Heart Rate Modulates the Disposition of Neurally Released Norepinephrine in Cardiac Tissues

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SUMMARY. We determined the effect of heart rate on the disposition of neurally released norepinephrine from the cardiac tissues in open-chest, anesthetized dogs with complete atrioventricular block. After injecting desipramine to block the neuronal uptake of norepinephrine, we stimulated the cardiac sympathetic nerves supramaximally for about 3 minutes at a mean frequency of 2.7 Hz. In one series of experiments, we allowed coronary sinus blood flow to change spontaneously in response to cardiac pacing and sympathetic stimulation. The mean coronary sinus blood flows just before cessation of sympathetic stimulation were 67.4 ± 4.2 (SE) and 37.4 ± 2.7 ml/min during pacing at a high (150/min) and a low (60/min) frequency, respectively. The mean norepinephrine overflow into the coronary sinus blood during steady state sympathetic stimulation was 45.0 ± 14.2% greater during high frequency pacing than during low frequency pacing. After cessation of neural stimulation, the norepinephrine overflow decayed more rapidly during high frequency pacing than during low frequency pacing. This difference in decay rates was mainly ascribable to the difference in coronary blood flows. In a second series of experiments, we adjusted the coronary sinus blood flow during sympathetic stimulation so that the flows were not significantly different during pacing at the two frequencies. During the initial 30-second period, after cessation of sympathetic stimulation, the mean decrements of norepinephrine overflow were 95.9 ± 21.4 and 59.2 ± 17.3 ng/min at the high- and low-pacing frequencies, respectively. In view of the equivalent coronary blood flows, the more rapid decay of the norepinephrine overflow during pacing at the higher frequency suggests that a cardiac massaging action may also facilitate the transport of the neurally released norepinephrine from the neuroeffector gaps into the myocardial capillaries. (Circ Res 57:19-27, 1985)

WE recently studied the cardiac responses to trains of sympathetic stimulation in open-chest, anesthetized dogs with complete atrioventricular (AV) block (Masuda and Levy, 1983). We found that when we paced the heart at different frequencies, the rate at which the ventricular inotropic response decayed immediately after cessation of sympathetic nerve stimulation varied inversely with the pacing frequency. We postulated that the norepinephrine (NE) released from the sympathetic nerve endings was removed from the neuroeffector gaps in the myocardium at a rate that varied directly with the contraction frequency.

In the present series of experiments, we tested this hypothesis by measuring the rates of NE overflow into the coronary sinus blood during and immediately after periods of tonic sympathetic stimulation. We found that the NE overflow did decay more rapidly after cessation of sympathetic stimulation when the heart was paced at a relatively high frequency than it did when paced at a relatively low frequency. The coronary blood flows were greater during pacing at the high frequency than during pacing at the low frequency. To determine whether these differences in coronary blood flow were entirely responsible for the disparities in NE overflow at the two different pacing frequencies, we carried out a second series of experiments in which we held the coronary blood flows at equivalent levels during pacing at the two different frequencies.

Methods

Surgical Preparation

Experiments were conducted on 25 mongrel dogs with a mean body weight of 20.0 ± 3.3 (SD) kg. The animals were anesthetized with sodium pentobarbital, 30 mg/kg, iv. A tracheal cannula was inserted through a midline cervical incision, and intermittent positive pressure ventilation was begun. The chest was opened bilaterally through a transverse incision in the 4th intercostal space. Both cervical vagi and the upper poles of both stellate ganglia were crushed by tight ligatures, in order to interrupt almost all of the tonic neural activity to the heart (Levy et al., 1966).

Arterial blood pressure was measured from a femoral artery by means of a Statham transducer (P23AA). A Walton-Brodie strain gauge arch was used to measure right ventricular contractile force. The strain gauge was attached to the ventricular wall at a site about halfway between the apex and base of the heart. The long axis of the strain gauge was parallel to the anterior descending coronary artery and about 1 cm lateral to it. The cardiac cycle length was derived electronically from the strain gauge output.

