Labeled Catecholamine Uptake in the Dog Heart

Interactions between Capillary Wall and Sympathetic Nerve Uptake

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SUMMARY The kinetics underlying the uptake of tracer amounts of norepinephrine and isoproterenol by the heart were studied in a pentobarbital-anesthetized dog with the multiple indicator dilution technique. The circumflex coronary artery was perfused with blood from the femoral artery, with a pressure-dependent system. A small bolus containing labeled albumin (a tracer confined to the vascular space), labeled sucrose (which penetrates into the extracellular space in a barrier-limited fashion), and labeled norepinephrine or isoproterenol was injected into the artery and outflow dilution curves were obtained from the coronary sinus. Analysis of data enabled us to assess separately the myocardial capillary permeability for norepinephrine or isoproterenol, and their rate constants for uptake by the interstitial sympathetic fibers (the process is essentially unidirectional over the time of a single passage, because of the highly concentrative nature of the uptake). We found a major resistance to catecholamine transfer at the capillary surface (approximately half of the label emerged at the outflow without leaving the circulation) and a neuronal uptake process beyond the barrier large enough that, after steady infusion, it would be expected to reduce the tracer concentration of norepinephrine to a value the order of one-sixth that in the plasma space. The injection of desmethylimipramine selectively diminished the apparently unidirectional flux of labeled norepinephrine into the neuronal terminals, and this uptake was found to be significantly lower for isoproterenol than for norepinephrine. The capillary-interstitium-concentrative uptake mechanism, documented here, partially explains the quantitatively different cardiac responses to infused and locally released catecholamines. Circ Res 47: 329–338, 1980

THE ability of the myocardium to extract catecholamines was first documented in vitro with labeled epinephrine (Raab and Gigee, 1955; Axelrod et al., 1959; Stromblad and Nickerson, 1961). The heart is abundantly innervated with sympathetic fibers (Sachs, 1970) and the whole of the adrenergic neurone takes up and concentrates exogenously administered catecholamines (Hamberger et al., 1964). The concentrative tissue uptake would lead one to expect to find evidence for substantial net uptake across the coronary circulation. With the development of a sensitive radioenzymatic assay procedure for measuring catecholamines, aortic-coronary sinus extractions of the order of one-third were found in dogs directly following bilateral thoracotomy and bilateral vagotomy (Yamaguchi et al., 1975; Yamaguchi et al., 1977), whereas, after maximum stimulation of the right cardio-accelera-

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different catecholamines in the heart, experiments designed to provide data concerning their passage through the capillary wall and uptake from the extracellular space are essential. For this purpose the single injection multiple indicator dilution technique (Chinard et al., 1955) is the method of choice. This was previously adapted for use in the in situ working heart (Ziegler and Goresky, 1971a; Rose and Goresky, 1976; Rose et al., 1977). It has been used in the liver to characterize the uptake of galactose and glucose (Goresky et al., 1973a; Goresky and Nadeau, 1974) and in the heart to obtain an understanding of the nature of free fatty acid uptake (Rose and Goresky, 1977).

The use of the multiple indicator dilution technique carries with it the need to model the process under examination, so that the outflow as a whole can be described in terms of both its throughput or nonexchanging components and its returning components, which have left the capillary and later returned to it (Goresky et al., 1970). In this case, where the neuronal mechanism sequesters the catecholamines from the extracellular space in a concentrative fashion, the removal process may be regarded as unidirectional over the short time of a single passage (Ziegler and Goresky, 1971b; Goresky et al., 1973b).

With this approach we have been able to outline the major sites underlying the uptake of labeled norepinephrine by the heart. A substantial barrier to passage has been demonstrated at the level of the capillary and the rate of removal from the extracellular space quantified. Desmethylimipramine was found to inhibit the uptake of labeled norepinephrine from the extracellular space. Similarly, the neuronal uptake of labeled isoproterenol, a catecholamine with a bulky N-substitution in comparison to norepinephrine (Corrodi et al., 1966), was found to be substantially less than that of norepinephrine.

