Myocardial Kinetics of Carbon-11-Meta-Hydroxyephedrine: Retention Mechanisms and Effects of Norepinephrine

Timothy R. DeGrado,* Gary D. Hutchins,† Steven A. Toorongian, Donald M. Wieland and Markus Schwaiger

Department of Internal Medicine, Division of Nuclear Medicine, University of Michigan Medical Center, Ann Arbor, Michigan

Carbon-11-labeled meta-hydroxyephedrine (HED, *N*-methyl-metaraminol) is a catecholamine analog developed for the PET imaging of sympathetic nerve terminals of the heart. The retention mechanisms of this tracer and interactions with norepinephrine were investigated in isolated working rat hearts. Externally monitored time-activity curves showed a strong uptake process in control hearts (K_1 = 2.66 ± 0.39 ml/g/min) and relatively slow monoexponential clearance rates (k_2 = 0.011 ± 0.003 min^{-1}). Comparative studies with the neuronal uptake inhibitor desipramine indicated little extraneuronal distribution and a strong dependence of clearance rate on neuronal reuptake of tracer. Norepinephrine (≤10 n*M*) increased HED clearance rate without affecting initial uptake rates. This effect may be related to competitive inhibition of neuronal reuptake and/or accelerated neuronal release of HED. These results indicate that the uptake and retention of HED by the myocardium is highly specific to sympathetic nerve terminals. However, its retention in the myocardium is not directly related to neuronal processing of catecholamines (i.e., metabolism and vesicular turnover). Thus, important differences may exist in the physiologic information indicated by retention measurements of HED and radiolabeled catecholamines. The finding of increased clearance rates with NE in the perfusion medium recommends the consideration of potential effects of circulating and endogenous catecholamines on PET measurements of myocardial retention of HED, especially in subjects with elevated plasma catecholamines or high sympathetic tone.

J Nucl Med 1993; 34:1287–1293

Positron emission tomography (PET) in conjunction with radiolabeled catecholamine analogs allows noninvasive assessment of sympathetic neuronal integrity in the human heart (*1–3*). The development of radiotracers and techniques has followed one of two general strategies. In the first approach, epinephrine (EPI), norepinephrine (NE) and dopamine (DA) have been labeled with ^{11}C or ^{18}F to mimic neuronal uptake (uptake-1), metabolism and vesicular storage of these catecholamines (*1,4–7*). Metabolism of radiolabeled catecholamines by monoamine oxidase (MAO) or catechol-O-methyl-transferase (COMT) give rise to metabolites that clear the myocardium. Therefore, the retention of radioactivity in the myocardium primarily reflects storage of labeled catecholamines within adrenergic vesicles in sympathetic nerve terminals. Qualitative interpretation of PET images may provide useful information concerning the uptake and disposition of catecholamines (*1*).

In a second approach, ^{11}C- and ^{18}F-labeled catecholamine analogs, many of which are false adrenergic transmitters, have been developed as tracers of NE uptake by sympathetic nerve terminals (*8–11*). These radiotracers are thought to share the same uptake, storage and release pathways as NE. Because they are not metabolized by MAO or COMT in the myocardium, their more simple disposition in comparison to the natural catecholamines may allow the application of tracer kinetic modeling techniques to PET data (*12*). Of this group of tracers, ^{11}C meta-hydroxyephedrine (HED, *N*-methylmetaraminol) has shown excellent characteristics for PET imaging of the heart (*3,13*). Preliminary studies with HED in an experimental model of ischemia (*14*) and in transplanted human heart (*3,13*) indicate that HED uptake and retention by the myocardium specifically reflects the functional integrity of the sympathetic neurons. Metabolites of HED are present in blood after intravenous administration in the guinea pig but are formed outside the heart and do not significantly accumulate in the myocardium (*10*).

Although preliminary in vivo studies indicate neuronal uptake of HED (*10*), questions remain regarding the distribution and turnover of the tracer in the neuron and the processes that critically influence the overall clearance rate from tissue. Furthermore, the relationship of HED kinetics to the uptake and utilization of catecholamines by the sympathetic nerve terminal has not been clarified. Since HED competes with catecholamines for carrier-facilitated transport processes, effects due to variation in circulating cate-

Received Dec. 3, 1992; revision accepted Apr. 1, 1993.

For correspondence or reprints contact: Timothy R. DeGrado, PhD, Duke University Medical Center, Radiology Dept., Box 3808, Durham, NC 27710.

*Present address: Duke University Medical Center, Radiology Dept., Box 3808, Durham, NC 27710.

†Present address: Indiana University Medical Center, Dept. of Radiology, 541 Clinical Dr., Indianapolis, IN 46202-5111.

cholamine levels or sympathetic nervous tone potentially complicate the interpretation of HED kinetics in cardiac patients studied with PET.

In this work, an isolated working rat heart preparation is utilized to study the mechanisms of HED uptake and retention and the effects of competition with NE, the true adrenergic transmitter. The isolated rat heart is an established experimental model for studying catecholamine uptake and retention mechanisms (*15–18*). It allows complete control of experimental conditions (i.e., concentration of substrates and inhibitors) and avoids systemic recirculation of tracer and radiolabeled metabolites. HED kinetics in the heart are directly monitored by an external detection technique.