After heparin, 500 units/kg, iv, was administered to...
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the determination of the basal NE concentration. Both

eration, a coronary sinus blood sample was withdrawn for

from the coronary sinus was led through the extracora-

real probe of an electromagnetic flowmeter (Biotronex,

model BL615), and the blood was returned to the venous

system through a cannula in the right external jugular

vein. Coronary sinus blood samples (5 ml/sample) were

obtained from a T-tube in this external line. The arterial

blood pressure, right ventricular contractile force, cardiac

cycle length, and coronary sinus blood flow were recorded

on a direct-writing oscillograph (Brush, Mark 260).

A small amount of formaldehyde was injected into the

AV node region in order to produce complete AV block

(Steiner and Kovalik, 1968). This procedure enabled us to

pace the ventricles at a relatively low frequency (60/min)
as well as at a more rapid frequency (150/min). Pacing

was achieved via a bipolar electrode that was attached to

the surface of the right ventricle. We separated the elec-

trode poles by only 2–3 mm, and we used a stimulus

strength just above threshold to minimize the amount of

norepinephrine that would be released in the myocardium

by the pacing stimuli.

The atra were maintained in fibrillation by a continu-

ous train of electrical stimuli delivered at a frequency of

30 Hz and at a voltage that was just above threshold.

Atrial fibrillation was induced to avoid the irregular vari-

ations in ventricular filling that would otherwise have

prevailed in these animals with complete heart block. Such

irregularities would have diminished the precision of our

estimations of the ventricular inotropic response decay

times.

Experimental Protocols

Near the beginning of each experiment, desipramine

hydrochloride, 1 mg/kg, was injected intravenously as a

bolus, and then was infused at a constant rate of 13 μg/

kg per min throughout the remainder of the experiment

(Levy and Blattberg, 1978). The desipramine was given to

block the neuronal uptake of norepinephrine (Iversen,

1975).

Two series of experiments were conducted. In the first

series, coronary blood flow was permitted to vary sponta-

neously in response to sympathetic stimulation and to

pacing. In the second series, the coronary blood flow was

controlled to ensure that the flows would be equal during

pacing at the two frequencies.

Series I: Coronary Blood Flow Not Controlled

This series consisted of two sets of experiments. In the

first set (seven dogs), the cardiac responses to sympathetic

stimulation were not abolished. In the second set of ex-

periments (six dogs), the cardiac responses were blocked

by propranolol.

First Set: Mechanical Responses Intact. In each experi-

ment, the ventricles were paced at frequencies of 60 and

150/min. The order of applying these pacing frequencies

was randomized. About 5 minutes after a given pacing

frequency had been instituted, but prior to neural stimu-

lation, a coronary sinus blood sample was withdrawn for

the determination of the basal NE concentration. Both
decaentralized ansae subclaviae were then stimulated with

a 3-minute train of square wave pulses (Grass stimulator,

model 59); each pulse was 2 msec long, and of supramax-

imal voltage (usually 15 V). In each experiment, we used

a stimulation frequency (mean, 2.7 Hz) that had elicited a

2-fold increase in right ventricular contractile force during

the preliminary phase of the experiment. In each animal,

the same sympathetic stimulation frequency was used
during pacing at each of the two frequencies.

The changes in right ventricular contractile force, car-
diac cycle length, coronary sinus blood flow, and arterial

blood pressure evoked by the 3-minute train of sympa-
thetic nerve stimulation were determined during pacing

during the two frequencies. Coronary sinus blood samples were

withdrawn for NE analysis before stimulation, and during

and after cessation of stimulation.

Second Set: β-Receptor Blockade. We performed a similar

set of experiments after propranolol had been infused.

Prior to any experimental procedure, propranolol hydro-

chloride (1 mg/kg) was injected as a bolus, and then it

was infused continuously at a rate of 16.7 μg/kg per min

throughout the remainder of the experiments. After a

complete AV block had been induced, the ventricles were

paced at a frequency of either 60 or 120/min. The exper-

imental procedures were the same as in the previous set,

except that the ansae subclaviae were stimulated at a fixed

frequency of 3 Hz for 3 minutes. Coronary sinus blood

samples were withdrawn prior to sympathetic stimulation,

and during and after cessation of stimulation.

