Decreased Basal Cardiac Interstitial Norepinephrine Release after Neuronal Uptake Inhibition in Dogs

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SUMMARY The effect of neuronal uptake inhibition on basal interstitial release of norepinephrine in the canine heart was examined by use of the multiple tracer dilution-bulk balance technique. A kinetic model incorporating the effects of flow, capillary permeability-surface product for norepinephrine, the interstitial uptake rate constant for neurotransmitter, and plasma norepinephrine input and output values was used to estimate rates of uptake from and release of norepinephrine into the interstitial space. The intravenous injection of the neuronal uptake inhibitor desipramine in anesthetized dogs under basal conditions reduced interstitial uptake of tracer norepinephrine in the heart, without significant changes in plasma concentration of norepinephrine in aorta and coronary sinus. The lack of change in the arterial venous balance for norepinephrine across the heart, in the face of the lowered uptake for this amine, suggested that the liberation of norepinephrine by cardiac sympathetic fibers was reduced. Analysis of the data with the norepinephrine tracer kinetic-bulk model showed that, after desipramine, the interstitial release of norepinephrine was reduced to the same extent as uptake was diminished. As a result, the concentration of norepinephrine in the extracellular space of the heart did not increase significantly. The findings indicate the presence of a presynaptic neuronal feedback inhibition of release, which serves to fine tune the myocardial interstitial concentration of norepinephrine in the basal state, with this, after desipramine, both norepinephrine uptake and release are correspondingly diminished (Circ Res 58: 859–866, 1986)

ACTIVATION of the peripheral sympathetic system results in the release of the neurotransmitter norepinephrine into the interstitial space of the heart. Since part of this will overflow into the venous outflow, it has been postulated that its measurement in the venous effluent of the heart might provide an index of the level of cardiac sympathetic activation (Cousineau et al., 1978). Multiple indicator dilution studies have shown, however, that the capillaries in the heart impose a major resistance to the diffusion of norepinephrine across the endothelial membrane (Cousineau et al., 1980), and this myocardial capillary barrier, coupled with an efficient interstitial neuronal norepinephrine uptake mechanism, considerately restrains the passage of norepinephrine from the interstitial to the vascular spaces, so that norepinephrine in the coronary sinus represents only a small fraction of the neurotransmitter initially released into the interstitial compartment. Study of the interactions between cardiac norepinephrine release and the capillary-interstitial uptake system also indicate that recruitment or derecruitment of capillaries, with corresponding changes in the capillary surface (Cousineau et al., 1983), or pharmacological inhibition of neuronal uptake of this amine, during high levels of stimulation (Cousineau et al., 1984), can modify the overflow of norepinephrine into the coronary sinus, independent of any change in the rate of liberation of norepinephrine by cardiac sympathetic fibers, whereas under similar circumstances, with α-receptor stimulation (Cavero et al., 1979) or blockade (Cousineau et al., 1983), coronary sinus overflow varies with the rate of release. The findings indicate the limited fashion in which coronary sinus plasma norepinephrine and norepinephrine overflow reflect sympathetic events in the myocardial interstitial space.

Inhibition of the neuronal norepinephrine uptake in the heart would be expected to increase interstitial norepinephrine levels at all levels of release and, hence, norepinephrine coronary sinus plasma levels, assuming that both interstitial release of this amine and capillary flow and surface are unchanged. At high levels of stimulation, these expectations appear to be fulfilled: overflow measurements show increased coronary overflow after desipramine (Cousineau et al., 1984); and, with this, there is a further increase in heart rate (Cavero et al., 1979). In contrast, the measurement of plasma catecholamines in aorta and coronary sinus in dogs at rest has shown that, after desipramine, arterial and venous levels of these amines across the heart are not different (Yamaguchi et al., 1977). The lack of change in the values for plasma catecholamines after desipramine at rest could have resulted from an accompanying reduction in the cardiac release of norepinephrine or from a reduced exchange at the capillary barrier due to capillary derecruitment, with reduction in the
exchange surface (a decrease in norepinephrine spill from the interstitium into the coronary sinus would have occurred, in either case). It is not possible, from measurements only of plasma catecholamines, to determine which of these possibilities applies. A more detailed approach is necessary.