METHODS

Synthesis of Radiotracer

Carbon-11 HED was synthesized in our laboratory as previously described (*10*). Radiospecific activity of this tracer was >1500 Ci/mmol.

Perfusion of Isolated Working Rat Hearts

Hearts were excised from pentobarbital-anesthetized, female, Sprague Dawley rats (225g–275g). Following cannulation of the aorta, retrograde perfusion of the hearts was initiated (aortic pressure = 60 mmHg). Without delay, the left atrium was cannulated and working perfusion established as previously described (*19*). Hearts were perfused at moderate workload (preload = 7.4 mmHg; afterload = 74 mmHg). The perfusion medium was a modified Krebs-Henseleit (K-H) 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 and gassed with 95% O_2 and 5% CO_2. Sodium ascorbate (20 mg/liter) and EDTA (10 mg/liter) were added to prevent auto-oxidation of catecholamines in the perfusion medium (*16*). The perfusion apparatus utilized two parallel perfusion circuits. One circuit was utilized for administration of radiotracer to the hearts (washin). The other circuit did not contain radiotracer and was used for perfusion before and after the washin period. Input to the heart was switched between the two circuits by means of a three-way valve just preceding the left atrial cannula. The apparatus utilized water-jacketed vessels and a heater/circulator to deliver the medium to the heart at a temperature of 37°C. Aortic pressure was monitored by a pressure transducer and allowed measurement of heart rate, diastolic and systolic pressures. Coronary and aortic flows were measured manually. Neither coronary nor aortic outputs were recirculated to the hearts. Hearts were not externally paced.

Hearts were divided into five groups according to the following additives to the perfusion medium: Group 1 (n = 5), none; Group 2 (n = 5), 40 n*M* desipramine (DMI) throughout perfusion; Group 3 (n = 4), 40 n*M* DMI during washout period only; Group 4 (n = 5), 5 n*M* NE throughout perfusion; and Group 5 (n = 5), 10 n*M* NE throughout perfusion. The range of NE concentration in our studies encompassed the typical plasma NE levels in cardiac patients studied with HED at our institution (0–7 n*M*). Norepinephrine concentrations exceeding 10 n*M* were not used in this study as a result of preliminary experiments showing the preparation to be physiologically unstable at high catecholamine concentrations.

The perfusion protocol consisted of a 20-min stabilization period, a 10-min HED washin period and a 50-min washout period in succession. Because Group 3 hearts showed rapid and extensive clearance, the washout period was shortened to 20–30 min. Before each perfusion, the HED perfusion circuit was filled with ~500 ml of perfusion medium containing 0.1–0.2 μCi/ml HED. The radioactivity concentration of the washin medium, C_p (CPM/ml), was measured in a NaI well-counter. Coronary and aortic outflows were discarded at all times. At the end of perfusion, hearts were chilled in 0°C K-H buffer and the ventricles were opened and gently blotted on paper toweling. Hearts were then placed in a pre-weighed tube, weighed and counted in the NaI well-counter. To account for all of the radioactivity that was monitored by the coincidence probes (described below), the paper toweling was also counted. This radioactivity was typically <2% of that in the myocardium. As a consequence of the extended periods of tracer washout, there was no radioactivity remaining in the left heart chambers or perfusion cannulae. Accounting for ^{11}C decay, the total apparent distribution volume (ADV) of HED in the heart at the end of perfusion was calculated from the sum of radioactivity in the myocardium and paper toweling (R_h), the mass of the heart (M_h), and C_p:

$$\mathrm{ADV}\left(\frac{\text{CPM/g heart}}{\text{CPM/ml perfusate}}\right) = \frac{R_h}{M_h C_p}. \qquad \text{Eq. 1}$$

Acquisition and Normalization of Time-activity Curves

Carbon-11 radioactivity was externally monitored by gamma, gamma-coincidence counting of the whole heart using two lead-collimated 2.54 × 2.54 cm bismuth germanate scintillation probes. The heart was suspended between the detectors by the aortic and left atrial cannulae. Single, coincidence and random coincidence events were sampled every second and stored on an IBM PC/AT computer. The true coincidence count rate (counts/sec) was calculated as measured coincidence minus random coincidence count rates. The entire timecourse of true coincidence count rate was referred to as the time-activity curve.

Each time-activity curve was normalized to the concentration of HED in the wash in perfusate and the mass of the heart. This was accomplished by rescaling the curve by multiplication by a calibration factor, f_c, determined for each study:

$$f_c\left(\frac{\text{(CPM/g)/(CPM/ml)}}{\text{counts/sec}}\right) = \frac{\mathrm{ADV}}{A_e}, \qquad \text{Eq. 2}$$

where ADV is obtained from Equation 1 and A_e is the average true coincidence count rate over the last 10 sec of data. The validity of using a single calibration constant to normalize the entire curve depends on the assumption that the efficiency of detection of the radioactivity in the myocardium and the "vascular" spaces in the field of view is independent of time. This is a valid assumption since the heart is stationary throughout the study. However, small biases could occur in the time-activity curves at times associated with washin and early washout of tracer because the detection efficiency of the "vascular" space (heart chambers and cannulae) could be slightly different than that of tissue. We chose to perform this normalization procedure for each study rather than calibrating the coincidence count rate with a representative source (*20*) because we considered the latter technique to be more susceptible to errors resulting from misalignment of the heart or variability of heart size and shape.