Series II: Coronary Blood Flow Controlled

The preparations and protocols were similar to those

described in the first set of experiments in series I, except

that in series II we adjusted the coronary blood flow so

that the flows would be equivalent during pacing at the

two frequencies. Just as in series I, we randomized the

order in which we paced at the two frequencies. The

protocols were slightly different, depending on which

stimulation frequency was to be used first. In sequence A,

the ventricles were paced first at 150/min, whereas in

sequence B, the ventricles were paced first at 60 min.

Sequence A: In those experiments in which the

ventricles were paced first at 150/min, a control sample

of coronary sinus blood was withdrawn for NE analysis

at t₀, prior to ansal stimulation (Fig. 1A). Both ansae

subclaviae were then stimulated (horizontal bar) at a fre-

quency of 2 Hz for 3 minutes, and coronary sinus blood

samples were withdrawn during and after cessation of

stimulation.

The ventricles were then paced at 60/min (Fig. 1A). A

coronary sinus blood sample was withdrawn at t₀, and

then a constant train of sympathetic stimulation (2 Hz)

was begun. After the ventricular response reached a steady

state level (at t₀), we gradually tightened a screw clamp

around the descending thoracic aorta. The clamp was

adjusted until the coronary sinus blood flow attained the

same level that had prevailed previously during ansal

stimulation and ventricular pacing at 150/min. Coronary

blood samples were withdrawn during and after cessation

of sympathetic stimulation (Fig. 1A).

Sequence B: In these experiments, we determined

the NE concentrations in the coronary sinus blood first

while the ventricles were being paced at a frequency of

60/min (Fig. 1B). However, we did in fact briefly stimulate

the sympathetic nerves during a preliminary period while

the ventricles were being paced at a frequency of 150/
Sampling Coronary Sinus Blood Flow, ml/min
(No Preliminary Period)

Sampling Coronary Sinus Blood Flow, ml/min
(Preliminary Period)

The heart had recovered from this preliminary stimulation, the ventricles were paced at 60/min, and the definitive portion of the experiment was begun (Fig. 1B). The protocol was identical to that used for subgroup A, except that (1) the order of applying the two pacing frequencies was reversed, and (2) the coronary sinus blood flow that prevailed during the preliminary procedure constituted the criterion for blood flow adjustment during ventricular pacing at a frequency of 60/min.

Norepinephrine Assay

Each blood sample was placed in a test tube that contained sodium metabisulfite. The plasma was separated by centrifugation, and the catecholamines were adsorbed on aluminum oxide and then eluted with perchloric acid (Anton and Sayre, 1962). The NE content of the eluate was determined by high performance liquid chromatography (Varian, model 5000), with electrochemical detection (Bioanalytical Systems, model LC-17). The mobile phase consisted of monochloroacetic acid, Na₂EDTA, and sodium octylsulfate. The pH of this phase was adjusted to 3.0 by titration with NaOH.

Statistical Analysis

The data were analyzed by means of a three-way, mixed-model analysis of variance (Sokal and Rohlf, 1969). The fixed factors were the pacing frequency and the elapsed time, and the individual animals comprised the random factor. The single degree of freedom test was used for a priori comparisons (Sokal and Rohlf, 1969). Scheffé's test was used for a posteriori comparisons and for all other comparisons for which the single degree of freedom test was not applicable (Scheffé, 1953).

Results

Series I: Coronary Blood Flow Not Controlled

Representative Experiment

Figure 2 shows the changes in right ventricular contractile force and coronary sinus blood flow that were evoked by a 3-minute train of ansal stimulation. The ventricles were being paced at a frequency of 150/min. The contractile force and coronary sinus blood flow responses to ansal stimulation reached a steady state within about 1 minute. The contractile force increased to about 250% of control, and the coronary sinus blood flow was 43 ml/min greater than the control value. The NE overflow (i.e., the product of the coronary sinus blood flow and NE concentration in the coronary sinus blood) varied between 101 and 107 ng/min between the 1st and 3rd minutes of neural stimulation.

After cessation of neural stimulation, the contractile force and coronary blood flow returned to the control level over the subsequent 2 minutes. Thirty seconds after cessation of stimulation, the NE overflow had decreased to 30 ng/min.