**Methods**

The main stem or circumflex branch of the left coronary artery of a pentobarbital-anesthetized mongrel dog was cannulated via a carotid artery (Rose and Goresky, 1976) and perfused through a pressure regulated extracorporeal circuit (Rose et al., 1980) with blood from a femoral artery. The coronary sinus was catheterized via the right jugular vein. Perfusion pressure, flow rate, intraventricular or aortic pressure, and lead 2 of the electrocardiogram were monitored continuously. The dog was ventilated by a constant volume respirator, after secure closure of the chest.

The materials to be injected were made up as follows: 6 ml of the injection mixture, adjusted to the same hematocrit as that of the dog, contained 0.05 mCi I-125-labeled albumin (Charles E. Frost), a reference substance that does not leave the coronary circulation within a single passage (Ziegler and Goresky, 1971a); 0.1 mCi sucrose[14C(U)] (New England Nuclear), a diffusible substance that leaves the circulation to enter the extracellular space during its passage through the heart; and 0.2 mCi d,L-norepinephrine[7-3H(N)] (specific activity 15.2 Ci/mmol) or d,L-isoproterenol[7-3H(N)] (New England Nuclear). When the preparation was in a steady state, 0.25 ml of the mixture was suddenly injected into the perfusion tubing. Simultaneously a collection rack was started and samples were collected from the coronary sinus at a rate of 1 to 1.25 samples/sec.

In 10 dogs, baseline studies were carried out with 3H-labeled norepinephrine. In a second group of six animals, desmethylimipramine, a compound known to inhibit the in vitro uptake of norepinephrine (Hertting et al., 1961), was given in a dose of 1 mg/kg 20 minutes prior to a norepinephrine study. In a third group of six dogs, the uptake of 3H-labeled isoproterenol was examined.

A volume of 0.1 ml from each heparinized blood sample was diluted in 1.5 ml of saline, pipetted into a counting tube, and assayed for radioactivity in a γ-ray spectrometer set for the photopeak characteristic of 125I. The proteins were then precipitated with 0.2 ml of trichloracetic acid, and 0.2 ml of the supernatant fluid was pipetted into a scintillation cocktail and assayed for 14C and 3H activity in a liquid scintillation counter. Samples from the injected mixture and cross-over standards were treated identically. To normalize the activity resulting from each of the tracers with respect to the others, the activity of each was divided by the total activity injected for that species. The resulting value is a fraction of the total injected per milliliter of venous blood.

**Analysis of Data**

Normalized coronary sinus outflow curves for the three tracers before and after administration of desmethylimipramine are shown in Figure 1. The events shaping the relations between outflow curves, previously described in detail (Goresky et al., 1970; Rose and Goresky, 1976; Rose et al., 1977), will be summarized here. Since labeled albumin does not leave the capillary to any significant degree during a single passage, its outflow is shaped solely by the distribution of transit times in the large or nonexchanging vessels and the capillaries or exchanging vessels. The upslope and peak of the sucrose curve are reduced with respect to the albumin curve because some of it permeates the capillary barrier via aqueous channels and enters the interstitial space. The remaining sucrose, the throughput component, traverses the capillaries without leaving. Later in time, the sucrose that has left the capillary, the returning component, returns to the capillary, and flow carries it to the outflow. The sucrose curve then crosses over the albumin curve and, if collections were carried out for a long time, all of the sucrose eventually would be recovered. Labeled norepinephrine is handled in sim-
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In a similar fashion at the capillary surface, but in the interstitial space we would expect an additional process, the removal and concentration of tracer by the sympathetic nerve fibers which run parallel to the capillaries. The returning component, of course, is reduced by this uptake process.

While the form of the curves makes it apparent that a large fraction of the norepinephrine bolus does not leave the capillary, further analysis of these data necessitates a mathematical model of the capillary and surrounding tissue. Since the sympathetic nerve fiber concentrates norepinephrine highly, we assume that flux across this membrane is unidirectional within the time for single passage of the vascular reference. The appropriate single-capillary model is therefore one in which there is bidirectional transfer at the capillary, with unidirectional removal from the interstitial space by the adrenergic neurones. The model is essentially identical to that previously developed to describe rubidium transport in the heart (Ziegler and Goresky, 1971a) and galactose uptake by the liver (Goresky et al., 1973a). We assume plug flow in the capillary, dimensions and flows such that the radial gradient in the interstitial space is negligible, and negligible longitudinal diffusion (Goresky et al., 1970).