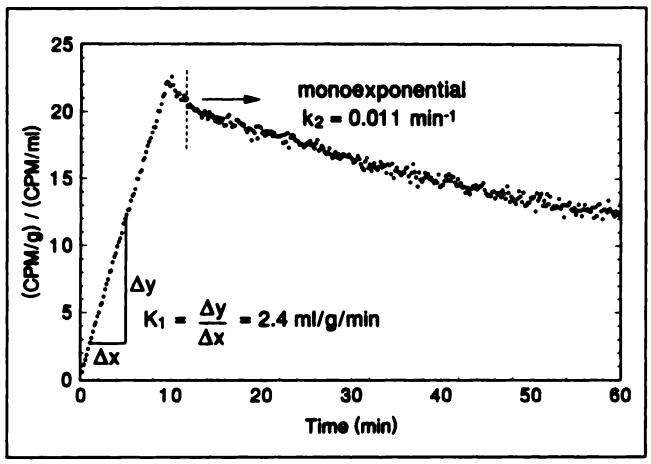

FIGURE 1. Kinetics of HED in a representative normal working rat heart. Estimates of tracer uptake rate (K_1) and monoexponential clearance rate (K_2) are shown. See text for details.

Estimation of HED Uptake and Clearance Rates

Figure 1 illustrates the derivation of HED uptake and clearance rates from time-activity curves. During the 10-min washin period, HED was administered to the hearts in the perfusate at a constant concentration (correcting for ^{11}C decay). The "vascular" spaces in the field of view of the detectors equilibrated rapidly (10–15 sec) with the input perfusate as determined by the high cardiac output (30 ml/min). After this short period, the uptake of HED by control hearts was linear to at least 10 min. The HED uptake rate constant, K_1 (ml/g/min), was obtained as the least-squares fitted slope of the normalized time-activity curve between 0.5 and 5 min. The fractional clearance rate of HED from the tissue, k_2 (min^{-1}), was determined by a monoexponential weighted least-squares fit to the unnormalized time-activity curves beginning at 2 min after the onset of the washout period. The weights for the fitting procedure were statistical, assuming a variance equal to the number of true coincidence counts in each sampled interval.

Estimation of HED Distribution Volume

The capacity of the myocardium to take up and retain HED is indicated by its equilibrium distribution volume, V_d (ml/g), defined as the ratio of ^{11}C concentration in tissue to that in perfusate at equilibrium. V_d was derived from estimates of tissue uptake and clearance rates. It is assumed that (1) HED distributes within the extravascular spaces of the isolated rat heart as a single, well-mixed pool, (2) the rates of tracer influx and turnover for this pool are given by K_1 and k_2, respectively and (3) the system is in steady-state over the period of the tracer kinetic study. V_d is calculated as:

$$V_d = \frac{K_1}{k_2}. \qquad \text{Eq. 3}$$

Statistical Analysis

Data are expressed as means ± standard error. The t-test (two-tailed) for unpaired samples was used to compare means. Values of $p < 0.05$ were considered significant.

RESULTS

Hemodynamic Parameters

All hemodynamic parameters were stable over the tracer kinetic study period. Data are shown in Table 1 for working rat hearts in Groups 1 (control), 4 (5 n*M* NE) and 5 (10 n*M* NE). Groups 2 (DMI throughout) and 3 (DMI during washout only) showed no deviation from the control group. An increase in heart rate with increasing NE concentration was observed. The positive inotropic effects of NE were evidenced by decreases in diastolic pressure and increases in systolic pressure. These effects were statistically significant only for the 5 n*M* group. Coronary flow and cardiac output were not affected by NE at levels up to 10 n*M*.

Neuronal Selectivity of HED

The normalized time-activity curve from a representative control study is shown in Figure 1. A very short time (10–15 sec) is necessary for HED to equilibrate in the "vascular" spaces. Subsequently, the curve becomes linear, showing a strong uptake process that does not approach equilibrium within 10 min. Uptake rates were 2.7 ± 0.4 ml/g/min (Table 2) in control hearts. Upon washout, the clearance kinetics in most hearts were monoexponential. An additional small, fast clearance component was apparent in the first 1–2 min of washout in some hearts (Fig. 1). This likely reflects initial washout of tracer from "vascular" and extraneuronal spaces. The monoexponential clearance rate constant, k_2, was estimated from the data after 2 min of washout (Table 2).

To define the specificity of HED for sympathetic neurons, neuronal uptake was blocked by pretreatment with desipramine (DMI). Uptake and retention of HED were dramatically reduced in comparison to control hearts (Fig. 2). Accumulation of HED was not linear in uptake-1 blocked hearts but appeared to saturate, approaching values of 4–5 ml/g. Ten minutes of tracer washin was insufficient to reach a true equilibrium and thus indicate the

TABLE 1
Hemodynamic Effects of Norepinephrine on the Isolated Working Rat Heart

Group	[NE] (n*M*)	Coronary flow (ml/min/g)	Cardiac output (ml/min/g)	Heart rate (min^{-1})	Diastolic pressure (mmHg)	Systolic pressure (mmHg)
1	0	11.3 ± 3.1	34.4 ± 3.1	280 ± 33	61 ± 4	87 ± 4
4	5	11.5 ± 1.8	33.7 ± 6.4	317 ± 18	54 ± 2*	96 ± 2*
5	10	12.6 ± 2.8	30.4 ± 8.2	337 ± 41	58 ± 4	90 ± 3

*$p < 0.05$. Measurements were averaged for each heart over the duration of the tracer kinetic study. Five hearts were perfused at each NE concentration.

