11-labeled epinephrine (^{11}C-EPI) was synthesized at the University of Michigan with the specific activity of 500–1000 Ci/mmol and radiochemical purity >95% (*16*).

Perfusion of Isolated Rat Heart

Female Sprague-Dawley rats (250–300 g) were anesthesized by intraperitoneal injection of sodium pentobarbital (15 mg/100 g body weight). The hearts were then quickly excised and the aorta cannulated for initiation of retrograde perfusion. The left atrium was then immediately cannulated and the working heart established. Hearts were perfused at a moderate work load (7.4 mmHg preload and 74 mmHg afterload) with Krebs-Henseleit bicarbonate buffer (118 m*M* NaCl, 4.7 m*M* KCl, 2.55 m*M* $CaCl_2$, 1.2 m*M* $MgSO_4$, 1.2 m*M* KH_2PO_4 and 25 m*M* $NaHCO_3$ containing 5 m*M* glucose, 20 mg/liter sodium ascorbate and 10 mg/liter EDTA) and gassed with a mixture of 95% O_2 and 5% CO_2 without recirculation. Rat hearts were perfused in the same manner as previous studies with HED by De Grado et al. (*21*).

The perfusion apparatus consisted of two parallel-perfusion circuits. One was used for perfusion with radiotracers in the perfusate during the wash-in period and the other was used for perfusion without radiotracers during the washout period. The two circuits were controlled by a three-way valve leading to the left atrial cannula. The entire apparatus was heated with circulating water at 37°C. Aortic pressure and heart rates were monitored. Measurements of coronary and aortic flows were taken every 10 min throughout the study. The hearts were not externally paced.

To study the metabolism of ^{11}C-EPI in the heart, effluent samples were collected during the study at 1, 2, 5, 10, 15, 20, 22 and 25 min after the initiation of ^{11}C-EPI perfusion. Each sample was applied to Alumina-N SEP-PAK cartridges (Millipore-Waters) and rinsed with 3 × 3 ml tris buffer to separate ^{11}C-labeled metabolites from ^{11}C-EPI, which remains on the SEP-PAK cartridge. Radioactivity in collected buffer washes and the cartridge was determined by assay in a gamma well counter. As a control, a sample of the perfusate containing only ^{11}C-EPI was also assayed for each study. Analysis of samples of perfusate and ^{11}C-EPI showed approximately 1%–2% of total radioactivity in the buffer washes (breakthrough). These values were subtracted from the fractions determined in the effluent samples.

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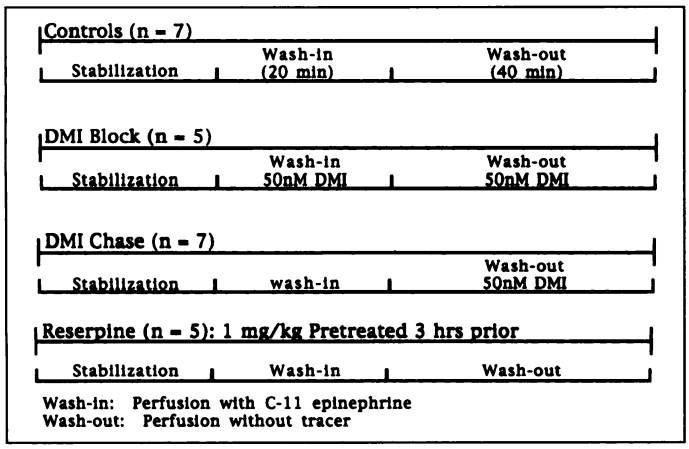

FIGURE 1. Experimental protocol. Rat hearts were perfused in four groups: control, DMI block, DMI chase and reserpine-treated hearts.

Perfusion Protocol

The study was divided into four groups: controls, DMI (desipramine) block, DMI-chase and reserpine treated. Figure 1 illustrates the experimental protocol. All hearts were initiated with a period of normal perfusion (20 min) to allow stabilization of the hearts. The control hearts were perfused with buffer without any additives. The DMI block group was perfused with desipramine chloride (50 n*M*) added to the buffer for the wash-in and washout periods. The DMI-chase group was perfused with desipramine chloride (50 n*M*) added to the buffer during the washout period only. The reserpine group was pretreated by intraperitoneal. Injection of reserpine (1.0 mg/kg) 3 hr before the study. All hearts were perfused with ^{11}C-EPI (300–450 μCi/liter) added to the buffer for 20 min (wash-in period) followed by 20 min of washout with buffer without tracer.

At the end of the study, hearts were placed immediately in chilled buffer, the ventricles were opened and excess fluids were gently blotted on a paper towel. The hearts were then weighed and counted in a NaI well-counter (CPM/g heart). The radioactivity of the perfusate (CPM/ml perfusate) was also obtained by well counting. To account for all the radioactivity, the paper towel was also counted.

