The Effect of the Pattern of Cardiac Sympathetic Activity on Myocardial Contractile Force and Norepinephrine Overflow in the Dog Heart

MATTHEW N. LEVY, M.D., AND BENJAMIN BLATTBERG

With the technical assistance of Herrick Finkelstein

SUMMARY The left or right cardiac sympathetic nerves in open-chest, anesthetized dogs were stimulated at mean frequencies of 2 or 4 Hz. The stimuli were applied intermittently, in patterns with repetition rates of either 60/min or 15/min, to simulate the spontaneous patterns of sympathetic neural activity that occur synchronously with the cardiac or respiratory cycles, respectively. With either repetition rate, intermittent stimulation of the left sympathetic nerves was about 10-20% less effective in enhancing myocardial contractile force (CF) and about 10% less effective in increasing coronary sinus blood flow than was steady stimulation at the same mean frequency. With right-sided stimulation, there was no appreciable difference between steady and intermittent stimulation patterns with respect to the effect on heart rate. With either left-or right-sided stimulation, the rate of norepinephrine (NE) overflow into the coronary sinus blood was 20-40% less with intermittent than with steady stimulation. Cocaine administration did not materially affect this difference in NE overflow. It was concluded that the higher instantaneous frequencies that prevail during intermittent stimulation result in a reduction in the rate of NE release at the sympathetic postganglionic nerve endings in the heart.

THE PATTERN of efferent activity in sympathetic nerve fibers varies considerably. In some fibers and under certain conditions, the activity appears to be random. Frequently, however, there are distinct groupings of activity, synchronous with either the heart beat or the respiration.15-19 Aside from introducing some rhythmical variations in heart rate,15-19 myocardial contractility,16,17 and peripheral resistance,12,13 the influence of the grouping of efferent impulses in sympathetic neurons has not been established. It is not known, for example, whether a given number of sympathetic neural impulses will produce different responses depending upon whether the activity is steady or intermittent. The present study was designed to answer this question with respect to certain cardiac responses. The changes in ventricular contractile force (CF) and heart rate were measured with different patterns of sympathetic stimulation, and these changes were correlated with the rates of overflow of norepinephrine (NE) into the coronary sinus blood. Two repetition rates were arbitrarily selected for the stimula-

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Methods

All experiments were conducted on mongrel dogs with an average weight of 22.4 ± 2.8 (SD) kg. The mean heart weight was 157 ± 24 (SD) g. The dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv) and the chest was opened through an incision in the 4th intercostal space. Heparin (500 U/kg, iv) was injected to prevent blood coagulation, and a modified Morawitz cannula was introduced into the coronary sinus via the azygos vein. The tip of the cannula was fixed in position by means of a suture placed in the posterior wall of the right atrium and around the coronary sinus, within 1 cm of the ostium of the coronary sinus. The coronary venous blood was conducted from this cannula through the extracorporeal probe of an electromagnetic flowmeter (Biotronix) and was returned to the venous system by way of the right external jugular vein. Both stellate ganglia were decentralized and bipolar platinum electrodes were placed about the two limbs of either the left or the right ansa subclavia. In most experi-
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Methods

All experiments were conducted on mongrel dogs with an average weight of 22.4 ± 2.8 (SD) kg. The mean heart weight was 157 ± 24 (SD) g. The dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv) and the chest was opened through an incision in the 4th intercostal space. Heparin (500 U/kg, iv) was injected to prevent blood coagulation, and a modified Morawitz cannula was introduced into the coronary sinus via theazygos vein. The tip of the cannula was fixed in position by means of a suture placed in the posterior wall of the right atrium and around the coronary sinus, within 1 cm of the ostium of the coronary sinus. The coronary venous blood was conducted from this cannula through the extracorporeal probe of an electromagnetic flowmeter (Biotronix) and was returned to the venous system by way of the right external jugular vein.