An analogous problem has been studied, in an in vitro preparation. In an isolated, recurrently stimulated canine saphenous vein preparation, the administration of a neuronal uptake inhibitor (cocaine) has been found to concomitantly inhibit not only uptake but also release of norepinephrine in response to stimulation (Shepherd and Vanhoucke, 1981). In this isolated vessel, cocaine administration is followed immediately by a decrease in the rate of liberation of previously stored tritiated norepinephrine, in response to continuing stimulation. The authors have proposed that, following the primary effect of the drug—namely, inhibition of neuronal uptake with a local rise in concentration—there is a prompt negative feedback inhibition of norepinephrine release which then maintains the local concentration of the neurotransmitter within relatively narrow limits, despite the lower uptake.

Our present study is designed to evaluate whether a similar regulatory control mechanism is active in vivo, in the basal state, in the in situ working heart. In pursuit of this, we have carried out under basal conditions, both in a control state and after the injection of the neuronal uptake inhibitor desipramine, a set of tracer norepinephrine experiments, with simultaneous measurements of plasma norepinephrine, in closed-chest dogs, as described previously (Cousineau et al., 1981). A kinetic analysis incorporating coronary flow, the capillary permeability-surface product for norepinephrine, and the rate constant for neuronal uptake of tracer norepinephrine is used to obtain, via plasma norepinephrine bulk input and output concentrations, the rates of bulk norepinephrine release into and uptake from the interstitial space (Cousineau et al., 1984). The anatomical compartmentalization of the myocardial interstitial space makes it virtually impossible to measure rates of local interstitial norepinephrine release and uptake in vivo in any other way, so that the feedback inhibition hypothesis has not previously been able to be appraised, in the working in situ heart. The combined multiple indicator tracer dilution-norepinephrine balance approach provides a way of undertaking this.

**Methods**

Mongrel dogs weighing between 17 and 21 kg were anesthetized with pentobarbital (30 mg/kg). The main stem or circumflex branch of the coronary artery was catheterized via a carotid artery under fluoroscopy with an angiographic catheter, and the coronary sinus was catheterized via the right jugular vein. Heart rate and aortic blood pressure were monitored continuously.

The injection mixture used in the multiple indicator dilution study consisted of blood matched to the hema-

circulation of the dog, containing $^{125}$I-labeled albumin (Charles E. Frosst), a reference substance that does not leave the coronary circulation within a single passage (Ziegler and Goresky, 1971), $[^{14}C]$sucrose (New England Nuclear), a diffusible substance that leaves the circulation to enter the interstitial space during its passage through the heart, and $[^{1,7-3}H(N)]$norepinephrine (specific activity, 30.0 Ci/mmol; New England Nuclear). When the preparation was in a steady state, 0.75 ml of the mixture (containing 6 μCi $^{125}$I-albumin, 12 μCi $[^{14}C]$sucrose, and 50 μCi $[^{3}H]$norepinephrine) was rapidly flushed through the input catheter with blood. Simultaneously, a collection rack was started and 40 sequential samples were collected from the coronary sinus at a rate of 0.5–1.0 samples/second. Immediately after each experimental run, 5 ml of blood were sampled simultaneously from aorta and coronary sinus, for the measurement of plasma catecholamines and blood hemoglobin and oxygen content. Multiple indicator dilution studies were carried out in two groups of eight dogs under basal conditions, 20 minutes after the intravenous administration of either saline (control) or desipramine (an uptake inhibitor), at a dose of 1 mg/kg.