Time-Activity Curves

Carbon-11 radioactivity was measured using a pair of BGO detectors interfaced with a personal computer. Coincidence events as well as single and random events were sampled every second. The time-activity curves were generated by subtraction of random events from the coincidence events. Each curve was corrected for radioactive decay. In addition, the curves were normalized to the radioactivity of the perfusate using a procedure previously described by De Grado et al. (*21*). Each curve was multiplied by a calibration factor f_c determined from the individual study:

$$f_c = ((\text{CPM/g heart})/(\text{CPM/ml perfusate}))/A_e$$

and

A_e = average coincident activity over the last 10 sec of data acquisition.

The accumulation of ^{11}C radioactivity rate constant, K_i, was determined by the slope calculated from the linear regression of the normalized time-activity curves from 10–20 min during wash-in. Clearance rate constant, k_2, was determined from the exponential fit of the curves from 10–30 min during washout.

Metabolite Analysis

SEP-Pak analysis of ^{11}C-EPI and metabolites gives the fraction of the ^{11}C activity of the venous effluent as ^{11}C-EPI (f_e) and metabolites (f_m). Since the a steady state was reached after only a few min, the accumulation rate ^{11}C in the isolated rat heart can be described by the equation:

$$R_i = R_u - R_m, \quad \text{Eq. 1}$$

where R_i = accumulation rate of ^{11}C radioactivity in the rat heart at steady state = K_i from time activity curve,

R_u = steady-state uptake rate

R_m = steady-state metabolic rate.

It is assumed that no ^{11}C-EPI metabolites are retained by the myocardium, and no back-diffusion of ^{11}C-EPI as indicated by DMI chase studies. The fraction of metabolites in the coronary effluent is given by:

$$f_m = R_m/F_c, \quad \text{Eq. 2}$$

where F_c is the coronary flow rate (ml/g/min). From Equations 1 and 2,

$$R_m = f_m F_c \quad \text{Eq. 3}$$

$$R_u = R_i + f_m F_c. \quad \text{Eq. 4}$$

Thus, the ratio of metabolic rate to uptake rate of ^{11}C-EPI in the isolated rat heart is estimated as:

$$R_m/R_u = f_m F_c/(R_i + f_m F_c). \quad \text{Eq. 5}$$

Statistical Analysis

Results are expressed as mean ± s.d. Statistical significance of the differences among the study groups were tested by Student's t-test. Values at $p \leq 0.05$ were considered significant.

RESULTS

Hemodynamic Data

Hemodynamic parameters are shown in Table 1 with heart rate, pressure, coronary flow and cardiac output defined as the sum of measured coronary and aortic flows. Hemodynamic data shown are the averaged measurements during the study. There were no significant differences in the hemodynamic data in the treated groups as compared with the control groups. However, in a small number of experiments, increases in heart rate were observed during tracer perfusion and returned to baseline values during washout. Cardiac output, diastolic and systolic pressures did not change significantly during ^{11}C-EPI perfusion in all hearts.

Neuronal Selectivity of EPI

Figure 2 illustrates representative time-activity curves of control, DMI-chase, DMI blocked and reserpine treated hearts. The time-activity curve of the control heart (Fig. 2A) shows that ^{11}C-EPI is taken up without approaching equilibrium during the 20 min of tracer perfusion. During the wash-in period, accumulation of ^{11}C radioactivity is linear over the interval of 10–20 min with K_i of 0.60 ± 0.20 ml/g/min. During washout, a brief period of fast clearance of tracer in the first few minutes reflects clearance of tracer from the vascular and extraneuronal spaces. This is followed by a much slower clearance of radioactivity from the myocardium. The monoexponential clearance rate constant, k_2, was estimated using data from 10–30 min of washout. The slow clearance of the tracer, 0.00121 ± 0.00114 min^{-1} during washout in control hearts, indicates avid retention of ^{11}C-EPI in the myocardium.