Both stellate ganglia were decentralized and bipolar platinum electrodes were placed about the two limbs of either the left or the right ansa subclavia. In most experi-
ments the ansa subclavia was stimulated at mean frequencies of 2 and 4 Hz, each for a total of 14 minutes, with a 10-minute rest period between stimulations. All stimulus pulses were 2 msec in duration, and of supramaximal voltage (usually 10 V). In each experiment the order of application of the two mean frequencies was determined by chance. Each 14-minute interval of stimulation was subdivided into seven equal periods, as illustrated at the top of Figure 1. The stimulation patterns were generated by a different "duty cycle" of stimulation was applied, as defined below. During the first, fourth, and seventh periods, steady stimulation (100% duty cycle) was always employed. For periods 2 and 3, intermittent stimulation was applied in 25% and 50% duty cycle patterns. The assignment of the sequence of these two duty cycles to periods 2 and 3 was made randomly in each experiment. Whichever sequence was used for periods 2 and 3, the opposite order was employed for periods 5 and 6.

The duty cycle patterns were arranged according to so-called "subperiods," as shown in the bottom section of Figure 1. The stimulation patterns were generated by a parallel logic analog computer (EAI 580). An impulse counter in the computer ensured that, for a given mean frequency, the same number of impulses per minute was delivered to the ansa subclavia, regardless of the stimulation pattern to be employed. In a given experiment, either a 1-second or a 4-second subperiod was employed, to stimulate the impulse groupings for cardiac or respiratory cycles, respectively, in the resting, unanesthetized animal. Thus, a 2-minute period consisted of either 120 consecutive subperiods of 1 second each, or 30 subperiods of 4 seconds each. In a 25% duty cycle, all the stimuli to be delivered at the prevailing mean frequency were delivered during the first 25% of each subperiod, and no stimuli were given during the remaining 75% of that subperiod. In the example shown in the bottom section of Figure 1, at a mean frequency of 4 Hz and with a subperiod of 1 second, a 25% duty cycle consisted of 4 stimuli given in 0.25 seconds. Similarly, a 50% duty cycle consisted of 4 stimuli in 0.5 seconds and no stimuli during the remaining 0.5 seconds.

Right ventricular CF was recorded from a Walton-Brodie strain gauge arch sutured about halfway between the apex and base of the heart and about 1 cm lateral to the anterior descending coronary artery. The R-R interval of the electrocardiogram was measured at each beat by means of the analog computer. CF, femoral arterial blood pressure, R-R interval, and coronary sinus blood flow were recorded on a Brush Mark 260 oscillograph. The CF gauge was not calibrated in these experiments, since only the fractional changes produced by neural stimulation were of interest. Therefore, measurements were made in terms of oscillographic pen deflection; the amplitude of such deflections has been shown to be proportional to CF.

NE was assayed spectrofluorimetrically in 10-ml samples of femoral arterial and coronary sinus blood by the methods of Anton and Sayre and Laverty and Taylor. Each 10-ml blood sample was withdrawn slowly over the last 30 seconds of each of the seven 2-minute periods (top section, Fig. 1). Each dog received about 400 ml of dextran 70 by intravenous drip during the experiment, to minimize the effects of withdrawal of blood for chemical determinations. The rate of endogenous NE overflow into the coronary sinus blood was computed by multiplying the coronary sinus blood flow by the difference between the concentrations of NE in the coronary sinus and arterial blood. During cardiac sympathetic neural stimulation, the NE concentration in arterial blood was a relatively small fraction (mean = 15%) of that in the coronary sinus blood. No significant changes in arterial blood NE concentration were ever detected as a consequence of a change in sympathetic stimulation pattern, at a given mean frequency.

Student's t-test was used to determine the significance of the difference between mean values. The t-test for unpaired data was used for intergroup comparisons. When two measures (e.g., a control and an experimental value) were obtained in each dog and it was desired to determine the significance of the mean difference between these values, the t-test for paired data was used.

Results

STIMULATION OF THE LEFT ANSA SUBCLAVIA

The effect of a change in the pattern of cardiac sympathetic stimulation on right ventricular CF is shown in Figure 2. The subperiod duration in this experiment was 1 second. With steady supramaximal stimulation (second panel, 100% duty cycle) of the left ansa subclavia for 2 minutes at 4 Hz,
the amplitude increased to 167% of the prestimulation control value (first panel). When the stimulation pattern was changed to a 50% duty cycle, the amplitude diminished to 150% of control. Thus, the increment (50%) in CF with a 50% duty cycle was only 1/4 as great as the increment (67%) with a 100% duty cycle. Reduction in the duty cycle to 25% was attended by a further diminution in CF to 143% of control. After 2 minutes at each of these lower duty cycles, the stimulation pattern was returned to a 100% duty cycle. The CF increased to 160% of control, which was slightly less than that obtained during the first period at a 100% duty cycle. After shifting from one duty cycle to another, a new steady state was usually attained in about 20–30 seconds.