Composite Data

First Set: No Propranolol. The mean changes in coronary sinus blood flow, NE concentration in the coronary sinus blood, and NE overflow into the coronary sinus blood evoked by ansal stimulation are shown in Figure 3. Coronary sinus blood samples were withdrawn for norepinephrine analysis just
FIGURE 2. The changes in right ventricular contractile force, coronary sinus blood flow, and norepinephrine overflow into the coronary sinus blood produced by a 3-minute train of supramaximal stimuli (15 V, 2 msec, 1 Hz; between the arrows) to the decentralized ansae subclavias in a representative experiment. Complete atrioventricular (AV) block had been induced, and the ventricles were paced at a frequency of 150/min. Desipramine (1 mg/kg) had been given to suppress the neuronal uptake of norepinephrine. Time scale, 1 minute.

Prior (t₀) to a 3-minute train of ansal stimulation, and at 2 minutes before (t₀-2), 1 minute before (t₀-1), and immediately before (t₀) cessation of ansal stimulation. Blood samples were also collected at times of 0.5 (t₀+0.5) and 1 min (t₀+1) after cessation of stimulation. The contractile forces (not shown) during sympathetic stimulation were substantially greater at the high pacing frequency than at the low pacing frequency. After cessation of sympathetic stimulation, the 50% recovery time of the contractile force response was significantly less (P < 0.01) during cardiac pacing at the high frequency than during pacing at the low frequency, in confirmation of our previous results (Masuda and Levy, 1983).

Coronary Sinus Blood Flow: The basal value (at t₀) of the coronary sinus blood flow was greater (P < 0.01) during pacing at the high frequency than during pacing at the low frequency (Fig. 3A). Ansal stimulation increased the coronary sinus blood flow; the increments were considerably greater (P < 0.001) during high frequency pacing than during low frequency pacing (Fig. 3A). At either pacing frequency, the flows were virtually constant during the last 2 minutes (from t₀-2 to t₀) of sympathetic stimulation, which indicates that a steady state had been attained. After cessation of stimulation, the coronary sinus blood flows fell more rapidly (P < 0.001) during high frequency pacing than during low frequency pacing.

Norepinephrine Concentration and Overflow: The NE concentration in the coronary sinus blood increased substantially in response to sympathetic nerve stimulation. The concentration

FIGURE 3. The mean coronary blood flows (panel A), norepinephrine (NE) concentrations in the coronary sinus blood (panel B), and NE overflows into the coronary sinus blood (panel C) evoked by a 3-minute train of cardiac sympathetic stimulation in seven animals. Complete AV block had been produced, and desipramine (1 mg/kg) had been given. The cardiac responses to sympathetic stimulation were determined when the ventricles were paced at frequencies of 60 (open bars) and 150 (solid bars) per minute. The coronary sinus blood samples were withdrawn prior to sympathetic stimulation (t₀), at 2 minutes before, 1 minute before, and just before (t₀-2, t₀-1, and t₀, respectively) cessation of stimulation, and at 0.5 and 1 minute after cessation (t₀+0.5 and t₀+1, respectively) of stimulation. The vertical lines through the tops of the bars denote the standard errors of the means.
reached a virtual steady state within 1 minute, regardless of the pacing frequency (Fig. 3B). The NE concentrations were greater during low frequency pacing than during high frequency pacing, but the differences were not significant. After cessation of ansal stimulation, the NE concentrations decreased rapidly toward the basal levels (Fig. 3B).

In response to ansal stimulation, the NE overflows increased rapidly from the low basal rates that prevailed prior to ansal stimulation, and they reached a virtual steady state within 1 minute (Fig. 3C). The mean NE overflows were 45.0 ± 14.2% greater (P < 0.05) during high frequency pacing than during low frequency pacing, even though the sympathetic stimulations were equivalent.

The NE overflows fell rapidly toward their basal values after cessation of stimulation (Fig. 3C). To correct for the disparities in the NE overflows at the two pacing frequencies just before cessation of stimulation, single exponential equations were derived for the NE overflow data for each animal, and the time constants were calculated. The NE overflows decayed more rapidly during high frequency pacing than during low frequency pacing; the mean time constants were 21.6 ± 1.3 and 32.3 ± 2.3 sec, respectively (P < 0.05).