Let $u$ and $v$ be the concentrations of tracer at a place $x$ and time $t$, in intravascular and extravascular spaces, respectively; $W$, the plasma bulk flow velocity in the capillary; $x$, the distance along the capillary; $\gamma$, the ratio of accessible extravascular to intravascular volume; $k_1$ and $k_2$, the permeability surface products for efflux from and influx into the capillary per volume of accessible extravascular space (both are equal when the exchange is passive); $k_s$, the unidirectional influx into tissue cells (equal to zero, for sucrose). The partial differential equations describing the distribution of tracer within this system include an equation of conservation

$$\frac{\partial u}{\partial t} + W \frac{\partial u}{\partial x} + \gamma \frac{\partial v}{\partial t} + \gamma k_s v = 0 \quad (1)$$

$$\frac{\partial v}{\partial t} = k_{1u} u - k_2 v - k_s v \quad (2)$$

The time-domain solution to these equations has been described previously (Ziegler and Goresky, 1971b; Rose et al., 1977). It consists of two parts: a throughput and an exchanging component.

To utilize a computer to construct a predicted sucrose or norepinephrine curve, it is necessary to know how to approach distribution of capillary transit times in the coronary circulation (capillary exchange occurs only during time spent in the capillary). This can be found from the relation between the labeled albumin and labeled sucrose curves. Rose and Goresky (1976) have found that, when the tone in the coronary circulation is normal, the upslope part of a labeled sucrose curve can be fitted without systematic bias only if it is assumed that the capillary transit time $r_c(t)$ increases along the outflow dilution curve. Early in time, the labeled sucrose curve is composed virtually completely of throughput material which, from the solution to Equations 1 and 2, may be represented mathematically as $\exp(-k_{1y} r_c(t))$ times the relative magnitude of the simultaneously injected vascular reference, labeled albumin. If $C(t)_{ab}$ is the fractional outflow of the albumin reference and $C(t)_{suc}$, the fractional outflow of the labeled sucrose, it is found that $\ln[C(t)_{ab}/C(t)_{suc}]$ begins with a finite value and increases linearly with time over the upslope and peak of the dilution curve. Since this function equals $k_{1y} r_c(t)$ and $k_{1y}$ is fixed, $r_c(t)$ increases linearly with time. In fitting the data, two parameters related to the heterogeneity will be found: $a'$, the finite value for $k_{1y} r_c$ at the appearance time; and $b'$, the rate at which $k_{1y} r_c(t)$ increases with time.

With this background, the modeling can be used to synthesize from the reference albumin curve whole organ outflow dilution curves for the exchanging tracer. The parameters arising from the physical model of the capillary-tissue exchange process can then be optimized by seeking the closest possible fit to the experimental outflow exchanging tracer curve. Although the optimization procedure can be accomplished in the time domain, the amount of computing time often is prohibitory, in terms of its expense. We have therefore used an alternate approach, a recently developed frequency-domain optimization procedure (Rose et al., 1980). The parameters optimized in the whole organ model (Rose et al., 1977) are the following:

1. for sucrose (with albumin as the intravascular reference):
   $$k_s = \text{the capillary permeability surface product per unit interstitial space (sec}^{-1}).$$
   Sucrose exchange is assumed to occur passively, so that, in terms of the modeling, $k_s = k_1 = k_2$.
   $$\Phi = \text{plasma flow per unit interstitial space (sec}^{-1})$$
   $$= \sum_{i=1}^{\infty} \frac{w_i}{\gamma_s T_{ci}}$$
   where $\gamma_s$ is the ratio of extravascular to intravascular space for sucrose in a given capillary unit and $w_i$ is the fraction of the total number of units represented by the index $i$.
   In general, $\gamma_s$ cannot be defined as an element separate from the $\gamma_s T_{ci}$ products, when the coronary tone is normal, and
   $$a' \text{ and } b' = \text{the parameters which essentially describe the heterogeneity of capillary transit times.}$$

2. for norepinephrine or isoproterenol:
   $$k_c = \text{the capillary permeability surface product per unit interstitial space for the tracer catecholamine. Again the exchange will be assumed to be non-
concentrative, in terms of free tracer in the plasma and interstitial spaces, so that $k_c = k_1 = k_2$.