A volume of 0.1 ml from each heparinized blood sample collected during the dilution study was diluted with 1.5 ml of saline, pipetted into a counting tube, and assayed for radioactivity in a gamma ray spectrometer set for the photopeak characteristic of $^{125}$I. The proteins then were precipitated with 0.2 ml of trichloroacetic acid, and 0.2 ml of the supernatant fluid was pipetted into a scintillation cocktail and assayed for $[^{14}C]$ and $[^{3}H]$ activity in a liquid scintillation counter. Samples from the injection mixture, diluted with blood, and crossover standards were treated identically. To normalize the activity resulting from each of the tracers with respect to the others (for the purposes of our later analysis), we divided the activity of each by the total activity injected for that species. The resulting value is a fraction of the total injected per milliliter of venous blood.

For determination of the catecholamine levels, 3 ml of blood were transferred to ice-cold tubes that contained glutathione and EGTA, as described by Peuler and Johnson (1977). Norepinephrine and epinephrine levels were determined in duplicate in 50 μl of plasma by the radiometric method outlined by these authors, with only minor modification (Cat-a-Kit, Upjohn). Blood hemoglobin and oxygen contents were measured with a Lex O2-Con-K analyzer (Lexington Instruments).

**Analysis of the Data**

Normalized coronary sinus outflow curves obtained in the same animal under control conditions before and after desipramine are shown in Figure 1. The relationships between the outflow dilution curves of the set can be interpreted qualitatively in the following way. Labeled albumin, which occupies the plasma space in blood and serves as the vascular reference substance, does not leave the capillary to any significant degree during a single passage; its outflow pattern is shaped solely by the distribution of transit times in the large or nonexchanging vessels and the capillaries or exchanging vessels. Labeled sucrose, which occupies a space in plasma identical to that of labeled albumin but permeates the capillary barrier via aqueous channels, to enter the interstitial space, serves as an interstitial or extracellular space reference. In early samples, the sucrose curve is reduced in relation to the albumin curve because of loss from the capillary and, later
in time, the interstitial tracer returns to the capillary, to emerge in delayed fashion at the outflow; the later components of the sucrose curve, emerging at the outflow after most of the labeled albumin has left the organ, then cross over and are found above the tail of the labeled albumin curve. Labeled norepinephrine is handled in a similar fashion at the capillary surface, but its removal and concentration by the sympathetic nerve fibers in the interstitial space, which run parallel to the capillaries, reduce the returning components of its dilution curve. The effect is most prominent in the control run. Since Chidsey et al (1962) have shown previously that, 3 minutes after the injection of labeled norepinephrine into the coronary artery of the canine heart, the metabolites of norepinephrine constitute only a barely perceptible proportion of the total radioactivity measurable in the coronary sinus, we have assumed that the injected material obtained at the outflow in our experiments, over periods up to 50 seconds, has emerged unchanged. After desipramine, a neuronal uptake inhibitor, there is, in comparison with the control run, an obvious change in the shape of the labeled norepinephrine curve. There is a marked decrease in the rate of decay of the late downslope of the norepinephrine curve. The change in shape corresponds to what would be expected with inhibition of the process which normally sequesters the tracer norepinephrine which has escaped from the capillary and entered the interstitial space. As a result of this, the later part of the norepinephrine tracer outflow has increased in magnitude and, with this, the total area under the outflow curve (the outflow norepinephrine tracer recovery) has increased.

The methodological and mathematical background necessary for modeling cardiac outflow tracer curves has been described both for sucrose (Rose and Goresky, 1976) and for norepinephrine (Cousineau et al., 1980). For sucrose, the parameters optimized in the whole organ model are: $k_\text{s}$, the capillary permeability-surface product for sucrose per unit sucrose interstitial space (ml/sec per ml); $\phi$, the plasma flow per unit sucrose interstitial space (ml/sec per ml), and $a'$ and $b'$, parameters that describe the heterogeneity of capillary transit times. The optimized parameters for norepinephrine are $k_\text{n}$, the capillary permeability-surface product for sucrose per unit sucrose interstitial space (ml/sec per ml), so that it will be directly comparable to the sucrose data; and $k_\text{u}$, the rate constant for unidirectional uptake from interstitial space, again with dimensions corresponding to a permeability-surface product per unit sucrose interstitial space (ml/sec per ml).

