Cardiac Norepinephrine Stores
and the Contractile State of Heart Muscle

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ABSTRACT

In order to assess the role played by endogenous norepinephrine (NE) stores in the intrinsic contractile state of cardiac muscle, the right ventricular papillary muscles from normal cats and cats with cardiac NE depletion produced by chronic cardiac denervation or reserpine pretreatment were studied. The contractile state of NE-depleted ventricular myocardium was found to be normal. The resting and active length-tension curves, the force-velocity relations, and the augmentation of isometric tension achieved by paired electrical stimulation and by increasing frequency of contraction were not depressed in either group of NE-depleted muscles. Similarly, no changes in the absolute refractory period and electrical excitability were observed. It is concluded that cardiac stores of NE are not fundamental for maintaining the intrinsic contractile state of the myocardium. Further, release of endogenous NE from cardiac muscle does not appear to play an essential role in the mediation of the positive inotropic effects of increasing frequency of contraction or of sustained postextrasystolic potentiation.

ADDITIONAL KEY WORDS papillary muscle myocardial contractility length-tension curve excitability paired electrical stimulation frequency of contraction refractory period cardiac denervation cardiac contractility cat

The mammalian ventricle is richly supplied with adrenergic nerves, and the release of the neurotransmitter, norepinephrine (NE), from the endings of these nerves provides one of the fundamental mechanisms for modulation of the contractile state of the heart. Cardiac tissues contain substantial quantities of NE, which appear to be localized almost exclusively within the terminations of the sympathetic nerves, in close apposition to the myocardial fibers.

An increase in sympathetic activity augments myocardial contractility, and loss of the cardiac sympathetic neurons or removal of their NE stores abolishes that aspect of myocardial regulation which is provided by this system. However, there is no agreement concerning the importance of these nerves and their NE stores in maintaining the basal contractile state of the myocardium and its ability to respond to various inotropic interventions. It has been proposed that intact NE stores are required to maintain the basal contractile state of heart muscle, and its contractile responses to changes in the frequency of contraction; however, these hypotheses have been questioned by other investigators. It has also been shown that profound cardiac NE depletion occurs both in clinically and experimentally produced congestive heart failure, and a significant correlation between the contractile state of failing human heart muscle and its NE content has been observed. These latter findings raised the further question of a possible causal relation between cardiac NE depletion and myocardial failure.
The present investigation was undertaken to determine the manner in which removal of cardiac adrenergic nerves and NE stores affects the intrinsic contractile properties of the myocardium and its responses to alterations in the frequency of stimulation, to paired electrical stimulation, as well as to tyramine and exogenous NE. Two recent developments have permitted critical analysis of these problems. The first is the ability to produce essentially complete NE depletion of the mammalian heart by total chronic extrinsic denervation, thus avoiding the administration of NE-depleting drugs, which by themselves may exert a direct effect on the contractile properties of heart muscle. The second is the extension of methods for the quantitative analysis of the mechanical properties of skeletal muscle to isolated preparations of mammalian myocardium.

The cat papillary muscle was selected for these studies since extrinsic cardiac denervation is possible in this species, and considerable experimentation with this muscle has shown that under proper conditions its performance characteristics remain stable. Further, the levels of force attained by cat papillary muscle in vitro are generally greater than those observed in myocardial preparations from other species and are comparable to the levels achieved in the intact feline heart.

The basic experimental plan was to compare the behavior of muscles obtained from three groups of animals: (1) normal; (2) chronic cardiac-denervated, NE-depleted; and (3) reserpine-treated, NE-depleted.

The mechanical properties of the myocardium which were studied included the resting and active length-tension curves and the force-velocity relations. The responses of the muscles to certain inotropic interventions, including alterations in the frequency of contraction, sustained paired electrical stimulation and the addition of exogenous NE were also analyzed. In addition, the refractory period and the electrical excitability of the muscles were determined.

**Methods**

The muscles were obtained from 15 normal cats, 15 cats which had undergone total cardiac denervation 10 to 20 days previously and 8 cats which had been given 3 mg/kg of reserpine intraperitoneally 48 and 24 hr prior to sacrifice. Total extrinsic cardiac denervation was accomplished by mediastinal neural ablation, a surgical procedure described previously. The soft tissues which contain the cardiac neural plexuses and all nerves that enter or leave the heart were excised. The base of the pericardium was incised and the segments of the great vessels adjacent to the heart were stripped of their adventitia. Since this procedure can be carried out through a right thoracotomy in the cat, the postoperative morbidity associated with bilateral thoracotomy is avoided. Myocardial NE was determined spectrofluorometrically by the trihydroxyindoleacetic method as previously described.