To investigate the non-neuronal uptake of ^{11}C-EPI, neuronal uptake (uptake-1) was blocked with DMI, a known uptake-1 inhibitor. Compared to the control hearts, uptake and retention

TABLE 1
Hemodynamic Patterns

	F_c (ml/min/g)	CO (ml/min/g)	HR (wash-in) min^{-1}	HR (washout) min^{-1}	Diastolic pressure (mmHg)	Systolic pressure (mmHg)
Control group	13.36 ± 3.52	48.09 ± 11.22	243 ± 45	209 ± 55	45 ± 15	100 ± 11
DMI block	14.10 ± 1.26	48.38 ± 12.26	258 ± 65	193 ± 58	51 ± 4	103 ± 12
DMI chase	12.51 ± 7.86	41.82 ± 6.16	180 ± 89	170 ± 74	51 ± 7	103 ± 13
Resperpine	13.10 ± 6.58	47.08 ± 12.41	231 ± 37	174 ± 88	46 ± 8	105 ± 8

F_c = coronary flow; CO = cardiac output (aortic flow + coronary flow); HR = heart rate; All parameters are expressed as mean ± s.d.

of ^{11}C-EPI in DMI-treated hearts were significantly reduced (Fig. 2B, Table 2). DMI block hearts showed accumu lation of ^{11}C radioactivity approaching equilibrium after approximately 3–4 min of wash-in and rapid clearance of ^{11}C radioactivity during washout. The accumulation rate constant, K_i, was 14-fold slower and the clearance rate constant, k_2, was sixfold faster in DMI-blocked hearts compared to control hearts (Table 2). Myocardial retention of ^{11}C radioactivity among the different experimental groups at various time points during wash-in and washout periods are also presented in Table 2 to allow comparison of tracer accumulation and clearance. Hearts perfused with DMI consistently showed significantly lower retention compared to controls at all time points. After 30 min of washout, tracer retention in DMI blocked hearts was reduced by nearly 91% compared to the control hearts. A trend toward decreased steady-state uptake rate, R_u, was also observed with blockade of neuronal uptake by DMI, but the difference (1.21 ± 0.20 vs 1.64 ± 0.66) did not reach statistical significance (Table 3).

Retention Mechanism of Carbon-11-EPI

To further study the influence of uptake-1 on the retention of EPI, neuronal reuptake of the tracer was blocked with DMI. Blockade of uptake-1 during the washout period in the DMI-chase hearts did not affect clearance of ^{11}C radioactivity compared to control hearts (Fig. 2C versus Fig. 2A). Myocardial retention values as well as kinetics parameters (Table 2) also were not significantly different from control hearts at all time points, indicating that neuronal retention of ^{11}C radioactivity is independent of uptake-1 activity.

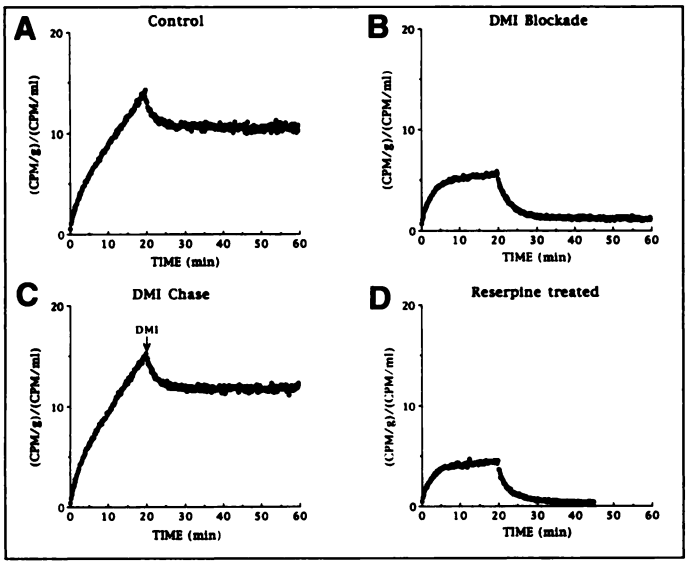

FIGURE 2. Representative time-activity curves of (A) a control heart, (B) a DMI-blocked heart, (C) a DMI-chase heart and (D) a reserpine-treated heart. All curves are decay-corrected and normalized to the radioactivity of the perfusate. Perfusion with ^{11}C-EPI during wash-in period and without tracer during washout period.

To observe the role of vesicular storage of ^{11}C-EPI, rats were pretreated with the vesicular blocker reserpine. Time-activity curves of reserpine treated hearts show dramatic reduction in uptake and retention of ^{11}C radioactivity even greater than that of DMI blocked hearts (Fig. 2D, Table 3). Tracer uptake approaches saturation after approximately 3–4 min of tracer perfusion. Retention value for reserpine-treated hearts after 30 min of washout was 0.53 ± 0.17 ml/g/min, representing a 95% reduction compared to control hearts (10.31 ± 2.62 ml/g/min). In addition, K_i value was 18-fold slower and k_2 was 26-fold faster compared to control hearts. These results suggest that accumulation of ^{11}C radioactivity by the heart is mainly dependent on vesicular storage of ^{11}C-EPI in the sympathetic neuron.