In computing the values to be used in the composite data for all experiments, the increments in CF during the 25% and 50% duty cycles were each expressed as a fraction of the average increments above control for the two periods of steady stimulation (100% duty cycle) that just preceded and just followed these periods of intermittent stimulation. Referring to the top section of Figure 1, the increments in CF during periods 1 and 4 were each expressed as a fraction of that average value. Similarly, the increments in CF for periods 4 and 7 were averaged, and the increments for periods 5 and 6 were each expressed as a fraction of that average value. Finally, the values for periods 2 and 6 (50% duty cycle) and for periods 3 and 5 (25% duty cycle) were each averaged.

The composite data for the eight dogs with subperiod of 1 second are displayed in the left sections of Figures 3 and 4 and in Table 1. With steady stimulation (100% duty cycle) at 2 and 4 Hz (Fig. 3), there was a 48.6 ± 7.0% (SE) and a 69.3 ± 10.1% increase in CF, respectively; both increases were significant (P < 0.001). The coronary sinus blood flow increased by 32% at 2 Hz (P < 0.02) and by 64% at 4 Hz (P < 0.01). The NE overflow into the coronary sinus blood was negligible prior to sympathetic stimulation at either frequency. It increased to 24.4 ± 8.3 (SE) ng/min at 2 Hz (P < 0.001) and 45.6 ± 9.2 ng/min at 4 Hz (P < 0.001); the overflow rate at 4 Hz was significantly greater than at 2 Hz (P < 0.001). The control heart rate was 128 ± 7.4 beats/min. The heart rate increased by 10.5 ± 4.3 beats/min at a stimulation frequency of 2 Hz, and by 22.0 ± 3.1 beats/min at a frequency of 4 Hz. The increase at 4 Hz was significantly greater than that at 2 Hz (P = 0.02); the heart rate data are not included in Figures 3 and 4.

For this same group of eight dogs, the effects of changing to an intermittent pattern of stimulation are shown in the left half of Figure 4. At a mean frequency of 2 Hz, the increment in CF during intermittent stimulation at a 50% duty cycle was 90 ± 2% (SE) of that obtained during steady stimulation (P = 0.001). At a 25% duty cycle, the increment was 86 ± 3% of that during steady stimulation (P = 0.001). The increment was significantly less at the 25% than at the 50% duty cycle (P = 0.05). Directionally similar findings were also obtained at a mean frequency of 4 Hz, although the fractional differences were somewhat less pronounced than those at a mean frequency of 2 Hz. The changes in coronary sinus blood flow appeared to parallel the changes in CF. Changes in duty cycle were not associated with any detectable alterations in heart rate.

A change in the pattern of sympathetic stimulation had a disproportionately greater effect on the rate of NE overflow than on either CF or coronary sinus blood flow (Fig. 4). At a mean frequency of 2 Hz, the NE overflows for duty cycles of 50% and 25% were 63.2 ± 8.3% and 57.8 ± 9.4%, respectively, of the overflow rates during steady stimulation. These values were both significantly less (P = 0.001) than that obtained with steady stimulation. However, the values for the 25% and 50% duty cycles were not significantly different from each other.

With a mean frequency of 4 Hz (Fig. 4), shifting from steady to intermittent stimulation produced changes in CF,
coronary sinus blood flow, and NE overflow that resembled those at a mean frequency of 2 Hz. However, the changes tended to be somewhat less pronounced at the higher than at the lower mean frequency.