\[
\frac{\gamma_c}{\gamma_s} = \text{the ratio of the interstitial space size for catecholamines to that for sucrose, and}
\]

$k_n = \text{the permeability surface product for unidirectional uptake of the catecholamines by the nerve fibers per unit interstitial space. In the modeling this is } k_3, \text{ with dimensions of } \sec^{-1}.$

The model does not permit an estimation of absolute interstitial space size, but once the sucrose curve has been fitted, the relative space of distribution for catecholamines can be determined. When this ratio is not unity (as is the case here), then the parameters $k_c$ and $k_n$ must be multiplied by this ratio to provide relative permeabilities, at the capillary level. All permeabilities are then expressed in terms of the sucrose interstitial space size. This normalization has been carried out, for the values tabulated later.

Frequency-domain modeling requires that the total response of the system be known. Since our data collection must stop at a finite time, extrapolation of the data is required (Rose et al., 1980). This has been carried out by adding a monoeXponential decay to the tail of the labeled sucrose curves, such that complete recovery of the sucrose with respect to labeled albumin is attained, and by adding a monoeXponential decay to the catecholamine curves which is a best fit through the last three data points. The recovery of the catecholamine tracers is always less than total. Reported tracer extractions are calculated on the basis of the relative proportion of the catecholamine label not recovered. The values correspond to the steady state extraction expected for labeled catecholamine in the absence of reflux of tracer from the nerve fiber.

**Results**

Figure 1 shows normalized outflow dilution curves from a set of norepinephrine studies carried out before and after the administration of desmethylimipramine. In each instance, the upslope and peak extractions for labeled norepinephrine are larger than those for labeled sucrose. The salient change, following the desmethylimipramine administration, is an increase in tailing late in time, indicating an increased return to the circulation of tracer which has entered the interstitial space (or, conversely, a decrease in neuronal tracer uptake from the interstitial space). The dashed lines indicate the optimized model approximations to both labeled sucrose and norepinephrine curves, before and after desmethylimipramine. The fits, in general, agree well with the data. Deviations from the data, where they occurred, were not systematic.

Figure 2 shows resolved curves into throughput and exchanging components. The throughput or first component is relatively unchanged by the desmethylimipramine administration, whereas the later part of the returning or second component is increased. The magnitude of the desmethylimipramine effect is accentuated by the shading between the actual curve and

**Figure 1** Normalized coronary sinus outflow fraction per milliliter vs. time curves, obtained in the same preparation before and after desmethylimipramine (DMI). The dashed line corresponding to the sucrose data is that resulting from fitting the exchange sucrose model to the experimental data, and the dashed line corresponding to the norepinephrine data shows the results achieved by fitting the exchange-sequestration model to that data. The significant change in the norepinephrine curve after desmethylimipramine is the large increment in tracer returning to the circulation late in time.

**Figure 2** The results of fitting the modeling to the norepinephrine curves illustrated in Figure 1. The actual outflow data are represented by the filled circles, with the solid line passing through them. The throughput or non-exchanging component is represented by the hatched shading and the returning component, by the clear zone between the throughput component and the outflow dilution curve. The upper solid line is the theoretical outflow curve predicted for $k_n = 0$. It represents the outflow pattern predicted if all of the tracer had returned to the circulation. The dotted shading, which emphasizes the area between the outflow curve and the $k_n = 0$, thus indicates the effect which the neuronal uptake process has had on the form of the curve. The results of the analysis of the sequential runs indicate that the throughput component is not modified following desmethylimipramine administration, whereas, in contrast, the neuronal uptake mechanism is grossly reduced.
the $k_n = 0$ curve. It is clear that a substantial decrease in uptake of labeled norepinephrine from the interstitial space has occurred following desmethylimipramine administration.

The outflow curves from a typical isoproterenol experiment are illustrated in Figure 3. The upslope and peak extractions for labeled sucrose and isoproterenol are relatively similar, suggesting that the permeability of the capillary barrier for isoproterenol is of the same order of magnitude as that for sucrose (that is, that it is less than that for labeled norepinephrine, which permeates the capillary barrier rather better than sucrose). The results of the analysis of throughput and returning components, illustrated in the righthand panel, indicate that the proportional return of tracer from the interstitial space is rather larger than in the control norepinephrine experiment, illustrated in the lefthand panels of Figures 1 and 2. A larger proportion of the tracer which has entered the interstitial space has escaped neuronal sequestration.