Metabolic Data

Table 3 shows the metabolic data expressed as percent of metabolite (f_m) in the total radioactivity in the collected effluent samples. The relatively small fraction of metabolites detected in the effluent during the wash-in period primarily reflects appearance of intact ^{11}C-EPI in the coronary effluent that was not extracted by the heart. Metabolite level approaches a steady-state after 5 min as the increase in metabolite level is small between 5 min and 20 min during tracer wash-in.

Evaluation of Equation 5 for control conditions (f_m = 0.08 ± 0.03, f_c = 13.4 ± 3.5 ml/min/g, R_i = 0.60 ± 0.2 ml/min/g heart) gives the ratio of metabolic rate to uptake rate of ^{11}C-EPI (R_m/R_u = 0.61 ± 0.12). The estimated uptake rate of ^{11}C-EPI into control hearts is R_u = 1.64 ± 0.66 ml/g/min (Eq. 4). Thus, a major fraction of the tracer taken up by the isolated rat heart appears to be rapidly metabolized. Metabolite data of ^{11}C-EPI during the washout period where the radioactivity in the effluent is derived only from the activity released from the heart shows that the majority of the radioactivity leaving the heart is in the form of metabolites, suggesting that ^{11}C-EPI in the axoplasm and myocytes is metabolized and released. The remainder is stored within the neuronal storage vesicles, protected from metabolic enzymes.

Although the estimated rate of metabolism of ^{11}C-EPI, R_m, was not significantly different for the treated hearts compared to controls (Table 3), the ratios of metabolic rate to steady-state uptake rate, R_m/R_u, were signifcantly higher for both DMI-blocked and reserpine-treated hearts as compared to controls.

DISCUSSION

Neuronal Uptake and Disposition of Carbon-11-EPI

Figure 3 illustrates a conceptual model of the fate of ^{11}C-EPI in the isolated rat heart. Similar to norepinephrine, ^{11}C-EPI diffuses from blood vessels into the neuronal cleft and is avidly taken up by the sympathetic nerve terminals through the sodium

TABLE 2
Myocardial Retention of Carbon-11-EPI

	^{11}C-EPI (ml perfusate/g heart)					
	Wash-in		Washout			
	10 min	20 min	15 min	30 min	K_i (ml/g/min)	k_2 (10^{-3} × 1/min)
Control group	8.90 ± 2.51	14.08 ± 4.24	11.43 ± 3.97	10.31 ± 2.62	0.596 ± 0.198	1.21 ± 1.14
DMI block	4.95 ± 0.53*	5.41 ± 0.47*	1.21 ± 0.13*	0.99 ± 0.17*	0.043 ± 0.006*	7.70 ± 4.70*
DMI chase	9.04 ± 0.99	13.36 ± 2.81	10.25 ± 2.88	9.85 ± 2.73	0.531 ± 0.116	1.26 ± 1.17
Resperpine	5.31 ± 1.76*	4.78 ± 1.61*	0.61 ± 0.15*‡	0.53 ± 0.17*‡	0.034 ± 0.010*	31.02 ± 10.88*†

*$p < 0.0001$ compared to control group.
†$p < 0.005$.
‡$p < 0.0004$ compared to DMI block.
Retention of ^{11}C-EPI at 10 min and 20 min of wash-in period, and at 15 min and 30 min of washout period; K_i = accumulation of ^{11}C radioactivity rate constant; K_2 = clearance rate constant. All values are expressed as mean ± s.d.

dependent active uptake-1 mechanism. This is supported by the results from the DMI blocking study, which showed a 91% inhibition of tracer retention after 30 min of washout perfusion period. Previous in vivo investigations in rats pretreated with DMI also reported a 91% inhibition of ^{11}C-EPI uptake by the left ventricle compared to control rats (*28*). Similarly, PET studies using ^{11}C-EPI in patients with recent heart transplants, a model for denervation, showed significantly low uptake compared to normal volunteers (unpublished data). These results confirm the neuronal uptake of ^{11}C-EPI in the heart.

Inside the nerve terminals, ^{11}C-EPI appears to be either rapidly metabolized or taken up and retained by the storage vesicles. Unlike previous studies in the isolated rat heart with HED (*21*), tracer kinetics of ^{11}C-EPI appeared to be unaffected by DMI blockade during washout as illustrated by Figure 2C representing a DMI chase study. The results suggest that, unlike HED, the retention of ^{11}C-EPI is not dependent on neuronal reuptake but on the vesicular storage of the tracer. Reserpine-treated rat heart studies confirm the necessity of the functional neuronal vesicles to retain ^{11}C-EPI. While HED is a sensitive marker for uptake-1 activity, ^{11}C-EPI is likely more sensitive to vesicular storage function. The slower release of vesicular ^{11}C-EPI from the vesicle relative to HED is a consequence of the presence of the catechol function that decreases its lipophilicity and aids in the intravesicular binding.