TABLE 2
Effects of Norepinephrine on Kinetics of HED in the Isolated Rat Heart

Group	[NE] (n*M*)	Uptake rate K_1 (ml/g/min)	Clearance rate k_2 (min^{-1})	Distribution volume V_d (ml/g)
1	0	2.66 ± 0.39	0.011 ± 0.003	230 ± 52
4	5	2.81 ± 0.25	0.016 ± 0.009	154 ± 42 (−33%)
5	10	2.53 ± 0.67	0.034 ± 0.016*	91 ± 49* (−60%)

*$p < 0.05$. Values in parentheses are the change in V_d in comparison to Group 1. Five hearts were perfused in each group.

equilibrium distribution volume. Most of the radioactivity was rapidly cleared from the heart during the washout phase: retention of HED was <2% of control values after 15 min of washout.

Effects of Blockade of Neuronal Reuptake by DMI

To understand the relationship of uptake-1 to the retention of HED in the heart, neuronal reuptake of HED was blocked by DMI during the washout phase (DMI-chase, Fig. 3). Clearance was monoexponential and about 20 times faster than normal with DMI in the washout perfusate ($k_2 = 0.19 \pm 0.05$ min^{-1} versus 0.011 ± 0.003 min^{-1}, control, $p < 0.0001$), indicating that the retention of HED in control hearts is strongly dependent on uptake-1 activity.

Effects of Norepinephrine

To study the influence of circulating catecholamines on HED kinetics, hearts were perfused with NE at concentrations up to 10 n*M*. The time-activity curves from NE perfused hearts showed similar HED uptake to those of controls but increased clearance rate of tracer with increasing NE concentration (Fig. 4). The uptake rate (K_1) was not influenced by NE (Table 2). HED clearance rate (k_2) was roughly doubled as NE concentration was increased from 0 to 10 n*M*. (Table 2). The relatively high variability in k_2 (25%–58% of mean) and its dependence on NE concentration could not be explained by differences in coronary flow (Table 1). The estimated distribution volume of HED (V_d) in the isolated heart was 230 ± 52 ml/g under control conditions and decreased in proportion to the increase in NE concentration (Table 2). The high variability of k_2 was propagated into the calculation of V_d.

DISCUSSION

The use of PET with ^{11}C HED may provide important information for understanding the effects of various disease processes on the integrity of the sympathetic nervous system of the heart (*3*). The avid uptake and retention of HED by the heart is highly specific to sympathetic nerve terminals, as shown by the sensitivity of myocardial time-activity curves to block uptake-1 transport by DMI (Fig. 2).

Extraneuronal Distribution of HED

When neuronal uptake of HED was blocked by DMI, the total distribution volume of tracer approached 4–5 ml/g (Fig. 2). This accumulation was largely cleared after 15 min of washout. Accounting for the tracer present in "vascular" spaces (heart chamber and cannulae (0.5–0.7 ml/g) and extracellular space (0.3–0.4 ml/g, ref. 21), the distribution volume of HED in cellular spaces is estimated to

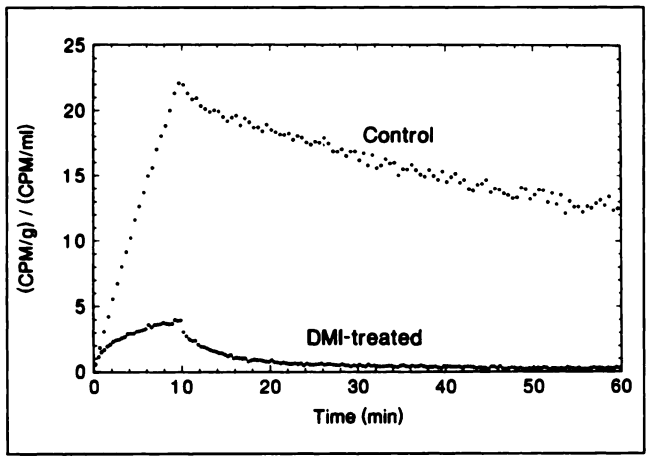

FIGURE 2. Representative normalized time-activity curves showing the effects of pharmacologic blockade of uptake-1 by DMI on HED kinetics in the isolated working rat heart. Uptake and retention of ^{11}C radioactivity was dramatically reduced in uptake-1 blocked (DMI-treated) heart, indicating high selectivity of HED for sympathetic nerve terminals.