In general, when a subperiod of 4 seconds was employed (right sections of Figs. 3 and 4), the results were similar to those obtained with a 1-second subperiod. During steady stimulation of the left ansa subclavia at 2 or 4 Hz in a group of eight dogs in which a 4-second subperiod was employed (Fig. 3, right half), the changes in CF, coronary sinus blood flow, and NE overflow were similar to those obtained in the dogs in which a subperiod of 1 second was used. Also, the changes produced by shifting to an intermittent pattern of sympathetic stimulation were directionally similar (Fig. 4, right half). Intermittent stimulation with a 25% or 50% duty cycle resulted in a 13–20% reduction ($P < 0.001$) in the increment in CF. At a mean frequency of 2 Hz, the reduction in CF was more pronounced with a 25% duty cycle than with a 50% duty cycle ($P = 0.02$). At a mean frequency of 4 Hz, however, the reductions in CF were not significantly different at these two duty cycles. Intermittent stimulation also produced significant reductions in NE overflow. The rates of NE overflow were 21–34% less ($P < 0.001$) than the rates obtained during steady stimulation at the equivalent mean frequencies. At either mean frequency, the reductions in NE overflow were not significantly different at the 25% and 50% duty cycles. The mean values of NE overflow at the various duty cycles are displayed in Table 1. Note that the means of the fractional changes during intermittent stimulation (shown in Fig. 4) are not identical to the fractional changes computed from the mean values (compiled in Table 1).

**STIMULATION OF THE RIGHT ANSA SUBCLAVIA**

In 7 dogs, the right ansa subclavia was stimulated supramaximally at mean frequencies of 2 and 4 Hz with the same types of stimulation patterns that were described above. Only 1-second subperiods were used. The changes in coronary sinus blood flow and in NE overflow were determined, as in the preceding experiments, but the cardiac cycle duration (R-R interval) was measured rather than the ventricular CF. The data are summarized in Figures 5 and 6 and in Table 2.

From a mean control level of about 520 msec (Fig. 5, left panel), the R-R interval decreased to about 300 msec with steady stimulation frequencies of either 2 or 4 Hz ($P < 0.001$). Also, at both frequencies, steady sympathetic stimulation caused coronary sinus blood flow approximately to double ($P < 0.001$). At a steady frequency of 2 Hz, the rate of NE overflow increased from nearly zero to a level of 36.3 ± 4.3 ng/min. At a frequency of 4 Hz, the NE overflow increased to 65.7 ± 13.7 ng/min, which was significantly greater than the value at 2 Hz ($P = 0.05$).

**TABLE 1** The Rates of Norepinephrine (NE) Overflow into the Coronary Sinus Blood during Supramaximal Stimulation of the Left Ansa Subclavia at Duty Cycles of 100%, 50%, and 25%

<table>
<thead>
<tr>
<th>Duty cycle</th>
<th>1-sec period</th>
<th>4-sec period</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>24.4 ± 8.3</td>
<td>45.6 ± 9.2</td>
</tr>
<tr>
<td>2 Hz</td>
<td>29.5 ± 7.5</td>
<td>62.2 ± 14.4</td>
</tr>
<tr>
<td>4 Hz</td>
<td>50%</td>
<td>19.3 ± 6.8</td>
</tr>
<tr>
<td>2 Hz</td>
<td>21.8 ± 6.2</td>
<td>34.7 ± 6.9</td>
</tr>
<tr>
<td>4 Hz</td>
<td>25%</td>
<td>18.0 ± 5.4</td>
</tr>
<tr>
<td>2 Hz</td>
<td>18.4 ± 5.4</td>
<td>50.0 ± 9.8</td>
</tr>
<tr>
<td>4 Hz</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The data represent the mean (±SE) rates of NE overflow for eight dogs in which the subperiod duration was 1 second, and for eight dogs in which the subperiod duration was 4 seconds. The mean stimulation frequencies were 2 and 4 Hz.

![Graphs showing changes in R-R interval, coronary sinus blood flow, and NE overflow](http://circres.ahajournals.org/doi/abs/10.1161/01.RES.39.3.344)
With intermittent sympathetic stimulation at 25% and 50% duty cycles, the R-R intervals were not significantly different from those observed during steady stimulation at the same mean frequency. Also, the coronary sinus blood flow diminished only slightly (3-5%) when the stimulation pattern was shifted from a steady to an intermittent pattern. The R-R interval and blood flow data are not included in the figures. The changes in NE overflow, however, were appreciably less during intermittent than during steady stimulation, as shown in the left panel of Figure 6. At a mean frequency of 2 Hz, the rates of NE overflow at duty cycles of 50% and 25% were 71.1 ± 9.0% (P < 0.001) and 56.7 ± 8.2% (P < 0.001), respectively, of the rates during steady sympathetic stimulation. The NE overflow during the 25% duty cycle was significantly less than that during the 50% duty cycle (P = 0.02). At a mean frequency of 4 Hz, shifting from a steady to an intermittent type of stimulation also caused a significant reduction in NE overflow (P = 0.02 for both 25% and 50% duty cycles), but the percentage differences were less pronounced than with a mean frequency of 2 Hz. The decrement tended to be greater with a 25% than with a 50% duty cycle, but the difference was not significant.