With uptake-1 transport intact in the reserpine-treated hearts, higher uptake of ^{11}C-EPI compared to DMI-blocked hearts would be expected. However, tracer retention values and K_i in resperpine-treated hearts were not significantly different from DMI-treated hearts during wash-in (Table 2). Moreover, retention were significantly lower and clearance rate, k_2, was significantly faster in reserpine hearts compared to DMI hearts during the washout period. One explanation for this discrepency is the possibility of increase in non-neuronal uptake due to DMI blockade. Studies using the dog model by Eisenhofer et al. (*26*) demonstrated that desipramine decreased cardiac spillover of adrenaline during sympathetic stimulation compared to measurements made before blockade with desipramine due to extraneuronal removal. Furthermore, without the neuronal storage vesicles, unstored epinephrine in the neurons also is vulnerable to metabolic enzymes, which is likely responsible for the rapid washout of ^{11}C-activity in the reserpine-treated hearts. Therefore, the combination of increased extraneuronal retention in DMI-blocked hearts and avid neuronal MAO degradation of unstored ^{11}C-EPI in reserpine hearts is likely the reason for the similarity in the uptake and the faster washout of the tracer in reserpine treated hearts compared to DMI hearts.

Metabolism of Carbon-11-EPI

Evidence of metabolism of ^{11}C-EPI is shown in results from assays of collected effluent samples during the study (Table 3). In previous studies, radio-HPLC analysis of heart tissue in rats for metanephrine, a metabolic product of epinephrine, showed that 16% of the radioactivity in the tissue is in form of metabolites 5 min after tracer injection; however, less than 2% was detected after 30 min (*28*). These results indicate that ^{11}C-EPI is metabolized in the heart, but the ^{11}C-labeled metabolites are not retained. Therefore, the retention of ^{11}C-EPI reflects neuronal retention and not accumulation of ^{11}C metabolites.

The two most predominant enzymes responsible for metabolism of norepinephrine and epinephrine are MAO and COMT, which are found in most tissues. Vesicular blockade by reserpine should have resulted in an increase in MAO metabolism of ^{11}C-EPI, since there would be more tracer in the neuron varicosities susceptible to metabolism by MAO. Although

TABLE 3
Metabolic Data

	Wash-in f_m (%)						Washout f_m (%)
	1 min	5 min	20 min	R_m (ml/g/min)	R_u (ml/g/min)	R_m/R_u	5 min
Control group	3.7 ± 3.1	9.0 ± 4.8	10.33 ± 2.3	1.04 ± 0.51	1.64 ± 0.66	0.61 ± 0.12	82.1 ± 10.1
DMI block	2.4 ± 0.9	5.2 ± 0.9	8.3 ± 0.6	1.17 ± 0.20	1.21 ± 0.20	0.96 ± 0.01*	85.8 ± 6.8
DMI chase	3.1 ± 2.5	6.3 ± 6.5	7.6 ± 4.9	1.00 ± 0.24	1.53 ± 0.27	0.65 ± 0.08	83.2 ± 9.3
Reserpine	2.8 ± 3.3	6.6 ± 3.5	11.6 ± 7.9	1.39 ± 0.15	1.42 ± 0.15	0.98 ± 0.01*	89.7 ± 3.4

*$p < 0.0001$ compared to control group.
f_m = percentage of ^{11}C radioactivity in coronary effluent samples in the form of metabolites of ^{11}C-EPI (mean ± s.d.); R_m = steady-state metabolic rate of ^{11}C-EPI; R_u = steady-state uptake rate of ^{11}C-EPI.