The individual values of the optimized parameters derived by the fitting procedures are given in Table 1; and the hemodynamic parameters pertaining to both the coronary perfusion circuit and sys-

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**TABLE 1** Individual Values for Optimized Parameters Derived by Best Fit Procedure (see Methods for Definition of Symbols) in the Norepinephrine Studies, the Norepinephrine Studies following Desmethylimipramine, and the Isoproterenol Studies

<table>
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<tr>
<th>Type of study</th>
<th>Experiment no.</th>
<th>$a$ (sec$^{-1}$)</th>
<th>$b$ (sec$^{-1}$)</th>
<th>$\Phi$ (sec$^{-1}$)</th>
<th>$k_n$ (sec$^{-1}$)</th>
<th>Area extraction $\gamma_1/\gamma_2$</th>
<th>$k_e$ (sec$^{-1}$)</th>
<th>$k_s$ (sec$^{-1}$)</th>
</tr>
</thead>
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<td>Norepinephrine</td>
<td>1a</td>
<td>0.183</td>
<td>0.014</td>
<td>0.130</td>
<td>0.034</td>
<td>0.316</td>
<td>2.65</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td>2a</td>
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<td>0.040</td>
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<td>0.048</td>
<td>0.473</td>
<td>3.43</td>
<td>0.055</td>
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<tr>
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* A difference from the norepinephrine group, significant at the $P < 0.05$ level.
† A difference from the norepinephrine group, significant at the $P < 0.01$ level.
The major and highly significant (P < 0.01) parameter change after desmethylimipramine was the reduction in $k_n$, the permeability surface product for unidirectional neuronal tracer norepinephrine uptake per unit interstitial space, normalized to the sucrose interstitial space. A second parameter change was also observed following the desmethylimipramine infusion: the $\gamma / \gamma_s$ ratio was reduced significantly. Thus, after desmethylimipramine, the capillary permeability to tracer norepinephrine did not change, but the neuronal unidirectional uptake rate is markedly reduced and the relative interstitial space accessible to tracer norepinephrine is also diminished.

As expected, the mean hemodynamic parameters and the tracer sucrose $k_s$ values in the control tracer norepinephrine and tracer isoproterenol experiments did not differ significantly. The $k_s$ value for tracer isoproterenol was close to the $k_s$ value for tracer sucrose [the average for $k_s/k_s$ was 0.97 ± 0.05 (SEM); and the average for the neuronal uptake constant $k_n$ was significantly smaller than that for the control tracer norepinephrine experiments. The $k_s$ value for tracer isoproterenol was of the same order of magnitude as the average for the tracer norepinephrine studies following desmethylimipramine. The values correspond to those expected, on the basis of the comparison of Figure 3 to Figure 1.

**Discussion**

The validity of using the capillary-interstitium-concentrative uptake model developed by Ziegler and Goresky (1971b) to describe rubidium uptake by the heart, as a description for catecholamine uptake, depends on the assumption that the neuronal uptake mechanism is a highly concentrative one and that, because of this, none of the tracer entering the sympathetic fibers returns to the interstitial space over the time of a single passage for a vascular tracer. The norepinephrine uptake process obeys Michaelis-Menten kinetics (Iversen, 1963) and is characterized by a $K_m$ of $10^{-9}$ M (Sachs, 1970). The concentration of norepinephrine used in our study was much lower, $10^{-10}$ M. This corresponds to basal endogenous circulating levels (Yamaguchi et al., 1975; Yamaguchi et al., 1977). It has been found that, after incubation with norepinephrine, the neuronal concentration reaches values 30,000 times higher than that in the external me-
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dium (Jonsson et al., 1969). With concentrative accumulations of this order, the kinetics of the process indicate that the return of tracer to the interstitial space from the neurone can certainly be neglected over single passage times (Goresky et al., 1973b), and the uptake of norepinephrine can be considered to be unidirectional, over these short times. The good fits to tracer norepinephrine data, obtained with the capillary-Interstitial uptake model, substantiate the reasonableness of the hypothesis.