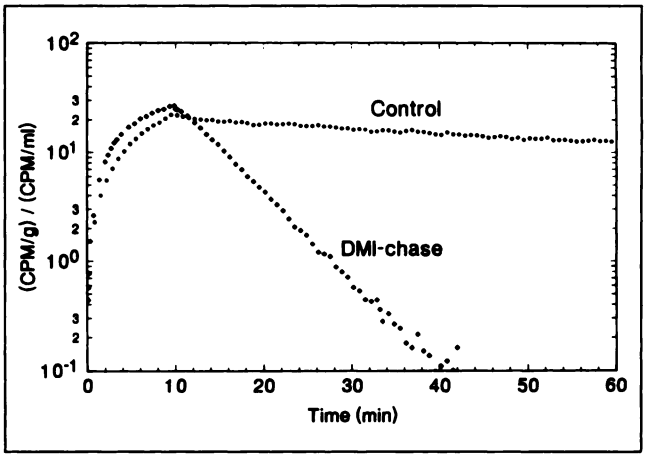

FIGURE 3. Representative normalized time-activity curves showing the effects of pharmacologic blockade of neuronal reuptake of HED on myocardial clearance rate. HED was administered to hearts under normal conditions and followed by washout with perfusate without DMI (control) or with 40 n*M* DMI (DMI-chase). The comparison shows a dramatic acceleration of HED clearance with DMI, indicating the strong dependence of myocardial retention on uptake-1 activity.

approach 3–4 ml/g. This is less than 2% of the estimated equilibrium distribution volume of HED in control hearts (Table 2). Thus, there is evidence for a limited, reversible uptake of HED into extraneuronal tissue components in the isolated heart. It should be noted, however, that the tissue-to-perfusate partition ratio of HED may be superficially high in this experimental model due to the absence of proteins (and cells) in the perfusion medium. Since the phenol moiety of HED would likely bind to plasma proteins with some affinity (*22*), the partition of tracer in extravascular tissues would therefore be expected to be lower in vivo. Similarly, our estimates of tissue distribution volumes of HED, regardless of the status of uptake-1, would also be higher than those determined in vivo.

The disposition of the very small fraction (~1% of initial accumulation) of HED remaining in the DMI-blocked hearts after 15 min of washout is not clear (Fig. 2). Interestingly, this level of retention was greater than that seen in hearts loaded under control conditions and washed out with DMI containing perfusate (DMI-chase, Fig. 3). A possible explanation for this discrepancy is that, as a consequence of uptake-1 blockade, concentrations of tracer in the interstitial space are higher in DMI-treated hearts than control hearts during the tracer washin period. This may allow greater amounts of tracer to diffuse into extraneuronal compartments with slow turnover rates.

HED Transport in the Myocardium

A model is proposed for the disposition of HED in the isolated rat heart as shown by Figure 5. HED may diffuse from the capillary bed to the neuroeffector junction via the interstitial space. The tracer is efficiently taken up into nerve terminals by uptake-1 transport. HED presumably distributes, as does metaraminol, in amine storage vesicles (*23*).

The acceleration of myocardial clearance of HED in DMI-chase experiments (Fig. 3) indicates that the tracer is readily released from nerve terminals and myocardial retention depends on uptake-1 mediated reuptake into the neuron. Evidently, HED undergoes continuous release and reuptake by nerve terminals of normal hearts. HED may be released from the nerve terminal by diffusion, outward transport through uptake-1 or vesicular exocytosis. Exocytotic release of vesicular HED is probably not significant in the isolated heart which lacks stimulation of the sympathetic neuron. Since outward transport through uptake-1 is inhibited by DMI (*24*), the release of HED from the neuron in DMI-chase studies may be primarily due to diffusion across the neuronal membrane. The overall clearance rate likely reflects diffusional loss (i.e., spillover) of tracer from the interstitial space into the perfusion medium. If the neuronal reuptake process is blocked by DMI, then the tracer is completely released from the neuron and clears the heart with a rate determined by its diffusional properties. Studies with ^{3}H-metaraminol show it to have a similar acceleration of myocardial clearance rate with uptake-1 inhibition (*25*). This is in contrast to the little effect that DMI has on the myocardial clearance of radioactivity following uptake of ^{3}H-NE (*26*; Wieland DM, *unpublished data*). As proposed by Almgren (*27*), the difference of metaraminol and NE with this respect may reflect higher lipid solubility and weaker binding in the adrenergic storage vesicles for metaraminol. Both of these factors may allow metaraminol (and likewise HED) to have higher extravesicular concentrations and a greater rate of diffusion across the neuronal membrane than NE.

The sensitivity of the clearance rate to DMI suggests that the turnover of HED in intraneuronal compartments (e.g., vesicles) and neuronal efflux are at least as rapid as the diffusion of HED from the junction. In analogous studies, clearance measurements could not allow the distinction of multiple pools of metaraminol within the neuron

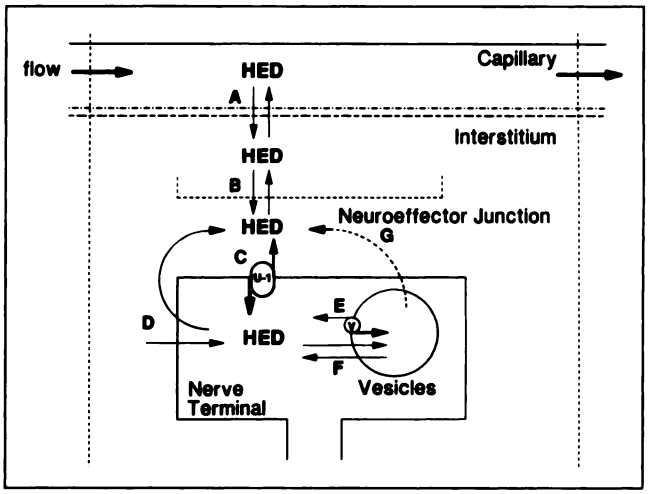

FIGURE 5. Proposed model of HED transport in the isolated rat heart. HED is transported within the bloodstream to the capillary bed. The subsequent transport processes are (A) diffusion across the endothelium, (B) diffusion into the sympathetic neuroeffector junction, (C) uptake-1 transport across neuronal membrane, (D) diffusion across neuronal membrane, (E) carrier-facilitated vesicular uptake and (F) diffusion across vesicular membrane. Exocytosis (G) is probably insignificant in the axotomized isolated rat heart.