In a similar series of experiments, the effects of changes in the pattern of stimulation of the right ansa subclavia were determined in a group of eight dogs after cocaine hydrochloride had been infused at a constant rate for 15 minutes at a total dose of 5 mg/kg. This dose of cocaine is the same as that which had been employed previously in the dog in order to reduce the reuptake of NE by the sympathetic postganglionic terminals in the heart. Steady stimulation at 2 Hz had less effect (P = 0.001) on the R-R interval after cocaine than it did at the same frequency in the dogs that did not receive cocaine (Fig. 5). The effects on coronary sinus blood flow and on NE overflow were very similar in the two groups, however. Koerker and Moran found that cocaine did not increase the NE overflow into the coronary sinus of dogs in response to cardiac sympathetic stimulation for periods longer than 30 seconds and they observed that cocaine had a marked inhibitory effect on the uptake of exogenous NE in their preparations. They postulated that cocaine also curtailed the release of NE, and that the diminution in release nullified the reduction in uptake to account for the virtual equality of NE overflow during sympathetic stimulation in the presence and absence of cocaine.

Shifting from steady to intermittent stimulation had virtually no effect on either the R-R interval or the coronary sinus blood flow, just as in the dogs that did not receive cocaine; these data are not included in the figures. The mean values for the R-R intervals and coronary sinus blood flows during intermittent stimulation in the dogs that received cocaine were all within 3% of the corresponding values during steady stimulation in these dogs. However, changing from steady to intermittent sympathetic stimulation did result in about a ½ reduction (P < 0.001) in the rate of NE overflow in the dogs that received cocaine (Fig. 6). The percentage diminution in NE overflow produced by intermittent stimulation was approximately the same as in the dogs that did not receive cocaine, at the same mean frequency (2 Hz) of stimulation (Fig. 6 and Table 2). After cocaine, however, the NE overflows were not significantly different at the two different duty cycles of intermittent stimulation.

The rate of restitution of the basal R-R interval after cessation of sympathetic stimulation was used as an index of the efficacy of suppression of NE reuptake by cocaine. The time course of the restitution of the R-R interval is illustrated in Figure 7 for a control experiment and for an experiment in which cocaine had been employed. In both experiments, the time at which sympathetic stimulation had been discontinued is denoted by an arrow. It is apparent that the R-R interval increases at a more rapid rate after cessation of stimulation in the control dog than in the dog that received cocaine.

The changes in R-R interval after cessation of stimulation in these same dogs are plotted semilogarithmically in Figure 8. The changes are expressed as percentages of the difference between the steady-state R-R interval near the end of sympathetic stimulation and the steady state R-R interval after cessation of stimulation. It is apparent that the data for both experiments are approximately linear when plotted semilogarithmically. The decay constant (k) was -2.16 x 10^{-2} sec^{-1} for the control dog and -1.07 x 10^{-2} sec^{-1} for the dog that had received cocaine. In six control dogs and six dogs treated with cocaine, the changes in R-R interval after termination of sympathetic stimulation were approximately linear when plotted on semilogarithmic coordinates. The mean decay constant was -3.76 x 10^{-2} ± 1.35 x 10^{-2} (SE) sec^{-1} for the control dogs and -1.22 x 10^{-2} ± 1.09 x 10^{-2} sec^{-1} for the dogs treated with cocaine (P = 0.05). Hence, cocaine significantly retarded the return to the control R-R interval after the cessation of sympathetic stimulation.