The uptake of norepinephrine by the adrenergic nerves is considered to be a very important inactivation mechanism for these amines and enzymatic degradation, to play only a minor role (Sachs, 1970). It has been shown that, 3 minutes after the injection of labeled norepinephrine in the coronary artery of the canine heart, metabolites of norepinephrine constitute only a minor proportion of the total radioactivity measured in the coronary sinus (Chidsey et al., 1963). These data make it reasonable to assume that the tritiated material obtained up to 50 seconds in our experiments is norepinephrine.

The data analysis provided by the modeling indicates that the capillary membrane is an important barrier to the passage of norepinephrine into the interstitial space. A major portion of the tracer norepinephrine initially present in arterial blood does not leave the capillary during the time of a single passage (Fig. 2). The capillary permeability surface product per unit interstitial space for nor-epinephrine $k_s$, corrected to the sucrose interstitial space, was higher than the $k_s$ for sucrose, as expected from the lower molecular weight of norepinephrine. The capillary rate constants for sucrose and norepinephrine, $k_s$ and $k_r$, did not change significantly after desmethylimipramine. The capillary permeability to isoproterenol is less than that for norepinephrine, apparently as a consequence of its larger molecular size and differing molecular structure.

The form of the data and the results of our analysis indicate that a proportion of the labeled norepinephrine that has entered the interstitial space returns to the capillary without undergoing uptake. After desmethylimipramine, the proportion of tracer returning is increased. The finding corresponds to previous in vitro observations that desmethylimipramine inhibits the neuronal accumulation of norepinephrine (Hertting et al., 1961). The data indicate that the uptake for labeled isoproterenol is less efficient than for labeled norepinephrine.

In the heart, radioautographic studies have shown the accumulative sites to be long thin filamentous adrenergic nerves, located between the muscle fibers (Marks et al., 1962). These fibers provide a potentially huge surface for exchange of catecholamines between the neurones and interstitial space. The neuronal uptake of norepinephrine is thought to occur over the whole of the post-ganglionic sym- pathetic neurone (Hamberger et al., 1964). It is this uptake which accounts for the kinetic uptake of tracer norepinephrine from the interstitial space, which we have observed, and it is this uptake which is diminished selectively by desmethylimipramine.

The significant reduction in the interstitial ratio $\gamma_c/\gamma_s$ after desmethylimipramine implies that a reduction in the apparent interstitial space of distribution available to tracer norepinephrine has occurred consequent to the administration of desmethylimipramine. Since the space ratio for both catecholamines (norepinephrine and isoproterenol) is always greater than one, they occupy an apparent interstitial space larger than that available to sucrose. A parallel set of phenomena have been found in another area, with indicator dilution methodology. Wolkoff et al. (1979) has shown that an increase in the concentration of a liver cell intracellular binding protein increases the apparent space of distribution available to a labeled probe binding to the protein; and Goresky et al. (1978) have shown that occupation of binding sites by unlabeled material diminishes the total sites available to the labeled probe, so that the apparent space of distribution becomes smaller. The magnitude of the $\gamma_c/\gamma_s$ space ratio implies that there are additional binding sites for catecholamines in the interstitial space, and the decrease in the $\gamma_c/\gamma_s$ ratio following desmethylimipramine administration suggests that the decreased neuronal unidirectional uptake of tracer norepinephrine was followed by a steady state interstitial accumulation of norepinephrine. Additionally, the statistically significant increase in heart rate following the administration of desmethylimi-primine provides support for this hypothesis. The phenomena following desmethylimipramine are not central to the main theme of this work, the definition of the capillary-Interstitial-neuronal concentrative uptake as the basic mechanism underlying catecholamine handling, but they do raise provocative and currently unanswerable questions concerning the identity of the interstitial binding sites with catecholamine receptors, and the degree to which the $\gamma_c/\gamma_s$ ratio values will provide a reflection of the degree of saturation of the interstitial binding sites.

