Heart rate modulates the slow enhancement of contraction due to sudden left ventricular dilation

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Heart rate modulates the slow enhancement of contraction due to sudden left ventricular dilation. Am J Physiol Heart Circ Physiol 280: H2136–H2143, 2001.—In isovolumic blood-perfused dog hearts, left ventricular developed pressure (DP) was recorded while a sudden ventricular dilation was promoted at three heart rate (HR) levels: low (L: 52 ± 1.7 beats/min), intermediate (M: 82 ± 2.2 beats/min), and high (H: 117 ± 3.5 beats/min). DP increased instantaneously with chamber expansion (Δ1DP), and another continuous increase occurred for several minutes (Δ2DP). HR elevation did not alter Δ1DP (32.8 ± 1.6, 33.6 ± 1.5, and 34.3 ± 1.2 mmHg for L, M, and H, respectively), even though it intensified Δ2DP (17.3 ± 0.9, 20.7 ± 1.0, and 26.8 ± 1.2 mmHg for L, M, and H, respectively), meaning that the treppe phenomenon enhances the length dependence of the contraction component related to changes in intracellular Ca2+ concentration. Frequency increments reduced the half time of the slow response (82 ± 3.6, 67 ± 2.6, and 53 ± 2.0 s for L, M, and H, respectively), while the number of beats included in half time increased (72 ± 2.9, 95 ± 2.9, and 111 ± 3.2 beats for L, M, and H, respectively). HR modulation of the slow response suggests that L-type Ca2+ channel currents and/or the Na+/Ca2+ exchanger plays a relevant role in the stretch-triggered Ca2+ gain when HR increases in the canine heart.

length-dependent activation; slow force response; Bowditch effect; intact canine heart

IT HAS BEEN PREVIOUSLY REPORTED that contractile strengthening due to a sudden myocardial stretch is followed by a time-dependent enhancement of performance during the next few minutes. This pattern of response to sudden stretch is essentially the same for intact ventricles (32, 33, 44, 45) and unicellular (20, 47) or multicellular (1, 4, 6, 10, 14, 21, 24, 26, 38, 42) preparations and has been described for dog (32, 33, 44, 45), rat (4, 6, 20, 24), cat (1, 14, 26, 38), rabbit (10), guinea pig (42, 47), and ferret (5, 12, 21) myocardium. These data support the concept that the biphasic myocardial force response to stretch is a phenomenon of cellular origin common to different mammalian species.

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It is admitted that the immediate heightening of contraction after a myocardial stretch is based on an increase in myofilament Ca2+ sensitivity and potentiation of maximum Ca2+-activated force due to changes in the myofibrillar force-Ca2+ relationship (4, 13, 18, 20, 24, 25, 28). Changes in myofilament Ca2+ responsiveness seem to be excluded as contributors to the slow increase in contraction (13, 20, 24).

Time-dependent contraction strengthening seems to entirely depend on an intracellular Ca2+ concentration ([Ca2+]i) increase (3, 13, 20, 21, 24). The mechanism of [Ca2+]i increase during the slow response is still under debate. Sufficient evidence has been accumulated to rule out any relevant contribution of the sarcoplasmic reticulum to the slow response (10, 13, 20, 24). Some reports favor the idea that [Ca2+]i gain depends on L-type Ca2+ channel transit intensification (1, 16, 24, 45); however, this possibility was contested by others (14, 33). Participation of stretch-activated ion channels in the [Ca2+]i elevation has been suggested (5, 16, 17, 30, 43, 48), and this possibility appears not to have been sufficiently tested until now. Todaka et al. (44), in isovolumic canine left ventricles, and Calaghan et al. (12), in ferret papillary muscles, described a striking parallelism between [Ca2+]i, and cAMP after myocardial stretch, suggesting that changes in [Ca2+]i may be primarily related to an increase in cAMP. A primary change of intracellular Na+ concentration ([Na+]i) determining the increase of [Ca2+]i, was previously theoretically predicted by Bluhm et al. (11). Alvarez et al. (6), studying rat ventricular trabeculae, reported a stretch-elicited intramyocardial paracrine mechanism, by which sequential activation of ANG II, endothelin, and Na+/H+ exchanger elevates [Na+]i. A [Ca2+]i increase should occur after the final action of the Na+/Ca2+ exchanger.

A number of fundamental points related to the [Ca2+]i gain elicited by stretch require more investigation for an unbiased understanding of the intimate Ca2+ transit changes evoked by stretch. Accordingly, there is no definitive evidence about whether 1) Ca2+ gain occurs during diastole, systole, or both, 2) L-type Ca2+ channel transit changes evoked by stretch.
Ca\textsuperscript{2+} channels, nonspecific stretch-activated ion channels, and Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger are structures effectively involved in the slow response, and 3) one or more mechanisms are involved in the [Ca\textsuperscript{2+}]i increase consequent to stretch. In addition, it is not fully understood to what extent the experimental conditions of cellular preparations act on subcellular functions, causing results that are not applicable to a blood-perfused, normal-temperature intact heart. Pragmatically, it may be stated that no point has been sufficiently validated to fully explain the [Ca\textsuperscript{2+}]i gain that follows myocardial stretch.