**FIGURE 7** The changes in R-R interval after cessation of supramaximal, steady stimulation of the right ansa subclavia at 2 Hz in a control dog and in one that had received cocaine hydrochloride, 5 mg/kg. The arrows denote the ends of the stimulation periods.

| TABLE 2 | The Rates of Norepinephrine (NE) Overflow into the Coronary Sinus Blood during Supramaximal Stimulation of the Right Ansa Subclavia at Duty Cycles of 100%, 50%, and 25% in Seven Control Dogs and in Eight Dogs that Received Cocaine Hydrochloride |
|------|---|---|
| **Duty cycle** | **NE overflow (ng/min)** | **Cocaine HCl (mg/kg)** |
| 100% | 36.3 ± 4.3 | 32.1 ± 8.0 |
| 50% | 21.7 ± 2.6 | 16.6 ± 5.7 |
| 25% | 16.6 ± 1.8 | 14.9 ± 5.4 |

The data represent the mean ± S.E. rates of NE overflow during cardiac sympathetic stimulation at a mean frequency of 2 Hz with a subperiod of 1 second.

**CARDIAC NOREPINEPHRINE OVERFLOW/Levy and Blattberg**
Discussion

In the present series of experiments, intermittent stimulation of the left cardiac sympathetic nerves (Fig. 4) was about 10–20% less effective than steady stimulation in augmenting myocardial CF, and about 5–10% less effective than steady stimulation in increasing coronary sinus blood flow. The differences between steady and intermittent stimulation were more pronounced (20–40%) with respect to the rate of NE overflow into the coronary sinus. The differences in efficacy of steady and intermittent stimulation on NE overflow during right-sided cardiac sympathetic stimulation (Fig. 6) were similar to those obtained with left-sided excitation. However, there were no detectable differences between steady and intermittent stimulation of the right sympathetic nerves with respect to the changes in heart rate.

Under steady state conditions, the quantity \( Q_v \) of NE appearing in the coronary venous blood may be represented by the equation \( Q_v = (Q_n + Q_r) - Q_u \), where \( Q_n \) is the quantity of NE in the coronary arterial blood that passes through the myocardial capillary network intact and enters the coronary veins, \( Q_r \) is the quantity released at the sympathetic postganglionic terminals, and \( Q_u \) is the quantity taken up again by neuronal and extraneuronal mechanisms. During cardiac sympathetic neural stimulation in the present series of experiments, the concentration of NE in the arterial blood was only a small fraction (approximately 15%) of that in the coronary sinus blood. Furthermore, most of the endogenous NE in the coronary arterial blood would probably be extracted from the blood during its passage through the myocardial capillaries, and only a small quantity \( Q_n \) would remain to appear in the coronary venous blood. Hence, during cardiac sympathetic neural stimulation, \( Q_n \) would be much less than the quantity, \( Q_r \), released at the nerve endings. The quantity, \( Q_r \), of NE that would overflow into the coronary venous blood, therefore, would represent essentially the disparity between the quantity released, \( Q_r \), and the quantity taken up again, \( Q_u \).

In the present study, intermittent sympathetic stimulation was associated with an overflow of NE that was about 20–40% less than that obtained with steady neural stimulation (Figs. 4 and 6). Over a given full period of stimulation, the same number of supramaximal stimuli were delivered to the sympathetic nerves, regardless of the stimulation pattern employed. The fact that the NE overflow diminished with the duty cycle signifies that either (1) the rate of NE release decreased, (2) the rate of NE reuptake increased, or (3) some combination of these processes occurred as the stimulation pattern became more discontinuous.

At a given mean frequency, the instantaneous frequency within each stimulus train changed with the value of the duty cycle. As shown in the lower half of Figure 1, the frequency within a stimulus train during the 25% duty cycle was 4 times that during steady stimulation. It has been found in various tissues that the quantity of NE released per stimulus changed with the frequency of stimulation. In the cat spleen, the quantity of NE released per stimulus increased up to some optimum frequency, after which the quantity released per stimulus diminished as frequency was further increased beyond this optimum. In different experimental series, the optimal frequency varied from 4 to 30 Hz. In the rat portal vein, the quantity of NE released per stimulus rose slightly as the stimulation frequency was increased to an optimal value of 4 Hz; beyond that value, there was a substantial reduction in the quantity of neurotransmitter released per impulse as frequency was elevated further. A similar relationship was observed in the rabbit pulmonary artery except that the optimal frequency was found to be 16 Hz. In the rabbit portal vein and vas deferens, the output of NE per impulse increased linearly with the logarithm of frequency over the frequency range 1–16 Hz. The quantity of NE released per impulse in the guinea pig vas deferens increased with the frequency of stimulation up to a maximum frequency of 30 Hz.