The bulk norepinephrine balance, in pmol/(ml sucrose interstitial space) per sec, is equal to $\phi(C_{\text{ven}} - C_{\text{ar}})$, where $C_{\text{ven}}$ and $C_{\text{ar}}$ are venous and arterial plasma norepinephrine concentrations, expressed in picomoles per milliliter (pm). From the simultaneous determination of plasma norepinephrine in aorta and coronary sinus and the mea-
measurement of the norepinephrine tracer extraction (calculated on the basis of the relative proportion of the tracer norepinephrine not recovered at the outflow), the net overflow to the outflow of unlabeled norepinephrine released into the interstitial space can be determined (Cou-
sineau et al., 1986). The net overflow of norepinephrine is equal to the difference between the bulk norepinephrine emerging in the coronary sinus and that predicted on the basis of the proportion of the input tracer escaping the myocardial extraction, that is, norepinephrine net overflow
\[ \text{Norepinephrine net overflow} = \phi[C_{\text{en}} - C_{\text{art}}(1 - \text{tracer extraction})] \] (1)

Values for norepinephrine net overflow, when calculated like this, are also expressed in terms of pmol/(ml sucrose interstitial space) per sec.

From the arterial and coronary sinus plasma levels of circulating bulk norepinephrine and the optimized parameters derived from the tracer norepinephrine curve, we can now use expressions for conservation of mass to determine the rate of release R of this amine into the interstitial space from cardiac sympathetic fibers, which is expressed in terms of pmol/ml interstitial space) per sec.

The modeling and expressions utilized for quantification of the local rate of release of unlabeled norepinephrine are those outlined previously (Cousineau et al., 1984). The underlying assumption is that, with concentrative uptake at the neuronal surface, and with intraneuronal compartmentation of secreted norepinephrine in vesicular and granular forms, prior to its release, the secreted norepinephrine can be regarded as essentially unlabeled, over the times of an indicator dilution study.

The modeling also provides, with the estimated norepinephrine release rates, estimates of the interstitial concentrations of norepinephrine in the heart. These are common at the inflow, but vary at the outflow with the underlying sinusoidal transit time. For each of the underlying profiles, the appropriate average interstitial concentration is the logarithmic average concentration (Goresky and Groom, 1984) These are, in each case, suitably weighted for the whole heart to provide an average interstitial concentration. This is the value reported below, in Results.

Finally, a value can be derived for the local rate of neuronal uptake of unlabeled norepinephrine. This is equal to the product of \( k_u \) and the local interstitial norepinephrine concentration. We report below the average value for this, the product of \( k_u \) and the overall average of the interstitial concentrations. This, again, is expressed in pmol (ml interstitial space) per sec.

Relative myocardial \( O_2 \) consumption in these experiments has been calculated by use of the following relation:

\[ V_{O_2} = \frac{1}{1 - Hct} \times \phi \times (C_{O_{art}} - C_{O_{cs}}) \times \frac{60}{100} \] (2)

where \( V_{O_2} \) is the myocardial oxygen consumption per unit interstitial space, expressed in units of ml/min per ml interstitial space; Hct is the hematocrit, expressed as a fraction; and \( C_{O_{art}} \) and \( C_{O_{cs}} \) are the oxygen contents of arterial and coronary blood, respectively, in units of ml/100 ml blood.

For statistical evaluations, Student's t-test was used; levels of significance at which the null hypothesis can be rejected are described, in each case.

**Results**

Mean values are given in Table 1 for plasma norepinephrine concentrations in aorta and coronary sinus, mean aortic blood pressure, myocardial oxygen consumption, and for the optimized parameters derived by best fit procedures from norepinephrine and sucrose curves, acquired in dogs under basal conditions after intravenous injection of either saline (control) or desipramine (uptake inhibitor).