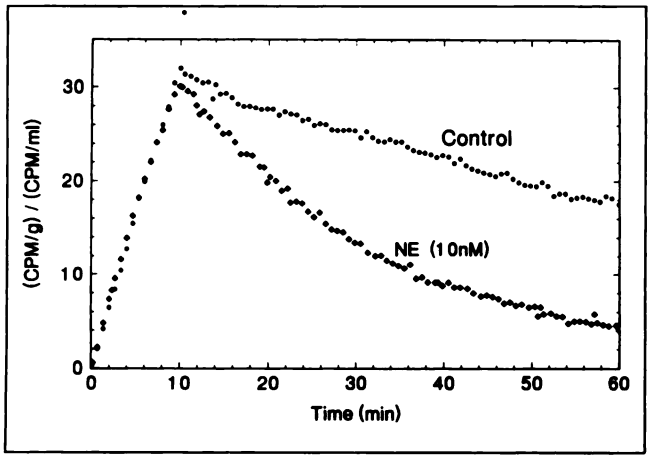

FIGURE 4. Effects of NE on HED kinetics in representative isolated working rat hearts. Myocardial clearance rate was increased with 10 n*M* NE, although the initial uptake rate was unchanged.

(*18*). The finding of monoexponential clearance with all but uptake-1 blocked hearts (Group 2) indicates that a "secular equilibrium" may occur for HED in nerve terminals, neuroeffector junctions and the interstitial space. Although the amount of tracer in these three distinct spaces is decreasing with time, there is probably a constant relationship between the concentrations of tracer in them. The rate of release of HED from the neuroeffector junction to the interstitium is presumably proportional to the concentration of HED in the junction. Similarly, axoplasmic HED may diffuse to the interstitium at a rate proportional to its concentration. Thus, the overall clearance rate of HED from tissue, reflecting spillover of tracer from the interstitial space into the perfusion medium, could be sensitive to a number of factors. These would include myocardial blood flow, membrane integrity, neuronal metabolic status, uptake-1 activity, vesicular transport and storage and interactions with catecholamines. In the innervated heart having sympathetic nerve stimulation, rates of vesicular exocytosis could also potentially influence the myocardial kinetics of HED.

Effects of NE on HED Kinetics

Since HED shares common pathways of uptake and storage with endogenous catecholamines, the kinetics of HED may be sensitive to changes in catecholamine concentrations in the axoplasm and neuroeffector junction of sympathetic nerve terminals. Uptake-1 transport of NE into an isolated rat heart obeys Michaelis-Menten kinetics with a Km value for perfusate NE concentration that is much higher than the concentrations used in our study (Km = 266 n*M*) (*16*). Therefore, it is not surprising that we observed no change in HED uptake rate with perfusate NE concentrations up to 10 n*M* (Table 2). It is likely that the initial uptake rate of HED by the heart is rate-limited by the diffusion of the tracer from the capillary bed to the neuroeffector junction. This interpretation agrees with the detailed transport studies in dogs showing the major limitation of transfer of NE from the plasma to the nerve terminal is at the capillary barrier (*28*).

However, the myocardial clearance rate of HED does show a dependence on NE concentration. The increase of clearance rate with NE (Table 2) indicates that a greater fraction of the HED recycling in and out of the nerve terminals is being lost to the interstitial space. Our measurements could not isolate effects of NE on uptake-1 transport from those on processes within the nerve terminal. NE could competitively inhibit neuronal reuptake of HED at uptake-1. Alternatively, effects of NE within the neuron could cause an increase in the release from the neuron. Both of these mechanisms would have the same result of increasing the concentrations of HED in the neuroeffector junction and interstitium during the washout period, resulting in an increase of tracer spillover to the perfusion medium. These results are consistent with the finding that myocardial retention of the radioiodinated catecholamine analog, meta-iodobenzylguanidine (MIBG), is inversely related to plasma catecholamine levels (*29*). The sensitivity of MIBG retention to catecholamine levels may be related to competition with endogenous catecholamines for uptake into storage vesicles (*30*).

Limitations of the Experimental Model

We utilized the isolated working rat heart preparation in these studies rather than an in vivo model because it is physiologically stable (*19*) and allows control of exogenous substrate and inhibitor/effector concentrations while avoiding systemic effects. Investigation of the effects of NE on HED were limited by the physiologic instability of this model at high catecholamine concentrations (>10 n*M*). Further work is encouraged with an experimental model more appropriate to addressing the higher local concentrations of NE that may be associated with certain conditions. In particular, myocardial ischemia may give rise to high levels of catecholamines (1×10^{-6} *M*) in the neuroeffector junction (*31*).