Our study has revealed the presence in the capillaries of the heart of an important barrier to the diffusion of norepinephrine between the interstitial and intravascular spaces. Thus, just as tracer norepinephrine in the plasma does not equilibrate immediately with the interstitial pool, so norepinephrine released by peripheral sympathetic fibers into the synaptic clefts and extracellular space will not equilibrate immediately with the vascular compartment. It has been shown in humans, for instance that, after sympathetic stimulation, the immediate increase in heart rate and blood pressure is not accompanied by an elevation in circulating norepinephrine, and only after several minutes is a steady
state elevation of circulating norepinephrine achieved which can then be related with hemodynamic changes (Cryer et al., 1976). The barrier at the level of the capillary will diminish the accessibility of circulating catecholamines to the receptors in the extracellular compartment and, when release does not match uptake, the concentrative uptake will sequester catecholamines and lower its concentration in the interstitial space, and less plasma catecholamine will reach the receptors than if there were no barrier. It has been demonstrated in humans that, following steady infusion of norepinephrine, the minimal level needed to produce perceptible hemodynamic changes in heart rate is eight times higher than the endogenous levels associated with observable hemodynamic or metabolic change (Silverberg et al., 1978). To rationalize their observations, these authors assumed an unimpeached movement of norepinephrine from the plasma into the sympathetic synaptic clefts and an identity of concentration at the two sites under steady state conditions. From this assumption, they then estimated the minimum biologically effective synaptic cleft norepinephrine concentration to be eight-fold basal levels. Our data indicate, however, that it is likely that the high doses of norepinephrine were required because of the presence of an important capillary membrane barrier to norepinephrine, backed up by an effective extracellular removal process.

It is instructive to use the numerical parameters calculated from our modeling to calculate the predicted ratio of plasma to interstitial space concentrations of norepinephrine during a steady state tracer infusion. During a steady infusion, the concentrative neuronal accumulation of tracer will equal the tracer input to the heart. The ratio value, derived theoretically by Goresky et al. (1973a), is

$$\frac{v(x,\infty)}{u(x,\infty)} = \frac{k_c}{k_c + k_n}.$$

For the control norepinephrine experiments

$$\frac{v(x,\infty)}{u(x,\infty)} = \frac{0.070}{0.070+0.400} = 0.15.$$ 

Thus we can predict that, in the steady state, the interstitial space concentration of labeled norepinephrine will be only 15% of the plasma concentration. In the absence of concomitant release of unlabeled material, interstitial space concentrations for total norepinephrine would reach the same ratio value. Because unlabeled material is being released concomitantly, the expected ratio value will be higher than this. The expected relative effects will vary with the rates of infusion of unlabeled material. At low infusion levels, in the absence of sympathetic activation, the release will be significant, in relation to uptake, and the interstitial values will be significantly higher than the predicted labeled norepinephrine ratio value; whereas, at higher levels of infusion, where the relative rates of release are now low, the interstitial values for total norepinephrine will be expected to approach the predicted ratio [particularly because the Michaelis constant for the neuronal concentrative uptake mechanism (Sachs, 1970) is high enough that the sequestration constant $k_n$ will not saturate]. We can now begin to understand the relative insensitivity of the heart to infused norepinephrine.

After desmethylimipramine, the predicted ratio (interstitial:vascular concentration) for labeled norepinephrine during steady infusion rises to 0.34. The steady state interstitial concentration of total norepinephrine will also be expected to rise. This expectation correlates with the observed adrenergic effect, the increase in heart rate.

The expected effects of the capillary barrier thus will vary, depending on the source of the catecholamine. Whereas, with exogenously administered norepinephrine, the capillary barrier will retard access of this neurohumor to the interstitial space, where both the neuronal uptake mechanism and the adrenergic receptors are situated, so that its physiological effects are scaled down, the consequences of the barrier for locally released material will be quite different. The effect of the capillary barrier will then be both to magnify the local effects of the neurotransmitter by prolonging its local contact with adrenergic receptors, and to effectively enhance the local concentration of neurotransmitter by retarding its loss from the tissue (conversely, the barrier will, by its presence, improve the local conservation of neurotransmitter). With the mediation of sympathetic effects by local receptors situated at the site of norepinephrine release, one would expect the much-damped local changes in blood levels to correlate rather better with evidence of sympathetic activity than in the case of catecholamines carried to tissues via the systemic circulation. Thus, in the heart, very good correlations have been reported between the output of norepinephrine in the coronary sinus and changes in inotropic and chronotropic functions following stimulation of the left sympathetic stellate ganglion (Yamaguchi et al., 1975). In contrast, during prolonged exercise in dogs, circulating catecholamines increase steadily to very high levels, presumably due to their liberation from the adrenal medulla, but heart rate, after a rapid initial increase no longer parallels the important elevation in circulating catecholamines (Péronnet et al., 1977) suggesting that access of these hormones to the adrenergic receptors in the heart has been blunted. High levels of circulating catecholamines, released by the adrenal glands during prolonged physical activity (Péronnet et al., in press), have important metabolic functions necessary for the maintenance of exercise (Galbo et al., 1975), and it is possible that the important barrier at the level of the capillaries in the heart functions together with an efficient removal mechanism, to protect the extracellular space from a too large
concentration of catecholamines. In humans, over-dosage with desmethylimipramine causes arrhythmias (Bucher and Stucki, 1967), probably due to the effect of the buildup of norepinephrine that occurs when the axonal membrane uptake mechanism has been blocked (Sachs, 1969).