The mean values for plasma norepinephrine in aorta and coronary sinus were not significantly increased in the desipramine group, compared with the control group. The mean values for arterial blood pressure, myocardial oxygen consumption, coronary flow, and permeability-surface product for sucrose and norepinephrine were not significantly different between the groups of control and desipramine-treated animals, whereas the tracer norepinephrine extraction and the rate constant for norepinephrine uptake from interstitial space were significantly reduced in the desipramine treated group, as expected from Figure 1.

From the measurements of plasma norepineph-

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**Table 1**

Levels of Plasma Norepinephrine (NE) in Aorta and Coronary Sinus, Mean Arterial Blood Pressure, and Optimized Parameters Derived by Best Fit from Dilution Curves in Two Groups of Eight Dogs under Basal Conditions*<br><br>![Table](https://example.com/table1.png)

Results are expressed as mean ± se. \( \phi \) = plasma flow per unit sucrose interstitial space; \( k_c \) = capillary permeability-surface product for sucrose, per unit sucrose interstitial space; \( k_s \) = capillary permeability-surface product for norepinephrine, per unit sucrose interstitial space (normalized in this way, so that \( k_s \) can be compared to \( k_u \)); \( k_u \) = rate constant for neuronal uptake of norepinephrine, per unit sucrose interstitial space.<br><br>After the injection of either saline (control group) or desipramine (the uptake inhibitor group).<br><br>\( \dagger P < 0.01 \) vs control group.
rine in aorta and coronary sinus, and of norepinephrine tracer extraction, it was found that neither mean norepinephrine balance nor overflow differed significantly between the control and desipramine-treated groups of animals (Fig. 2). However, analysis of the data with the tracer kinetic-bulk model demonstrated that there was a significant reduction in the average rate of uptake of bulk norepinephrine after desipramine (Fig. 2). This is the finding expected, particularly in view of the change in the shape of the norepinephrine tracer curve and reduction in outflow tracer recovery after desipramine, and the major reduction in the calculated rate constant for the neuronal uptake of tracer norepinephrine. It also conforms to ordinary pharmacological precepts.

Since arterial and coronary sinus values for bulk norepinephrine in the desipramine groups did not differ significantly from control, one would infer that the rate of norepinephrine release in the desipramine-treated group must also have been lower. This is precisely what is documented by the model analysis. This revealed a significant reduction in norepinephrine release after desipramine (Fig. 3), indicating that the inhibition of neuronal uptake had concomitantly been accompanied by a decrease in the rate of liberation of the neurotransmitter by the cardiac sympathetic fibers, in this basal state. The mean values for norepinephrine uptake and release were closely similar in the control animals, as well as in the uptake inhibitor animals, but both values were significantly lower in the latter group (Fig. 3). The proportional decrease in norepinephrine release with the lower uptake of this amine after desipramine resulted in a lack of significant increase in the average estimated interstitial norepinephrine concentration in the uptake inhibitor group, compared with the control group (Fig. 4). The findings indicate that, in the basal state under steady state conditions, interstitial norepinephrine release was adjusted to match uptake, in the face of the pharmacologically induced decrease in the uptake of neurotransmitter. Figure 5 provides a graphic illustration of this phenomenon. Individual values for norepinephrine release in the groups of control and uptake inhibitor animals are closely related to corresponding values for norepinephrine uptake. The best fit relation is relatively closely related to the identity line.
The data from the present study indicate that the administration of desipramine, a neuronal uptake inhibitor, in a dose of 1 mg/kg to dogs under basal conditions, results in a decrease in both uptake and interstitial release of norepinephrine by cardiac sympathetic fibers. The diminution in norepinephrine liberation after the uptake inhibitor was similar to the reduction in norepinephrine uptake and, as a result, the concentration of the neurotransmitter in the interstitial space was not significantly increased after uptake inhibition, compared with the values found in the control group.