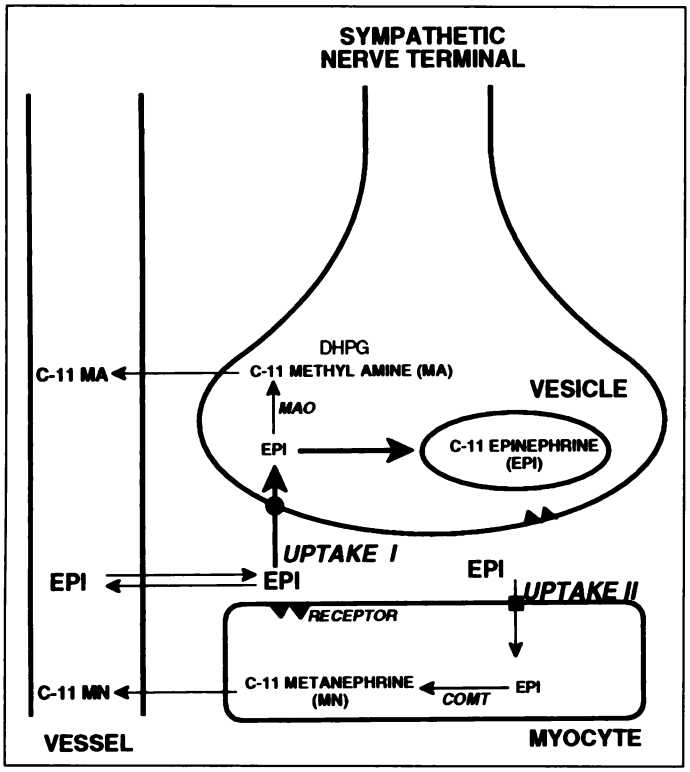

FIGURE 3. A conceptual model of the fate of ^{11}C-EPI in the sympathetic presynaptic nerve terminal. Carbon-11-EPI is taken up by the neuron by uptake-1 and retained in the storage vesicles. Carbon-11-EPI is also sensitive to metabolism and radiolabeled metabolic products are excreted.

levels of excreted metabolites (f_m) detected from reserpine-treated hearts did not differ significantly from control hearts, ratio of rate of metabolism to uptake rate was significantly higher. However, this ratio as well as rate of metabolism, R_m, were only slightly greater, but not significantly, than the values in DMI-blocked hearts (Table 3). These data suggest that extraneuronal metabolism plays a greater role than intraneuronal metabolism of ^{11}C-EPI by MAO, and that uptake is rate-limiting for the production and release of metabolites from the heart in this rat heart model. Reports by Iversen et al. (*23*) also showed that epinephrine is more sensitive than norepinephrine to COMT inhibition, and that epinephrine has a higher affinity for uptake-2 transport but lower affinity for uptake-1 in comparison with norepinephrine. Furthermore, metabolic rate estimated for the DMI-blocked hearts, which should have derived mainly from extraneuronal metabolism, were not significantly higher than values from control hearts (1.17 ± 0.20 versus 1.04 ± 0.51).

Due to limitations of the assay performed, identification of the type of metabolites excreted was not possible. Therefore, the location of ^{11}C-EPI metabolism in this study is inconclusive. Another limitation is that the metabolites were determined as fractions of total radioactivity in the effluent, which also contained ^{11}C-EPI in the perfusate that was not extracted by the heart. Thus, the fractions are relatively small. Further investigations are necessary to clarify the metabolic fate of ^{11}C-EPI in the heart. Despite these limitations, the ratio of metabolism to uptake rate in control hearts is approximately 61%. The important point of these results is that ^{11}C-EPI, unlike HED, is metabolized in the heart, which needs to be considered in the interpretation of results from scintigraphic applications. PET studies using ^{11}C-EPI in humans confirm these findings as high levels of ^{11}C metabolites detected in the blood, and correction for metabolite radioactivity in the arterial input function used in calculating tracer retention in the tissue was necessary (unpublished data).

Extra-Neuronal Uptake of EPI

Previous investigations have shown that extraneuronal uptake of epinephrine is greater than that of norepinephrine (*25,26*). Uptake of ^{11}C-EPI in DMI-blocked hearts appears to approach a saturation level of approximately 5 ml/g after 20 min of tracer wash-in period (Table 2). Since DMI is a highly efficient uptake-1 inhibitor, the accumulation of radioactivity during wash-in mainly reflects the accumulation of tracer in the heart chambers and cannulae in the field of view of the detectors and extraneuronal uptake. The vascular volume and extracellular space of the rat hearts have been approximated at 1.1 ml/g (*21*). Thus, extraneuronal uptake of ^{11}C-EPI is apparent in this isolated rat heart model. In previous study with HED, DeGrado et al. (*21*) explained that the absence of plasma proteins and blood cells in the perfusate may contribute to higher extraneuronal extraction of tracer in the isolated rat heart than would be expected in vivo. This effect is due to the superficially high partition ratio of tissue to perfusate in the isolated rat heart. The same study also suggests that uptake-1 blockade by DMI may lead to greater accumulation of tracer in the interstitial space during wash-in compared to control hearts, which may allow greater extraneuronal extraction of tracer. Moreover, Eisenhofer et al. (*26*) reported a decrease in plasma spill-over of epinephrine during sympathetic stimulation in dogs with DMI blockade compared to controls, suggesting greater efficiency in extraneuronal uptake with DMI blockade. Unfortunately, uptake-2 blockade was not available at the time of this study. Thus, additional studies are necessary to determine the extent of extraneuronal uptake of ^{11}C-EPI.