Second Set: Propranolol. In the animals that had received propranolol, sympathetic stimulation evoked only small increases in contractile force. The changes in coronary sinus blood flow, NE concentration in the coronary sinus blood, and NE overflow that were elicited by the sympathetic stimulation are shown in Figure 4. Coronary sinus blood samples were withdrawn prior to the 3-minute train of ansal stimulation, and at 1 minute before and just before (t<sub>c-i</sub> and t<sub>c</sub>) cessation of stimulation. Samples were also withdrawn at 0.33, 0.66, and 1.0 minute after cessation of stimulation. The coronary sinus blood flow changed only slightly during stimulation and after cessation of stimulation (Fig. 4A). The coronary sinus blood flows were somewhat higher during high frequency than during low frequency pacing (Fig. 4A).

Sympathetic stimulation increased the NE concentration in the coronary sinus blood (P < 0.01) and the NE overflow into the coronary sinus blood (P < 0.05; Fig. 4). The NE overflows during sympathetic stimulation were greater (P < 0.01) during high frequency pacing than during low frequency pacing. The mean time constants for the decay of the NE overflow after cessation of sympathetic stimulation were not significantly different at the two pacing frequencies.

Series II: Coronary Blood Flow Controlled

In the experiments illustrated in Figures 5 and 6, the coronary sinus blood flows during ansal stimulation were adjusted so that they would be equal during pacing at the two frequencies. During low frequency pacing, the initial increase in the coronary sinus blood flow (Fig. 5A) evoked by sympathetic stimulation (at time t<sub>b</sub>, before clamp adjustment) was not as great as the increases in flow evoked by the same sympathetic stimulation during high frequency pacing (at times t<sub>c-i</sub> and t<sub>c</sub>). By adjusting the screw clamp during low frequency pacing while sympathetic stimulation was maintained, we then increased the coronary blood flow (times t<sub>c-i</sub> and t<sub>c</sub>) to approximately the same level that prevailed during high frequency pacing. Note that this induced increase in coronary blood flow during low frequency pacing evoked a reduction in the NE concentration (Fig. 5C) in the coronary sinus blood (times t<sub>c-i</sub> and t<sub>c</sub>). The NE overflow (Fig. 5D) during low frequency pacing was significantly greater (P < 0.05) immediately after the coronary blood flow had been increased (time t<sub>c-i</sub>) than it had been prior to the adjustment of the coronary blood flow (t<sub>b</sub>). At comparable blood flows, the NE concentrations and NE overflows during high frequency pacing were...
not significantly different from the corresponding values during low frequency pacing (times \( t_{c-1} \) and \( t_c \)). The increases in contractile force (times \( t_{c-1} \) and \( t_c \)) were somewhat less during high frequency pacing than during low frequency pacing (Fig. 5B), but the differences were not significant.

In nine of the 12 animals in series II, we also determined the effect of pacing frequency on the rate of decline of the NE overflow during the first 30 seconds after cessation of sympathetic stimulation (Fig. 6). During low frequency pacing, the degree of aortic constriction that was applied during sympathetic stimulation was not altered until at least 1 minute had elapsed after cessation of anastomosis. Just before cessation (\( t_c \)) of anastomosis, the coronary sinus blood flows were not significantly different during cardiac pacing at the two frequencies, nor were they significantly different at the two pacing frequencies 0.5 minute (\( t_{c+0.5} \)) after cessation of stimulation (Fig. 6A).

The NE overflows at the end of sympathetic stimulation (time \( t_c \)) were not significantly different at the two pacing frequencies (Fig. 6B). Immediately after cessation of stimulation, the NE overflow declined more rapidly (\( P < 0.05 \)) during high than during low frequency pacing. Thirty seconds after the cessation of sympathetic stimulation, the mean decrements in the NE overflow were 95.9 ± 21.4 and 59.2 ± 17.3 ng/min during pacing at the high and low frequencies, respectively.

**Discussion**

The coronary sinus blood flow was not controlled in the first series of experiments. Sympathetic stimulation induced greater increments in coronary sinus blood flow and in NE overflow (Fig. 3) during cardiac pacing at the high frequency (150/min) than during pacing at the low frequency (60/min). Immediately after cessation of sympathetic stimulation, the coronary sinus blood flows and NE overflows...
decayed more rapidly toward their control levels during high frequency pacing than during low frequency pacing (Fig. 3). The faster decay of these responses at the higher pacing frequency suggests that a greater heart rate facilitates the removal of the neurally released NE from the cardiac tissues.