The experiments on the muscles from the three groups of animals were carried out concurrently rather than sequentially and in an identical manner. The cats were anesthetized with sodium pentobarbital (25 mg/kg) intraperitoneally and two papillary muscles from the right ventricle were removed rapidly and each was transferred to a myograph containing oxygenated Krebs solution.

The muscles were studied in detail previously. The papillary muscle was held at its lower nontendinous end by a spring-loaded clip, forming the end of a rigid pin that penetrated the bottom of the bath, and was attached directly to a Statham (GI-4-250) force transducer. The upper tendinous end of the muscle was attached to an isotonic lever for the measurement of muscle shortening, and the lever itself was mounted on a rigid Palmer stand. With this arrangement, when the position of the lever was fixed, the force of isometric contraction at any desired muscle length could be measured. The lever could also be freed and, by appropriate loading, the extent and velocity of shortening of the muscle at any preload (the small load that acts on the resting muscle and thereby establishes the initial length) and afterload (the load encountered by the contracting muscle when it attempts to shorten) could be measured. In this manner the length-tension and force-velocity relations could be obtained. The muscles were stimulated with square wave DC impulses of 9 msec duration and 1.5 times the threshold voltage, delivered through field electrodes placed parallel to the long axis of the muscle. Force, muscle length, the first derivatives of these variables, and the stimulus artifact were recorded on a multichannel oscillograph.

*American Electronics Stimulator, Model 104A.
All experiments were carried out with the bath temperature at 30°C. In order to maintain optimal performance of the muscles for prolonged periods of time, the frequency of contraction was set at 12 per min, except when the effects of changes in frequency were specifically studied. When the effects of paired electrical stimulation were determined, 12 pairs of stimuli per min resulting in 12 effective contractions per min were employed and the interstimulus interval did not exceed the muscle's absolute refractory period by more than 20 msec. To assure steady-state performance, a period of 1 hr was allowed between the time the muscles were placed in the myograph and the initial recordings were started. The function of the papillary muscles remained stable for periods of at least 3 to 4 hr.

**Results**

I. **RIGHT VENTRICULAR NE CONCENTRATIONS**

A. **Chemical Determinations**

Cardiac denervation and reserpine treatment essentially eliminated the cardiac NE stores. While the right ventricular NE concentration averaged 2.13 ± 0.30 µg/g (SEM) in 11 normal animals, this value averaged only 0.006 ± 0.003 µg/g, both in 14 chronic cardiac-denervated cats and in 8 reserpine-treated animals. In 3 animals from each group there was no detectable right ventricular NE. Left ventricular NE concentration in 4 denervated cats averaged 0.003 µg/g.

B. **Responses to Tyramine**

Tyramine, a sympathomimetic amine considered to exert its positive inotropic effects through the release of endogenous NE, was added to the bath while isometric tension was recorded at the apex of the muscle's length-tension curve. The concentration employed (10⁻⁶M) was found to induce the maximal response, which averaged 90 ± 20% above control values in 5 normal muscles, 5 ± 1% in muscles from 14 cats with chronic cardiac denervation, and 7 ± 2% in muscles from 8 reserpine-treated animals.

II. **MYOCARDIAL MECHANICS**

A. **Length-Tension Curves**

The average length-resting tension and length-active tension curves are shown in figure 1. The active tensions were calculated as the difference between the total and resting tensions, L_max was defined as that muscle length at which the isometric tension was maximum, and the increments of muscle length were expressed as percentages of L_max. Tensions were expressed as force per unit of cross-sectional area of each muscle. No significant differences among the average length-resting tension curves in the three groups of animals were found. At L_max, the resting tensions averaged 2.3 ± 0.4 g/mm² in the normal group; 1.6 ± 0.3 g/mm² in the denervated muscles; and 2.8 ± 0.8 g/mm² in the muscles obtained from cats that received reserpine.