The one-barrier model developed for the analysis of the norepinephrine data also gives an adequate description of our tracer isoproterenol data. The \( k_r \) for isoproterenol was smaller than that for norepi-

nephine, as expected for a compound with a larger molecular weight, and the neuronal removal rate constant \( k_n \) for isoproterenol was found to be much lower than that for norepinephrine. Histofluores-
cent techniques have shown that isoproterenol is taken up poorly by the adrenergic nerves (Corrodi et al., 1966). These in vitro observations are the correlate to our in vivo findings. It has been suggested that the low uptake is secondary to steric hindrance, consequent to the bulky-N-substitution (Hertting, 1964). It also has been proposed that the greater chronotropic activity of isoproterenol in the heart, when compared with that of an equimolar dose of norepinephrine, could be secondary to the less rapid neuronal uptake of this amine (Gauthier et al., 1974). Our findings document this phenomenon and emphasize that the rate of removal is an important factor in particularizing the activity of synthetic sympathetic amines.

The present study results in a new viewpoint, that catecholamine effects in the heart are modu-

lated not only by neuronal uptake and release processes, and the adrenergic receptors, situated in the interstitium, but also by the presence of a significant barrier at the level of the cardiac capillaries. The interactions between the capillary barrier, the inter-

stitium, and the concentrative neuronal uptake process provide not only a way of quantifying tracer kinetics, as explored in the experiments reported here, but also a qualitative way of accounting for the quantitatively different cardiac responses to catecholamines administered intravenously or re-

leased locally in the interstitium.

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Inhibition of Glycolysis in the Denervated Dog Heart

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SUMMARY We measured glucose metabolism in five dogs before and 3 weeks after cardiac denervation; after this time myocardial norepinephrine is depleted. The discharge by the myocardium, of $^{14}$CO$_2$ from infused $^{14}$C-D-glucose (U), decreased following denervation ($P = 0.05$). The ratio of $^{14}$CO$_2$ to total CO$_2$ production, which measured the proportion of glucose to total substrate oxidized, also decreased following denervation ($P = 0.05$). The inhibition of glucose oxidation by denervation was not due to an increase in arterial lactate concentration. There was an associated increase in myocardial content of fructose-6-phosphate in an additional seven dogs ($P < 0.01$). We postulate that myocardial tissue norepinephrine is one of the controllers of the activity of phosphofructokinase.

METHODS

Twelve male, mongrel dogs were studied. They were divided into two groups: series I, five dogs, weighing 15.0-17.0 kg for glucose oxidation studies, and series II, seven dogs, weighing 14.5-21.0 kg for tissue biopsy studies.

Series I (Glucose Oxidation Group)

The dogs were subjected to a standardized environment and diet for 3 weeks before as well as throughout the period of study. The first study was carried out 1 week prior to cardiac denervation. The dogs were anesthetized with intravenous methohexitone sodium (10 mg/kg for induction, wearing off in 5 minutes), followed by a standard dose of chloralose (Arfors et al., 1971). The left jugular vein was exposed through a small incision and a cardiac catheter inserted. The catheter was advanced and positioned in the coronary sinus under fluoroscopic control. The position of the catheter was checked by visualizing the coronary sinus with a bolus injection of 5.0 ml of Uro-
Labeled catecholamine uptake in the dog heart. Interactions between capillary wall and sympathetic nerve uptake.
D Cousineau, C P Rose and C A Goresky

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