The mean values for plasma norepinephrine concentrations in aorta and coronary sinus did not differ significantly, for the groups of control and desipramine-treated dogs, as previously reported by Yamaguchi et al. (1977). We now must determine what can be learned from the relation between the arterial and coronary sinus levels in the present set of experiments. When circulating norepinephrine plasma levels are similar in the aorta and the coronary sinus, there is no underlying net extraction along the myocardial capillaries, and vascular and interstitial levels of the hormone will be the same, from capillary entrance to exit. Under those conditions, circulating norepinephrine values will directly reflect interstitial space levels (Cousineau et al., 1981, 1984). The findings of essentially identical average input and output norepinephrine concentrations in both present sets of data lead one to anticipate that average interstitial concentrations would not differ significantly. This is indeed what is found. Desipramine reduced the extraction of tracer norepinephrine, as previously reported (Cousineau et al., 1981). The comparable values for interstitial norepinephrine concentration after the uptake inhibitor could have been achieved only by a reduction of the neurotransmitter release by cardiac sympathetic fibers comparable to the lowering of interstitial uptake. The comparable decrement was substantiated by our quantitative measurements of norepinephrine uptake and release, which showed that these processes had decreased to a similar extent after the uptake inhibitor.

It has previously been proposed from in vitro experiments that the reduction in norepinephrine release following the administration of a neuronal uptake inhibitor results from an increase in the local concentration of the neurotransmitter, which via a negative feedback mediates a decrease in the liberation of norepinephrine by sympathetic fibers and stabilization of the local concentration (Shepherd and Vanhoutte, 1981). In our in vivo study, the norepinephrine release after desipramine was decreased at interstitial concentrations of this amine, which had increased, but not significantly, suggesting that, if this is the manner in which the response is mediated, the negative feedback system operates with only small variations in the local concentration of norepinephrine. Thus, the similarity between the rates of norepinephrine uptake and release in the groups of control and uptake inhibitor animals indicates that there may be an autoregulatory system of high sensitivity and precision operating in vivo.

The mechanism underlying the reduction in neurotransmitter release in the heart is quite evidently presynaptic, but we cannot ascertain, from the present data, whether the mechanism is localized to the
presynaptic membrane, or whether via some afferent or central mechanism, it involves either ganglionic or central parts of the sympathetic pathway. For instance, the intravenous desipramine could have affected ganglionic or central sites directly. The inhibition of release response to the pharmacological uptake inhibitor has been demonstrated to be characteristic of adrenergic neurones at a number of sites. Thus, apart from the present demonstration in the heart, Shepherd and Vanhoutte (1981) have shown a similar effect involving the neurones innervating an isolated canine saphenous vein preparation, and Svenssen and Usdin (1978), one in which the adrenergic neurones of the locus caeruleus of the rat brain respond to desipramine by reducing their rate of firing. The finding of a similar response in the isolated canine saphenous vein preparation would suggest, by analogy, that the cardiac mechanism is more likely to be local than central in nature.

The primary pharmacological effect of desipramine, the reduction of the neuronal uptake of sympathetic amines, is clearly direct. On the other hand, the effect of desipramine on norepinephrine release may be direct or secondary. There is some evidence suggesting that the effect of desipramine on release might be direct. Desipramine is known to inhibit depolarization-induced calcium uptake in synaptosomes (Isenberg and Tamargo, 1985), and imipramine has recently been shown to reduce calcium conductance in another excitable tissue, the isolated ventricular myocyte (Aronstam and Hoss, 1985). These findings suggest that desipramine and related molecules could directly suppress calcium-dependent processes such as secretion. The observed inhibition of release could then result.