The length-active tension curves of muscles from normal cats and cardiac-denervated cats were also essentially identical, the maximum isometric tensions in the two groups averaging 6.2 ± 0.7 and 5.8 ± 0.9 g/mm² respectively. The length-active tension curve from the reserpine-treated group was slightly higher than the other two curves, having a maximum isometric tension which averaged 7.5 ± 0.6 g/mm², a value which, however, is not significantly different than that observed in the normal and denervated groups. The cross-sectional area of the papillary muscles averaged 0.88 ± 0.06 mm², 0.97 ± 0.12 mm², and 0.82 ± 0.12 mm² in the muscles from...
normal, cardiac-denervated and reserpine-treated cats respectively, values which are not statistically different. The maximum active tensions per unit of cross-sectional area were unrelated to the absolute cross-sectional area of the individual muscles.

B. Force-Velocity Relations

Force-velocity relations were determined in all three groups and the average values and their standard errors are shown in figure 2. Velocity of shortening was corrected for muscle length and was expressed in muscle lengths per second, while the force (or load) was expressed in g/mm². Initial muscle length was set by a small preload which was constant for the entire force-velocity curve. The effects on the velocity of shortening of progressively increasing afterload were then determined. The velocity of shortening at the smallest load (0.5 g/mm²) was used to approximate \( V_{\text{max}} \) in order to avoid errors which might result from extrapolation of the curve to a zero load.

Similar inverse relations between force and velocity were found in all three groups. There were no statistical differences for \( V_{\text{max}} \) at a load of 0.5 g/mm² (fig. 2); this variable averaged 0.90 ± 0.08 lengths/sec in the muscles from the normal cats, 0.98 ± 0.03 lengths/sec in the muscles from the denervated cats, and 0.99 ± 0.05 lengths/sec in the muscles from the reserpine-treated cats.

Hill’s equation was also used to describe these force-velocity curves. Analyzing them as displaced hyperbolae, the constants \( a \) and \( b \) and the actual \( V_{\text{max}} \) of the Hill equation were calculated (fig. 3). The ratio \( a/P_o \), which characterizes the steepness of the force-velocity curve, and \( b \), the velocity constant, were similar in all three groups. Since \( V_{\text{max}} = (P_o/a)b \), the calculated \( V_{\text{max}} \) was also similar in all three groups.

III. RESPONSES TO INOTROPIC INTERVENTIONS

A. Force-Frequency Relations

The effects of stepwise increments in the frequency of contraction, from 6 to 48 per min, on the tension at \( L_{\text{max}} \) are shown in figure 4. As frequency was increased, a small though progressive average increase in tension occurred in all three groups (fig. 4A); the differences in the augmentations result-

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\frac{P_o - P}{V} = \frac{1}{b}(P + a), \quad \text{where} \quad P = \text{load}, \ V = \text{velocity of shortening}, \ P_o = \text{maximum isometric force}, \text{and} \ a \text{ and } b \text{ are constants which may be derived from the force-velocity curve. Substituting the values from figure 2, the plot shown in figure 3 is obtained. The slope of the line equals } \frac{1}{b}, \text{ and the intercept on the } y \text{-axis equals } a \text{ in the Hill equation. The values thus obtained are shown in the insert of the figure.}
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Force-frequency relations in the three groups of muscles. Maximum isometric tension (A), rate of tension development (B), and time from stimulation to peak tension (C) are shown as functions of the frequency of contraction. The numbers in parentheses indicate the number of animals in each group.

From increasing the frequency from 6 to 48 per min in the three groups were not statistically significant. As frequency was increased, the rate of tension development (\( \frac{df}{dt} \)) increased (fig. 4B), while the time to peak tension (TTP) decreased (fig. 4C), both in linear fashion. The absolute levels of \( \frac{df}{dt} \) and TTP and the changes which occurred as a consequence of altering frequency did not differ significantly from one another.

B. Paired Electrical Stimulation

The effects of the sustained postextrasystolic potentiation produced by continuous paired electrical stimulation on the maximum isometric tension developed in the three groups of muscles are shown in figure 5. The average tensions observed during normal stimulation, the absolute levels achieved by paired electrical stimulation in the center, and the increments in tension produced by this inotropic intervention at the bottom.

The effects of the sustained postextrasystolic potentiation produced by continuous paired electrical stimulation on the maximum isometric tension developed in the three groups of muscles. The average tensions observed during normal stimulation are shown at the top, the absolute levels achieved by paired electrical stimulation in the center, and the increments in tension produced by this inotropic intervention at the bottom.