Despite the recognized dependence of the slow response on changes in Ca\textsuperscript{2+} kinetics and although rhythm decisively influences Ca\textsuperscript{2+} transit, there has been no systematic evaluation of the behavior of the slow enhancement of contraction after a sudden stretch when stimulation frequency varies.

Intimate mechanisms concerned with force-frequency relationships are reasonably well established. Steady-state and transient changes in stimulus frequency are associated with changes in [Ca], that parallel the force change. It is admitted (2) that, in most mammals, heart rate (HR) elevation within the physiological range is accompanied by contractile enhancement dependent on an increase in [Ca], due to accentuation of the reverse action of the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger. In addition, recent evidence has shown that slow Ca\textsuperscript{2+} channel currents are upregulated by increased stimulation rate (8, 9, 29, 31, 39, 40, 49).

Previous studies on isolated myocells have suggested some kind of interaction between rhythm of contraction and slow force response. Hongo et al. (20) reported that 44% of isolated rat ventricular myocytes bathed in 1 mM Ca\textsuperscript{2+}-Tyrode solution showed the slow response at a stimulation rate of 0.5 Hz. When stimulation frequency was increased to 1 Hz, the occurrence of the slow response was increased to 60%. On the other hand, White et al. (47) reported that reduction of stimulation rate of single guinea pig ventricular myocytes seems to increase the probability of producing the slow increase. Lakatta and Jewell (26) described an inverse relation between slow response half-time (T\textsubscript{1/2}) and frequency of stimulation and a dependence of the number of beats needed to complete T\textsubscript{1/2} on stimulation rate in cat papillary muscles. These data support the idea that stimulation rhythm may interfere with the time-dependent myocardial response to a sudden stretch.

The objective of the present study was to describe the influence of HR on the slow increase of left ventricular contraction after a sudden dilation. Isolated isovolumic blood-perfused dog hearts were subjected to sudden dilation at three different levels of HR. We found that the intensity of time-dependent contraction enhancement after a sudden dilation is proportional to stimulation frequency. In addition, the time course of the slow response and the number of beats needed to complete T\textsubscript{1/2} shifted in opposite directions: an increase in stimulation frequency reduced T\textsubscript{1/2} and raised the number of beats included in T\textsubscript{1/2}.

**METHODS**

Experiments were performed on isolated hearts from male dogs weighing 12–16 kg that were supported by the arterial blood of a second dog. The support dog (19–23 kg) was anesthetized with morphine hydrochloride (2 mg/kg im), chloralose (60 mg/kg iv), and urethane (600 mg/kg iv), heparinized (500 U/kg iv), and mechanically ventilated. Maintenance doses of chloralose (6 mg/kg iv) plus urethane (60 mg/kg iv) and heparin (50 U/kg iv) were administered hourly. Only data from experiments in which the mean arterial pressure of the support dog remained stable at >80 mmHg were utilized. Arterial blood pH and gases were periodically analyzed and corrected as needed (pH = 7.35–7.45, P\textsubscript{O2} > 100 mmHg, PC\textsubscript{O2} = 35–45 mmHg) by addition of bicarbonate or adjustment of ventilation.

A roller pump circulated the blood from the left femoral artery of the support dog to a reservoir to perfuse the isolated heart through a cannula fixed in the ascending aorta. The reservoir was placed at a height sufficient to maintain a perfusion pressure of 100 mmHg. The blood temperature, measured in the perfusion cannula, was maintained at 37°C by means of a heat exchanger. Coronary venous and thebesian flow returned from the isolated heart to the support dog through catheters inserted into the pulmonary artery and the apex of the left ventricle. Electrodes for epicardial electrocardiograms were sewn to the right ventricle.

Total atrioventricular block was induced by injecting 10% formalin (0.6–2.0 ml) into the region of the atrioventricular node through a right atrial incision, and the atrioseptum was closed by a purse-string suture. This procedure was performed during temporary occlusion of the coronary perfusion line; cardiac ischemia occurred for only 50–170 s during this period. Electrodes for artificial pacing were inserted into the right ventricular anterior wall, and an artificial stimulator was used to pace the heart at the lowest frequency that maintained a regular rhythm. The mitral apparatus was excised, and a soft distensible latex balloon mounted on a cannula (18 mm diameter) was placed in the left ventricle. A purse-string suture at the base of the left atrium was tightened around the cannula and held the balloon inside the left ventricular cavity. The balloon was sufficiently compliant, and its size was large enough so as not to contribute to pressure over the range of volumes studied. A rubber stopper traversed by two polyethylene catheters occluded the distal end of the mitral cannula. One catheter (15 cm long, 0.3 cm diameter) was attached to a P\textsubscript{23} ID pressure transducer, and the other (15 cm long, 0.3 cm diameter) was connected to a stopcock that allowed the control of the amount of saline inside the ventricular balloon. Left ventricular pressure was determined by means of the transducer signal conditioner (model 13-6615-50, Gould) of a Windofagr recordor. Left ventricular developed pressure (DP, i.e., systolic pressure minus diastolic pressure) was used for the analysis of ventricular performance. Perfusion pressure, measured at the level of the left ventricular midlevel cavity, was continuously monitored.