On the other hand, if the inhibition of release is a response to the resulting change in local norepinephrine concentration and is independent of the desipramine, the sensing mechanism may be α-adrenergic in character. In vivo studies have previously shown that presynaptic α-adrenergic receptors exert a modulatory effect on norepinephrine release in the heart. α-Adrenergic antagonists have been found to increase cardiac norepinephrine release at all levels of sympathetic activation, presumably by blocking a negative feedback inhibition which ordinarily suppresses the liberation of neurotransmitter by the sympathetic fibers (Yamaguchi et al., 1977; Cavero et al., 1979; Cousineau et al., 1984). In contrast, α-adrenergic agonists have been shown to suppress cardiac norepinephrine release by a presynaptic α-receptor-mediated mechanism. However, this suppression is effective only at low levels of sympathetic stimulation, and becomes no longer perceptible when cardiac adrenergic activation is intense; it appears to be inhibited at very high rates of release (Yamaguchi et al., 1977). In the present study, if this is the mechanism, we will see the local response to the natural agonist, norepinephrine. In this closed-chest study, plasma norepinephrine levels approach the values found in conscious trained dogs at rest (Péronnet et al., 1981), and the adrenal medullae are also relatively silent (aortic plasma norepinephrine values never exceed 1.5 nM). In this present set of animals, in a basal state with low sympathetic tone, there was, in response to desipramine, an effective presynaptic inhibition of norepinephrine release; the inferred small increase in the local concentration of the neurotransmitter (too small to reach levels of significance with the number of experimental animals utilized) was accompanied by a major and significant suppressive effect on norepinephrine liberation at sympathetic endings. This inhibition provides an apparent fine control, at a reduced level of transneuronal exchange, in the resting situation. At high levels of sympathetic activation, there is no longer need for such a control mechanism; it might therefore be expected that the presynaptic inhibitory action of α-adrenergic agonists would be less efficient, and the release of norepinephrine, essentially unimpaired. This is indeed what we recently were also able to demonstrate in a parallel study, characterizing the mechanism of action of desipramine in closed-chest animals during the intense cardiac sympathetic activation provoked by coronary sinus pacing (Cousineau et al., 1984). Desipramine, during this intense stimulation, inhibited the uptake of norepinephrine from the myocardial interstitial space; the concentration of norepinephrine in the interstitial space increased significantly, and norepinephrine overflow increased to a major degree. Despite this, the local rate of release of neurotransmitter was not diminished. The lack of inhibition of release at these higher levels of stimulation appears teleologically sensible. Inhibition of norepinephrine release during high levels of sympathetic stimulation would be redundant, since the physiological need is then for myocardial adrenergic functions to become maximally energized.

The unchanged values for norepinephrine balance and overflow observed in the present study after desipramine might, in the past, have suggested that liberation of norepinephrine by cardiac sympathetic fibers in the basal state was unaffected by desipramine. However, the analysis of the data with the tracer kinetic-bulk model shows a significant reduction in both norepinephrine uptake and release after neuronal uptake inhibition. It indicates that, at levels of activation corresponding to the conscious basal state, there may be a functional control mechanism operating in vivo.

The present set of findings also shows how sensitive the tracer kinetic-bulk norepinephrine approach is, in the quantification of the events underlying norepinephrine balance across the heart. The utility of this approach was also recently demonstrated in our study of the effects of β-adrenergic blockade on the interstitial release of norepinephrine in the canine heart (Cousineau et al., 1984). β-Adrenergic blockade reduced norepinephrine overflow, as previously reported by others (Yamaguchi
et al., 1977; Lavallée et al., 1981), but was found to have no effect on norepinephrine release. The lower norepinephrine overflow traditionally attributed to presynaptic β-inhibition of norepinephrine release was, in fact, secondary to capillary derecruitment, to a decrease in the capillary permeability-surface product for norepinephrine.

The in vivo approach to the measurement of cardiac norepinephrine uptake and release in the intact system means, ipso facto, that the structural integrity of the myocardium, especially that of its capillary membrane barrier, is respected. In this situation, with previous conventional approaches to sympathetic studies, events in the interstitial space were essentially inaccessible. Equally, in this same situation, the multiple indicator dilution-bulk balance method provides a nondestructive approach to the in vivo quantification of the interstitial uptake and release of norepinephrine. With this, in the present study, we have been able to elucidate, in the intact animal at basal levels of sympathetic activation, reduced norepinephrine release following desipramine which would not have been easily definable in vivo by any other approach. Similar problems in the future can also be approached in a similar fashion.

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