Implications for PET Imaging

PET imaging of the human heart with HED may allow identification of functioning sympathetic nerve terminals. HED exhibits a high affinity for uptake-1 transport into nerve terminals, slow myocardial clearance and very low uptake into and rapid clearance from extraneuronal tissues. These properties, along with its characteristic rapid blood clearance following bolus administration, allow excellent differentiation of normal and neuronally compromised tissue in relatively short periods of time (15–20 min) (*3,13*). HED may distribute in adrenergic storage vesicles but the release of tracer from vesicles appears to be relatively rapid and does not solely determine the clearance rate of radioactivity from the myocardium. By comparison, radiolabeled catecholamines concentrate more rapidly in storage vesicles as a result of extravesicular metabolism by MAO and COMT to diffusible species (*1*). Retention measurements obtained from PET imaging with HED will primarily reflect tracer delivery (myocardial perfusion) and uptake-1 transport. The high sensitivity of tracer uptake and retention on uptake-1 activity would give HED a particular advantage over radiolabeled catecholamine analogs for specific evaluation of effects of drugs or diseases on the neuronal catecholamine uptake process. Conversely, externally monitored kinetics of HED would be less sensitive than those of radiolabeled catecholamines to the status of vesicular catecholamine turnover (sympathetic tone).

The influence of the clearance rate of HED from the myocardium on indices of HED retention should be considered as PET data acquisition and analysis protocols are established. The clearance rate is probably determined by the concentration of tracer in the neuroeffector junction, which is in turn determined by a complex function of a number of active and passive transport processes available to the tracer (Fig. 5). Our studies suggest that catecholamines may accelerate the HED clearance rate by competitively inhibiting uptake-1 transport and/or vesicular accumulation. This is a concern for applications of PET-HED in

diseases that exhibit disturbed systemic or regional catecholamine homeostasis, including congestive heart failure (*32*), diabetes (*33*), myocardial ischemia (*31*), myocardial infarction (*34,35*) and cardiomyopathy (*36*). In the isolated rat heart, the HED distribution volume (V_d) decreases with increasing exogenous NE concentration (Table 2). If these data can be extrapolated to the in vivo situation, then a decrease would also be expected in estimates of V_d via compartmental modeling of dynamic PET data (*12*). Other indices of tissue retention of HED would also be sensitive to the influence of catecholamines. In particular, the time-dependence should be considered for model independent indices, such as tissue radioactivity concentration normalized to the integral of blood activity (*3*). All other parameters being equal, the effects of differences of clearance rates on such indices will be greater at later rather than earlier times. Thus, earlier PET image data would be less sensitive to the modulation of tracer clearance caused by catecholamines. This consideration is balanced by the limitation imposed by the time needed to clear the tracer from the blood after intravenous administration.

ACKNOWLEDGMENTS

The authors thank the staff of the Cyclotron Facility at the University of Michigan for the production of ^{11}C. This work was supported in part by grants from the National Institutes of Health, (R01 HL41047-01 and R01 HL27555-06), the American Heart Association of Michigan (88-0699-J1) and the Department of Energy (DE-FG02-90ER61091). Dr. Schwaiger is an established investigator of the American Heart Association. Dr. DeGrado was partly supported by the National Cancer Institute Training Grant NCI-5-P32-CA09015.