The NE released into the neuroeffector gaps in the heart is dissipated by the following processes: (1) uptake by sympathetic nerve terminals, (2) uptake by extraneuronal tissues, and (3) physical transport (e.g., diffusion) from the cardiac interstitium to the myocardial capillaries. The NE overflow into the coronary sinus blood is a measure of the physical transport process; the overflow represents the difference between the rates of release and reuptake of the adrenergic neurotransmitter. Extraneuronal uptake plays a relatively minor role in the heart (Junstad et al., 1973; Langer and Rubio, 1973; Iversen, 1975; Matsuda et al., 1979; Masuda et al., 1980; Mann and Yudilevich, 1984). Hence, the overflow of NE mainly reflects the balance between the neuronal release and the subsequent neuronal reuptake of neurotransmitter.

A change in pacing frequency may have affected the NE overflow in our first series of experiments by altering one or more of the following mechanisms: (1) the physical transport of NE in the interstitial space, (2) the rate of neuronal release of NE, (3) the rate of neuronal uptake of NE, or (4) the pattern of myocardial venous drainage.

An altered rate of physical transport (mechanism 1) is probably principally responsible for the finding that the NE overflows evoked by a given level of sympathetic stimulation were significantly greater during high frequency pacing than during low frequency pacing (Fig. 3C). The coronary sinus blood flows during tonic sympathetic stimulation were substantially greater during high than during low frequency pacing (Fig. 3A). The greater coronary blood flow at the higher pacing frequency is ascribable partly to the increased metabolic rate induced by the greater cardiac contraction frequency (Berne and Rubio, 1979; Feigl, 1983). Also, the greater contractile force elicited by the sympathetic stimulation during pacing at the high frequency must have contributed to the increment in coronary blood flow. Note that the differences between the coronary blood flows that prevailed at the two pacing frequencies were much less pronounced when the differences in the contractile force were virtually eliminated by the administration of propranolol (compare Figs. 3A and 4A).

The rate of decay of the overflow of NE into the coronary sinus blood after we halted a train of sympathetic stimulation supports the hypothesis that an augmented coronary blood flow facilitates the washout of NE from the cardiac tissues. The coronary blood flows were greater during high frequency pacing than during low frequency pacing after cessation of sympathetic stimulation (Fig. 3A). Concomitantly, the NE overflow decayed more rapidly during high frequency pacing than during low frequency pacing (Fig. 3C). Furthermore, when we augmented the coronary blood flow by aortic constriction during low frequency pacing, the NE overflow during sympathetic stimulation increased by about 30% (Fig. 5D; compare values at t_o and t_c).

Other investigators have also concluded that an increase in coronary blood flow enhanced the diffusion of neurotransmitters in the cardiac tissues (Starke et al., 1970; Lindmar et al., 1982; Cousineau et al., 1984; Fuder et al., 1984). Furthermore, in isolated perfused hearts, an increase in coronary perfusion rate accelerated the washout of certain small solutes from the cardiac tissues (Schafer and Johnson, 1964; Young, 1968).

With respect to mechanism 2, the electrical pulses that we used to pace the ventricles probably released some neurotransmitter from the nerve fibers in the region of the pacing electrodes (Schwartz et al., 1979; Euler, 1980; Cousineau et al., 1984). In our experiments, therefore, more neurotransmitter may have been released when we paced at the high frequency than when we paced at the low frequency. However, this phenomenon could not have accounted for more than a small fraction of the substantial differences in NE overflow that we observed during pacing at the two frequencies (Fig. 3C). The electrode poles were only 2 or 3 mm apart, and the pacing stimuli were only slightly above threshold. Therefore, such pacing stimuli could have released NE from only a very small fraction of the intramural sympathetic nerve fibers in the ventricles. Furthermore, the pacing electrodes were attached to the right ventricular wall, whereas the coronary sinus blood flow is derived almost exclusively from the left ventricular vasculature (Gregg and Shipley, 1947). Therefore, any NE released by the right ventricular pacing stimuli could not have contributed significantly to the NE overflow into the coronary sinus blood.