C. Exogenous NE

I-NE was added to the muscle bath at 5 min intervals in increasing concentrations, starting at \( 10^{-10} \)M, while isometric tension was recorded at the apex of the muscles' length-tension curve, and the maximum isometric tension following each addition was noted. As shown in figure 6, the muscles obtained from cardiac-denervated cats were supersensitive to NE, with the dose-response curve shifted to the left and upwards. The threshold concentration of NE was significant.
The effects of exogenous norepinephrine on isometric tension of the papillary muscle. The per cent increase in isometric tension is shown on the ordinate and the concentration of added norepinephrine on the abscissa. The number of muscles from which each average value was obtained is shown at the bottom. Several muscles developed spontaneous rapid pacemaker activity at the higher doses of norepinephrine.

ly lower, with 7 of the 10 denervated, but only 1 of the 8 normal muscles responding to $3 \times 10^{-9} \text{M NE} (P = < 0.02)$.

IV. REFRACTORY PERIOD AND EXCITABILITY

The absolute refractory period of the muscles, i.e., the shortest attainable time interval between a driven and propagated premature stimulus, was determined at a frequency of 12 contractions per min. This period averaged 424 ±30 msec in 5 normal muscles, 494 ±28 msec in 5 muscles from cardiac-denervated cats, and 440 ±22 msec in 8 muscles from reserpine-treated cats, values which did not differ significantly from one another.

The electrical excitability of the muscles was determined by measuring the threshold current required for activation, with impulses of 3, 6, and 9 msec durations. No significant differences among the three groups of muscles were observed for any of these three stimulus durations. For example, with a stimulus of 9 msec, the threshold current averaged 11.1 ±1.7 ma in 8 normal muscles, 12.9 ±2.0 ma in 11 muscles obtained from denervated cats, and 13.4 ±1.5 ma in 7 muscles obtained from reserpine-treated animals.

Discussion

There is no unanimity concerning the role played by the cardiac stores of NE in maintaining the intrinsic contractility of heart muscle. Lee and Shideman postulated that reductions of cardiac NE stores depressed cardiac contractility and therefore suggested that these stores are important in maintaining the normal contractile state of the heart. This view was based on experiments which showed that the amplitude of isotonic contraction of isolated papillary muscles obtained from cats which had undergone bilateral sympathectomy and cats which had been treated with reserpine were markedly depressed. These procedures reduced the cardiac NE concentrations to average values of 0.28 and 0.15 \( \mu \text{g/g} \) respectively, levels substantially higher than those observed in the present investigation. More recently, Maxwell and collaborators studied the effects of catecholamine depletion on the isometric tension developed by the entire isolated left ventricle of the rabbit. Reduction of catecholamine stores with guanethidine was associated with a minimal to moderate depression of the active tension, while more pronounced depletion with reserpine (to a NE level averaging 0.09 \( \mu \text{g/g} \)) resulted in only slight depression of force.

Withrington and Zaimis stated that severe heart failure was present in cats 24 hr after 1 mg/kg of reserpine had been administered and they described severe degenerative changes in the heart muscle histologically. Nayler observed that strips of ventricle removed from toads pretreated with reserpine developed abnormally low tensions, but did not attribute these depressions of myocardial function to the depletion of NE stores. On the other hand, Moore and Moran reported that in intact dogs pretreated with reserpine, the right ventricular contractile force recorded with calibrated strain gauge arches was not depressed, and Blinks and Waud showed that the left ventricular function of isolated heart-lung preparations from reserpine-treated dogs...
was normal. Similarly, Cairoli et al. showed that the isometric tension as well as the rate of deterioration of papillary muscles obtained from reserpine-treated cats was normal.

The present study provides a more comprehensive analysis of the contractile state of heart muscle than previous investigations did. Demonstration of considerable uniformity of performance in the muscles from cats within each group allowed meaningful comparison of the various mechanical properties among the groups. Establishing the relation between muscle length and both resting and actively developed tensions allows evaluation both of the diastolic compliance of the muscle and that aspect of the contractile state that is reflected by the isometric tension. Furthermore, the force-velocity relation defines what Hill has described as the most fundamental property of active muscle and reflects the intrinsic speed of the muscle, which in turn is a function of the rate of interaction of the contractile sites. NE depletion, whether produced by chronic denervation or reserpine pretreatment, did not depress any of these fundamental properties of the myocardium. Both chronic cardiac denervation and reserpine pretreatment lower cardiac NE stores to an average value of 0.3% of normal. Indeed, several right ventricles in each group had no detectable NE and their responses did not differ from those which had traces of NE remaining. Although the possibility cannot be completely excluded that residual trace amounts of NE exerted some physiologic effect, the values in the denervated and reserpinized hearts were so low that this appears quite unlikely. Furthermore, the marked reductions of the muscle's contractile response to tyramine demonstrate that the NE depletion following denervation and reserpine pretreatment involves the papillary muscles in addition to the free wall of the ventricle. The effects of reserpinization and denervation must be clearly distinguished. While both interventions produce profound depletion of cardiac NE stores, reserpine has also been noted to produce direct effects on the myocardium which are unrelated to its catecholamine depleting action. However, if these direct depressing effects of reserpine do exist, they were not reflected in the contractile properties of the myocardium which were studied.