REFERENCES

1. Goldstein DS, Chang PC, Eisenhofer G, et al. Positron emission tomographic imaging of cardiac sympathetic innervation and function. *Circulation* 1990;81:1606–1621.
2. 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.
3. 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.
4. Fowler JS, Ansari AN, Atkins HL, Bradley-Moore PR, MacGregor RR, Wolf AP. Synthesis and preliminary evaluation in animals of carrier-free ^{11}C-1-dopamine hydrochloride: X. *J Nucl Med* 1973;14:867–869.
5. Ding YS, Fowler JS, Gatley SJ, Dewey SL, Wolf AP, Schlyer DJ. 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.
6. Ding YS, Fowler JS, Gatley SJ, Dewey SL, Wolf AP. Synthesis of high specific activity (+)- and (−)-6-[^{18}F]fluoronorepinephrine via the nucleophilic aromatic substitution reaction. *J Med Chem* 1991;34:767–771.
7. Chakraborty PK, Gildersleeve DL, Toorongian SA, Kilbourn MR, Schwaiger M, Wieland DM. Synthesis of [^{11}C]epinephrine and other biogenic amines by direct methylation of normethyl precursors. *J Label Compounds Radiopharm* 1992;32:172–173.
8. Mislankar SG, Gildersleeve DL, Wieland DM, Massin CC, Mulholland GK, Toorongian SA. 6[^{18}F]fluorometaraminol: a radiotracer for in vivo mapping of adrenergic nerves of the heart. *J Med Chem* 1988;31:362–366.
9. Wieland DM, Rosenspire KC, Hutchins GD, et al. Neuronal mapping of the heart with 6-[^{18}F]fluoro-metaraminol. *J Med Chem* 1990;33:956–964.
10. 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.
11. Garg PK, Garg S, Welsh PC, Zalutsky MR. Fluorine-18-labeled analogs of meta-iodobenzylguanidine. *Proceedings of the 19th International Symposium on Radiopharmaceutical Chemistry* 1992, 544–546.
12. Hutchins GD, Schwaiger M, Haka MS, Rosenspire KC, Wieland DM. Compartmental analysis of catecholamine analogs in myocardial tissue [Abstract]. *J Nucl Med* 1989;30:735.
13. Schwaiger M, Hutchins GD, Kalff V, et al. Evidence for regional catecholamine uptake and storage sites in the transplanted human heart by positron emission tomography. *J Clin Invest* 1991;87:1681–1690.
14. Wolpers HG, Nguyen N, Rosenspire K, Haka M, Wieland DM, Schwaiger M. ^{11}C-hydroxyephedrine as marker for neuronal catecholamine retention in reperfused canine myocardium. *Coronary Artery Disease* 1991;2:923–929.
15. Axelrod J, Gordon E, Hertting G, Kopin IJ, Potter LT. On the mechanism of tachyphylaxis to tyramine in the isolated rat heart. *Br J Pharmacol* 1962;19:56–63.
16. Iversen LL. The uptake of noradrenaline by the isolated perfused rat heart. *Br J Pharmacol* 1963;21:523–537.
17. Burgen ASV, Iversen LL. The inhibition of noradrenaline uptake by sympathomimetic amines in the rat isolated heart. *Br J Pharmacol* 1965;25:34–49.
18. Enna SJ, Shore PA. Characteristics of storage and release of metaraminol in the rat heart. *Biochem Pharmacol* 1972;21:1535–1542.
19. Taegtmeyer H, Hems R, Krebs HA. Utilization of energy-providing substrates in the isolated working rat heart. *Biochem J* 1980;186:701–711.
20. Nguyen VT, Mossberg KA, Tewson TJ, et al. Temporal analysis of myocardial glucose metabolism by 2-[^{18}F]fluoro-2-deoxy-D-glucose. *Am J Physiol* 1990;259:H1022–H1031.
21. Morgan HE, Henderson MJ, Regen DM, Park CR. Regulation of glucose uptake in muscle. I. The effects of insulin and anoxia on glucose transport and phosphorylation in the isolated, perfused heart of normal rats. *J Biol Chem* 1961;236:253–261.
22. Judis J. Binding of selected phenol derivatives to human serum proteins. *J Pharm Sci* 1982;71:1145–1147.
23. Porter CC, Torchiana ML, Totaro JA, Stone CA. Displacement of norepinephrine from the rat heart by ^{14}C-metaraminol. *Biochem Pharmacol* 1967; 16:2117–2124.
24. Carlsson A, Abrahamsson T. Characterization of the inhibitory effect of some antidepressant drugs on the outward transport of norepinephrine in the ischemic myocardium. *J Pharmacol Exp Ther* 1988;247:715–720.
25. Carlsson A, Waldeck B. Release of ^{3}H-metaraminol by different mechanisms. *Acta Physiol Scand* 1966;67:471–480.
26. Titus EO, Matussek N, Spiegel HE, Brodie BB. The effects of desmethylimipramine on uptake of dl-norepinephrine-7-^{3}H in the heart. *J Pharmacol Exp Ther* 1966;152:469–477.
27. Almgren O. The effect of protriptyline on the disappearance and nerve impulse induced release of labelled metaraminol in the rat. *Acta Physiol Scand* 1971;82:539–544.
28. Cousineau D, Rose CP, Goresky CA. Labeled catecholamine uptake in the dog heart. *Circ Res* 1980;47:329–338.
29. Nakajo M, Shapiro B, Glowniak J, Sisson JC, Beierwaltes WH. Inverse relationship between cardiac accumulation of meta-[^{131}I]iodobenzylguanidine (I-131 MIBG) and circulating catecholamines in suspected pheochromocytoma. *J Nucl Med* 1983;24:1127–1134.
30. Gasnier B, Roisin M-P, Scherman D, Coornaert S, Desplanches G, Henry J-P. Uptake of *meta*-iodobenzylguanidine by bovine chromaffin granule membranes. *Mol Pharmacol* 1986;29:275–280.
31. Schömig A, Dart AM, Dietz R, Mayer E, Kübler W. Release of endogenous catecholamines in the ischemic myocardium of the rat. Part A: locally mediated release. *Circ Res* 1984;55:689–701.
32. Packer M. The neurohormonal hypothesis: a theory to explain the mechanism of disease progression in heart failure. *J Am Coll Cardiol* 1992;20:248–254.
33. Ganguly PK, Beamish RE, Dhalla KS, Innes IR, Dhalla NS. Norepinephrine storage, distribution, and release in diabetic cardiomyopathy. *Am J Physiol* 1987;252:E734–E739.
34. Manger WM. Catecholamines in normal and abnormal cardiac function. *Adv Cardiol* 1982;30:1–145.
35. Goldstein DS. Plasma catecholamines in clinical studies of cardiovascular diseases. *Acta Physiol Scand Suppl* 1984;527:39–41.
36. Opie LH, Walpoth B, Barsacchi R. Calcium and catecholamines: relevance to cardiomyopathies and significance in therapeutic strategies. *J Mol Cell Cardiol* 1985;17 Suppl 2:21–34.