Unfortunately, data are not available concerning the relationship of stimulus frequency to the output of NE per stimulus in the heart. Yamaguchi et al. reported recently that the overflow of NE into the coronary sinus blood in the dog increased linearly with the frequency of sympathetic stimulation, up to a frequency of 10 Hz. Unpublished observations on the dog heart in our laboratory confirm these findings; with frequencies above 15 Hz, on the other hand, NE overflow diminished with increasing frequency. These data suggest a constant output of NE per stimulus up to a frequency of about 10 Hz. However, until data are available on the reuptake of NE in the heart as a function of stimulation frequency, conclusions about NE release on the basis of overflow values remain tenuous.

At a given mean frequency of stimulation, the duration of the pauses between stimulus bursts varies inversely with the duty cycle (Fig. 1). As the duty cycle is diminished, for example, there is a greater interval between stimulus bursts. The longer pauses might provide a greater opportunity for
NE reuptake, thereby producing a lower tissue level of NE for a given mean frequency of stimulation. Therefore, more complete reuptake of NE could account for the reduction in CF and NE overflow as duty cycle is diminished (Fig. 4).

The experiments in which cocaine was administered suggest, however, that the alterations in cardiac response and in NE overflow with changes in duty cycle are probably related to changes in NE release rather than in reuptake. Cocaine is known to be a potent inhibitor of NE uptake by sympathetic terminals.\(^{13,14}\) One manifestation of the inhibition of NE uptake in the heart is a prolongation of the various cardiac responses.\(^{15-17}\) In the present study (Figs. 7 and 8), cocaine resulted in a significant increase in the time required for restitution of the control level of heart rate after cessation of right-sided cardiac sympathetic stimulation. In these same experiments, however, the NE overflow after the infusion of cocaine was about 35% less during intermittent stimulation than during steady stimulation (Fig. 6, right panel). In the absence of cocaine (Fig. 6, left panel), the differences in NE overflow between steady and intermittent stimulation were not significantly different from those obtained after cocaine, at equivalent mean frequencies (2 Hz) of stimulation. Although the extent of the suppression of NE reuptake by cocaine in these experiments cannot be ascertained, the similarity of the data in the presence and absence of cocaine suggest that changes in fractional reuptake are not primarily responsible for the differences in NE overflow obtained by shifting from steady to intermittent stimulation. By exclusion, therefore, it appears likely that the differences in NE overflow produced by changes in the pattern of sympathetic neural activity reflect alterations in NE release.

The postulated reduction in NE release consequent to shifting from steady to intermittent stimulation of the left ansa subclavia probably accounts for the associated reduction in ventricular CF and coronary sinus blood flow (Fig. 4). A shift in the pattern of stimulation of the left or right ansa subclavia did not affect heart rate perceptibly. It is conceivable that either the pattern of sympathetic stimulation does not influence the rate of NE release for those sympathetic terminals located in the relatively small region of the sinusial node, or the pacemaker cells in the sinusial node are not influenced materially by the magnitude of the local change in NE release engendered by the alteration in the stimulation pattern. Our present data do not provide an explanation for this phenomenon.

The present study suggests that the periodicity of sympathetic neural activity that occurs synchronously with the heart beat and with the respiratory cycle does affect the rate of release of NE at the neural terminals in the heart. There are at least two opposing mechanisms that determine the quantity of NE released from the nerve endings with each action potential as the frequency is varied.\(^{18}\) On the one hand, as the nerve impulses are more tightly grouped, there is a tendency for facilitation of the excitatory junctional potentials,\(^{19}\) thereby enhancing the release of neurotransmitter. On the other hand, the more closely grouped the nerve impulses, the greater the local concentration of NE at the nerve endings.\(^{20}\) The increased NE concentration then tends to curtail transmitter release by activating inhibitory \(\alpha\)-adrenoreceptors on the nerve terminals.\(^{21}\) Also, the number of secretory vesicles in a suitable location for release at the nerve endings appears to limit the rate of neurotransmitter liberation.\(^{22}\) Other factors being equal, the numbers of such vesicles in the proper location probably tends to diminish as the frequency of neural activity rises. In the present study, we have found a reduction in NE overflow when the stimulation pattern was changed from a steady to an intermittent one. This suggests that, in the heart, those mechanisms that act to diminish NE release per nerve impulse predominate over those that tend to facilitate NE secretion.