There has been disagreement about the role of myocardial NE in postextrasystolic potentiation and the positive inotropic effects of increased frequency of stimulation. Whalen and co-workers studied papillary muscles from cats which had undergone cardiac sympathectomy and found that the normal force treppe and postextrasystolic potentiation were diminished or absent. These authors suggested that endogenous NE is released from cardiac muscle by these two interventions and that this released NE may be responsible for the positive inotropic effect. Similarly, Penna et al. have reported that either cardiac sympathectomy by neural ablation or reserpine pretreatment abolished the augmentation of force developed by papillary muscles when the frequency of contraction was increased. Conversely, Furchgott and co-workers stated that adrenergic blockade with DCI or reserpine pretreatment did not alter the positive force treppe or postextrasystolic potentiation observed in cat papillary muscles or guinea pig atria. Koch-Weser has recently shown that neither reserpine pretreatment (1 mg/kg for 1 day) nor beta-adrenergic blockade with propranolol prevents the positive inotropic effects of an increase in frequency of contraction or paired electrical stimulation. Also, Ross and co-workers, using a right ventricular bypass preparation in the intact dog heart, found that reserpine pretreatment or beta-adrenergic blockade did not alter the inotropic response to paired electrical stimulation.

The findings of the present study agree with the results of these last three investigations. The changes in peak force, rate of force development, and time to peak tension with altering frequency of contraction were all normal in NE-depleted papillary muscles. Furthermore, both the increments and absolute levels of tension produced by the sustained postextrasystolic potentiation induced
by paired electrical stimulation were normal in both groups of NE-depleted muscles. From these data, it is concluded that intact cardiac stores of NE are not necessary for the positive inotropic effects of changes in the frequency and rhythm of contraction.

It has recently been observed that both clinical and experimental heart failure are associated with marked reduction of cardiac NE stores. If these endogenous catecholamine stores do contribute to maintenance of the intrinsic contractile state of the myocardium, then the reductions of cardiac NE stores in congestive heart failure could play an important role in the depression of the functional state of the failing heart. However, the present investigation clearly shows that the contractility of NE-depleted ventricular myocardium is not depressed. Thus, if one were to extrapolate the results of this study to the failing heart it would appear that the NE depletion occurring in congestive heart failure might impair cardiac performance, not by altering the intrinsic contractile state of the myocardium but by interfering with the augmentation of contractility provided by the adrenergic nervous system.

The finding that both groups of NE-depleted muscles exhibited marked reductions, though not complete abolition, of the positive inotropic effects of tyramine offers further support to the concept that the major action of this amine on the heart results from release of NE. The small residual positive inotropic effect of tyramine is of interest and suggests either that tyramine may have a small direct effect on the myocardium or that the release of the minimal quantities of NE remaining in the heart was responsible. Super-sensitivity to exogenous NE is a well-known response of sympathetically denervated tissues. However, the finding in this study of an upward and leftward shift of the NE-dose inotropic response curve represents the first demonstration that this phenomenon applies to the contractile properties of isolated cardiac muscle obtained from surgically denervated hearts.

Exogenous NE is known to decrease the absolute refractory period of ventricular myocardium in open-chest dogs and to induce automaticity in the cat papillary muscle. Roberts and Modell have shown, in dogs with surgically produced complete A-V block, that the spontaneous ventricular rate is decreased after cardiac sympathectomy or reserpine treatment. Thus, while the addition of exogenous NE and the absence of endogenous NE have been shown to influence these electrophysiological properties of the ventricles, the role of endogenous cardiac NE in determining the absolute refractory period or electrical excitability of ventricular myocardium has not been documented. The present investigation shows that in the isolated papillary muscle neither of these characteristics is dependent on cardiac stores of NE, and that, in the doses employed, reserpine pretreatment does not exert a direct effect on these properties.

References
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