Nevertheless, the maintained metabolite production in the presence of DMI indicates that extraneuronal uptake and metabolism are significant clearance pathways for ^{11}C-EPI. Careful consideration should be given to the influence of metabolic pathways on measurements of ^{11}C-EPI retention attributed to vesicular storage function; retention of ^{11}C-EPI by the heart may also be related in an inverse manner to the flux of ^{11}C-EPI through extraneuronal or neuronal metabolic pathways. It is conceivable that changes in the flux of metabolic pathways could confound differences in the vesicular retention of ^{11}C-EPI.

In addition, the low sensitivity of the estimated ^{11}C-EPI influx rate (R_u) to neuronal uptake blockade with DMI indicates rate limitation preceeding cellular uptake of the tracer. This observation is consistent with previous observations in the same isolated rat heart model with the catecholamine analog tracer metaiodobenzylguanidine, which is also a substrate of both uptake-1 and uptake-2 mechanisms in the rat heart (*29*).

CONCLUSION

The results of this study show that ^{11}C-EPI may provide unique information for the evaluation of sympathetic innervation of the heart by PET. Blocking studies with DMI show that uptake of ^{11}C-EPI utilizes the active neuronal transport mechanism, uptake-1. Results from DMI chase and vesicular blockade by reserpine indicate that retention of ^{11}C-EPI is strongly dependent on storage by neuronal vesicles. Furthermore, data from metabolite assay suggest that unstored ^{11}C-EPI undergoes metabolism, and metabolites are rapidly cleared from the heart. Carbon-11-EPI appears to share the uptake, metabolic and storage mechanisms as norepinephrine in the isolated rat heart. Therefore, ^{11}C-EPI appears to show potential as a tracer for the indication of neuronal uptake, metabolism and vesicular storage

of catecholamines by the heart using PET. However, the interplay between metabolism and storage of ^{11}C-EPI in normal and specific disease conditions warrants further investigation.