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Interaction of Capillary and Tissue Forces in the Cat Small Intestine

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SUMMARY We measured steady state capillary hydrostatic pressure \( P_{c,i} \), plasma and lymph protein concentrations, lymph and blood flow, and capillary filtration coefficients in an in situ loop of cat small intestine at venous outflow pressures \( P_v \) of 0, 5, 10, 15, 20, 25, and 30 mm Hg. The data were used to calculate colloid osmotic pressure of lymph and plasma, interstitial fluid pressure \( P_I \), pre- and postcapillary resistances, and a tissue pressure-volume curve of the intestinal interstitium. When \( P_v \) was elevated from 0 to 30 mm Hg, lymph protein concentration decreased from 3.8 to 1.9 g/100 ml (representing a change in colloid osmotic pressure of 6.2 mm Hg), lymph flow increased 7-fold (or an equivalent imbalance in Starling forces of 4.3 mm Hg), and the calculated \( P_I \) increased from \(-1.8 \) to \(-5.3 \). Because lymph flow draining the loop decreased during the determination of \( P_c,i \) at venous pressures between 15 and 30 mm Hg, the corresponding calculated \( P_I \) may be in error by 1–2 mm Hg. The tissue pressure-volume relationship calculated from the data indicates that the intestinal interstitial volume expands nonlinearly and this expansion is characterized by two distinctly different compliant components: (1) tissue compliance is low at \( P_v \) between 0 and 15 mm Hg \((0.4 \text{ ml/mm Hg})\), and (2) at \( P_v \) greater than 15 mm Hg the tissue compliance is relatively high \((4 \text{ ml/mm Hg})\). We found that when \( P_v \) was elevated from 0 to 15 mm Hg, increases in \( P_v \) are the major tissue adjustments that oppose the increased filtration pressures. Furthermore, at \( P_v \) of 20–30 mm Hg, tissue protein concentration decreases, lymph flow relative to the filtration coefficient \((\Delta P_{DPL})\) increases and, to a much lesser extent, \( P_I \) increases. Finally, the combination of these changes in tissue force at high filtration pressures represent a maximum tissue edema “safety factor” of 10 mm Hg; further increases in filtration pressures result in large volume movements into the intestinal lumen.

STARLING PROPOSED, in 1896,\(^1\) that the rate and direction of fluid exchange across the capillary membrane was a function of the capillary hydrostatic pressure \( P_c \) and the plasma colloid osmotic pressure \( \pi_{PL} \). \( P_c \) determined filtration and \( \pi_{PL} \) was responsible for absorption. Starling’s concept was later extended to include the hydrostatic pressure \( P_v \) and colloid osmotic pressure \( \pi_v \) of the interstitium, the pressure head driving the lymph [effective capillary filtration pressure \((\Delta P_{DPL})\)], and the physical properties of the capillary membrane.\(^2 \) Under normal conditions these forces are in a steady state with a small net filtration resulting in a low resting lymph flow. When this steady state is disturbed, an increase in net filtration or absorption is observed, and over a period of time the capillary bed and tissue spaces attain a new steady state in which the tissue is not gaining or losing weight.\(^4 \) This new steady state is achieved by the readjustment of the Starling forces in a direction to oppose the increases in filtration or absorption forces.

Pappenheimer and Soto-Rivera\(^3 \) indirectly measured the tissue forces, tissue colloid osmotic pressure, and tissue fluid pressure in an isolated hindlimb preparation. They found that the isovolumetric pressure, which is equal to the sum of the other Starling forces, \( \pi_{PL}, \pi_v, \) and \( P_v \), was equal to \( \pi_v - 2 \) (mm Hg); this finding indicates that \( P_v \) and \( \pi_v \) were very small in their preparation. Any major increase in capillary pressure would result in continuous filtration into the interstitium without any apparent adjustment of Starling forces to oppose filtration. In fact, these authors used the...
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