In support of this contention, the NE overflows that prevailed during cardiac pacing, but before sympathetic stimulation (time t_o), were very small (Figs. 3C, 4C, and 5D). Furthermore, before sympathetic stimulation, the disparity was slight between the mean values observed at the two pacing frequencies (Figs. 3C, 4C, and 5D). Blombery and Heinzow (1983) also found that the basal NE overflows did not change significantly over a wide range of pacing frequencies in open-chest dogs.

Therefore, the frequency of pacing probably did not have any appreciable direct effect on the rate of NE release during sympathetic stimulation in our experiments. However, differences in pacing frequency may have affected the NE release rates by an indirect mechanism. As explained above, an increased pacing frequency augments the coronary blood flow, which enhances the removal of NE from the interstitial spaces in the heart. This accelerated washout of NE probably lowers the NE concentra-
tion in the neuroeffector gaps. A potent negative feedback mechanism, mediated by presynaptic α-receptors, may increase the release of NE from the sympathetic terminals when the local concentration of NE declines (Langer, 1977; Starke, 1977).

With respect to mechanism 3, the neuronal uptake of NE was suppressed by desipramine in our experiments. Therefore, it is unlikely that a change in the neuronal uptake of NE could have been an important direct cause of the change in NE overflow that was induced by altering the pacing frequency (Figs. 3C and 5D). Although desipramine is a very effective inhibitor of neuronal uptake (Iversen, 1975), the inhibition was probably incomplete in our experiments. Cousineau et al. (1984) estimated that 1 mg/kg of desipramine (the same dose that we used) suppressed the rate of NE uptake of NE from the cardiac interstitial space by 67% in intact anesthetized dogs.

Because the neuronal uptake of NE probably was not completely suppressed, a change in pacing frequency may have indirectly affected the rate of NE uptake in our experiments. As explained, an increase in pacing frequency increases the coronary blood flow, which tends to lower the NE concentration in the neuroeffector gaps. The neuronal uptake of NE varies directly with the NE concentration in the vicinity of those nerve endings. Consequently, the NE uptake would be expected to vary inversely with the pacing frequency.

With regard to mechanism 4, the apparent increase in NE overflow into the coronary sinus blood that was elicited by the higher pacing frequency (Fig. 3C) may simply have been an experimental artifact. An increase in pacing frequency may not have altered the total overflow of NE into the myocardial venous blood. Instead, it may have caused a greater fraction of the coronary arterial inflow to be drained by the myocardial veins into the coronary sinus, thereby causing a greater fraction of the neurally released transmitter to appear in the coronary sinus blood. We do not believe, however, that such a potential change in the venous drainage pattern did account for the relationship that we observed between NE overflow and pacing frequency (Fig. 3C). In studies conducted by Gregg and Shipley (1947), the ratio between coronary arterial inflow and coronary sinus outflow remained remarkably constant, despite such interventions as increasing the arterial blood pressure, raising the right ventricular pressure, and infusing saline or blood into the left coronary artery.

Our second series of experiments was designed to determine whether an increase in pacing frequency per se facilitates the washout of NE from the interstitial spaces in the heart, by a mechanism that is independent of the associated change in coronary blood flow. We held the coronary blood flow constant by adjusting an aortic clamp, and we compared the effects of the cardiac pacing frequency on the decay of the NE overflow after cessation of sympa-

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A greater contraction frequency might assist the removal of neurotransmitter from the cardiac interstitium by virtue of a more effective mechanical massaging action. Evidence for the bulk transport of solutes from the interstitial space to the vascular compartment in the heart has also been adduced by other investigators. Stubbs and Widdas (1959) detected appreciable changes in interstitial fluid volume in the course of just one cardiac cycle in isolated, perfused rabbit hearts. Young (1968) observed that the washout of various solutes from the perfused, beating rat heart consisted of a slow and a fast component. The slow process appeared to involve pure diffusion. The fast component, on the other hand, appeared to be mediated by the bulk transport of interstitial fluid. The fast component was absent in the quiescent heart, and its contribution to the washout in the beating heart was augmented as the cardiac contractility was increased. Thus, the mechanical contraction of the heart may be able to influence the elimination of certain solutes, including neurotransmitters, from the cardiac interstitium by mechanisms that include not only true diffusion but also bulk transport.
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