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REFERENCES

1. Chierchia S, Davies G, Berkenboom G, Crea F, Crean P, Meseri A. Alphaadrenergic receptors and coronary spasm: an elusive link. *Circulation* 1984;69:8.
2. Watkins PJ, Mackay JD. Cardiac denervation in diabetic neuropathy. *Ann Intern Med* 1980;92:304–7.
3. Corr PB, Gillis RA. Autonomic neural influence on the dysrhythmias resulting from myocardial infarction. *Circ Res* 1978;43:1.
4. Lown B, Verrier RL, Rabinowitz SH. Neural and psychologic mechanisms and the problem of sudden death. *Am J Cardiol* 1977;39:890.
5. Lown B, Verrier RL. Neural activity and ventricular fibrillation. *N Engl J Med* 1976;294:1165.
6. Allman KC, Stevens MJ, Wieland DM, et al. Noninvasive assessment of cardiac diabetic neuropathy by carbon-11 hydroxyephedrine and positron emission tomography. *JACC* 1993;22:1425–1432.
7. Allman KC, Wieland DM, Muzik O, DeGrado TR, Wolfe ER, Schwaiger M. Carbon-11 hydroxyephedrine with positron emission tomography for serial assessment of cardiac adrenergic neuronal function after acute myocardial infarction in humans. *JACC* 1993;22:368–375.
8. Goldstein DS, Chang PC, Eisenhofer G, et al. Positron emission tomographic imaging of cardiac sympathetic innervation and function. *Circulation* 1990;81:1606–1621.
9. Schwaiger M, Guibourg H, Rosenspire K, et al. Effect of regional myocardial ischemia on sympathetic nervous system as assessed by fluorine-18-metaraminol. *J Nucl Med* 1990;31:1352–1357.
10. Schwaiger M, Kalff V, Rosenspire K, et al. Noninvasive evaluation of sympathetic nervous system in human heart by positron emission tomography. *Circulation* 1990;82:457–464.
11. Eisenhofer G, Hovevey-Sion D, Kopin IJ, et al. Neuronal uptake and metabolism of 2- and 6-fluorodopamine: false neurotransmitters and positron emission tomographic imaging of sympathetically innervated tissues. *J Pharmacol Exp Ther* 1989;248:419–429.
12. Fowler JS, Ansari AN, Atkins HL, et al. Synthesis and preliminary evaluation in animals of carrier-free ^{11}C-1-dopamine hydrochloride. *J Nucl Med* 1973;14:867–869.
13. Ding YS, Fowler JS, Gatley SJ, et al. Synthesis of high specific activity 6-[^{18}F]fluorodopamine for positron emission tomography studies of sympathetic nervous tissue. *J Med Chem* 1991;34:861–863.
14. Ding YS, Fowler JS, Gatley SJ, et al. Synthesis of high specific activity (+) and (−)-6-[^{18}F]fluoronorepinephrine via the nucleophilic aromatic substitution reaction. *J Med Chem* 1991;34:861–863.
15. Mislanka SG, Gildersleeve DL, Toorongian SA, et al. 6[^{18}F]fluoro-metaraminol: a radiotracer for in vivo mapping of adrenergic nerves of the heart. *J Med Chem* 1988;31:362–366.
16. Chakraborty PK, Gildersleeve DL, Toorongian SA, et al. Synthesis of [^{11}C]epinephrine and other biogenic amines by direct methylation of normethyl precursors. *J Lab Compd Radiopharm* 1992;32:172–173.
17. Wieland DM, Rosenspire KC, Hutchins GD, et al. Neuronal mapping of the heart with 6-[^{18}F]fluorometaraminol. *J Med Chem* 1990;33:956–964.
18. 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.
19. Schwaiger M, Hutchins GD, Kalff V, et al. Evidence for regional catecholamine uptake and storage sites in the transplatned human heart by positron emission tomography. *J Clin Invest* 1991;87:1681–1690.
20. Wolpers HG, Nguyen N, Rosenspire KC, et al. Carbon-11-hydroxyephedrine as marker for neuronal marker for neuronal carecholamine retetion in reperfused canine myocardium. *Coron Artery Dis* 1991;2:923–929.
21. DeGrado TR, Hutchins GD, Toorongian SA, Wieland DM, Schwaiger M. Myocardial kinetics of carbon-11-metahydroxyephedrine: retention and effects of norepinephrine. *J Nucl Med* 1993;34:1287–93.
22. Iversen LL. The uptake of noradrenaline by the isolated perfused rat heart. *Br J Pharmacol* 1963;21:523–537.
23. Iversen LL. The uptake of adrenaline by the isolated perfused rat heart. *Br J Pharmacol* 1965;24:387–394.
24. Iversen LL. Inhibition of noradrenaline uptake by drugs. *J Pharm Pharmacol* 1965;17:62–64.
25. Iversen LL, Glowinski J, Axelrod J. The uptake and storage of ^{3}H-norepinephrine in the reserpine-pretreated rat heart. *J Pharm Exp Ther* 1965;150:173–183.
26. Eisenhofer G, Smolich J, Esler M. Disposition of endogenous adrenaline compared to noradrenaline relased by caridac sympathetic nerves in the anaesthetized dog. *Arch Pharmacol* 1992;345:160–171.
27. Taegtmeyer H, Hems R, Krebs HA. Utilization of energy-providing substrates in the isolated working rat heart. *Biochem J* 1980;186:701–711.
28. Wieland DM, Chakraborty PK, Gildersleeve DL, et al. Comparison of true and false neurotransmitters in the heart: evaluation of R-(−)-[^{11}C] epinephrine in animals [Abstract]. *J Nucl Med* 1993;34(suppl):123P.
29. DeGrado TR, Zalutsky MR, Vaidyanathan G. Uptake mechanisms of meta-iodobenzylguanidine uptake and retention in isolated rat heart. *Nucl Med Biol* 1995;22:1–12.

Comparison of Motion Correction Algorithms for Cardiac SPECT

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Patient motion remains a significant source of unsatisfactory cardiac SPECT examinations. The extent to which image recovery can be achieved with correction algorithms is unknown. **Methods:** Nine subjects who had completed motion-free redistribution ^{201}Tl cardiac SPECT subsequently underwent simultaneous dual-isotope (^{201}Tl/^{99m}Tc) SPECT with a ^{99m}Tc cutaneous point source, while the imaging table was subjected to predefined nonreturning y-translation movements. Cardiac reconstructions, marker reconstructions and marker-compressed dynamic images were generated from the raw data after applying the following correction methods: diverging squares, cross-correlation of the cardiac data and cross-correlation of the marker. **Results:** Marker cross-correlation performed significantly better than all other methods with good-excellent results in all evaluations. This compared with good-excellent results in none of 27 for the raw data, in 13 of 27 for cardiac cross-correlation and in 7 of 27 for diverging squares ($p < 10^{-5}$). The superiority of the marker-based method was confirmed on analysis of bullseye difference maps and quantitation of residual motion in the point-source data. **Conclusion:** Motion artifacts can accurately be detected and corrected using cross-correlation of an external point-source. Furthermore, this technique provides useful independent information on the degree of image recovery.

Key Words: thallium-201; SPECT; motion artifacts; myocardial perfusion

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Patient motion can seriously degrade clinical SPECT imaging, and motion effect has been particularly well studied in relation to myocardial perfusion scintigraphy (*1–4*). Although many algorithms have been proposed for the identification of cardiac motion, some of these are strictly visual while others require operator intervention for motion correction. While the use of a cutaneous point-source has been advocated for the qualitative

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