**Protocol.** Eleven preparations were studied. After the equilibration period (30 min), the deflated volume of the balloon was determined for each heart. The deflated volume was defined as the minimal volume of saline in the balloon that allowed a stable level of diastolic pressure and a positive pressure without artifacts during contraction. The deflated volume was associated with a negative diastolic pressure in all cases. In each study, left ventricular pressure was recorded while the balloon was at the deflated volume and after sudden expansion until the highest final level of peak systolic...
pressure was reached. Experiments were conducted by promoting the same extent of ventricular dilation at three levels of HR.

For each study, in the first stage of the protocol, the HRs to be used and the volume of saline to be added to the balloon were previously determined. The lowest HR that allowed a regular control of heart rhythm was used as the low HR. Thereafter, the stimulation frequency was elevated in steps (~20 beats/min) to confirm that increments of ~60 beats/min increased left ventricular systolic pressure. The upper limit tested was defined as the highest value to be used as high HR, and an intermediate value was chosen as the intermediate HR. With the HR settled at the high value, the maximal volume of saline to be used during the experiment was defined, taking into account that peak systolic pressure did not exceed perfusion pressure (100 mmHg). The difference between maximal volume and deflated volume of the balloon corresponded to the volume of saline to be utilized in dilating the left ventricle. The ventricular volume was returned to the deflated condition, and a new 10-min equilibrium period was allowed to elapse. Thereafter, the heart was paced in a randomized manner at one of the three frequencies previously determined. After an equilibration period (~4–5 min), left ventricular pressure was continuously recorded, and ventricular dilation was produced by manually injecting the predetermined volume of saline into the balloon. Thereafter, the stimulation frequency was successively changed to the two other defined frequencies, and the ventricular responses to dilation were again analyzed. Between interventions, the preparations were allowed to stabilize for 10 min at the deflated volume.

Statistical analyses. Values are means ± SE. Repeated-measures ANOVA complemented by the Student-Newman-Keuls test was used for comparison of parameter changes. P < 0.05 was considered statistically significant.

RESULTS

The HRs at which ventricular dilations were studied were 52 ± 1.7 (low), 82 ± 2.2 (intermediate), and 117 ± 3.5 (high) beats/min.

Ventricular response to a sudden dilation reproduced the pattern previously described (44, 45) (Fig. 1). Ventricular volume expansion was promptly succeeded by an instantaneous increase in DP followed by a continuous increase in mechanical performance for several minutes. Immediately after intensification of the instantaneous contraction, there was a short period of modest decline of performance, described and discussed elsewhere (45). Sudden ventricular dilation frequently provoked bursts of premature beats. Experiments in which this results in the evaluation of immediate (Δ₁DP) and time-dependent (Δ₂DP) heightening of performance that was hazardous were excluded from analysis.

Inotropic effects of HR elevation were observed in the three analyzed conditions: 1) when the balloon was deflated, 2) immediately after sudden ventricular dilation, and 3) when DP attained the final values after slow contractile enhancement. In the deflated condition, DP increased (P < 0.001) from 12.8 ± 1.4 mmHg at the low HR to 15.0 ± 1.4 mmHg at the intermediate HR and 17.9 ± 1.6 mmHg at the high HR, and the same behavior was observed for DP values immediately after dilation (45.6 ± 2.2, 48.6 ± 2.0, and 52.2 ± 1.8 mmHg for low, intermediate, and high HR, respectively, P < 0.001) and for the final DP values (62.9 ± 2.5, 69.3 ± 2.3, and 79.0 ± 1.9 mmHg for low, intermediate, and high HR, respectively, P < 0.001). When the inotropic effects of HR elevation were analyzed with the DP values in deflated, dilated, and final conditions considered as relative values of DP observed at low HR, a previously described pattern of myocardial response to the frequency-resting length interplay was observed. Indeed, it has been previously established (26, 41) that the Bowditch effect induces a more pronounced contractile stimulation at shorter than at longer muscle length, and this peculiarity was observed in the present experiments (Fig. 2B). In Fig. 2, DP values at intermediate and high HR were taken as relative values of DP obtained at low HR, as described. With HR elevations, relative DP rose more strikingly in the deflated than in the dilated condition, showing that the Bowditch effect was more prominent at shorter than at longer length.

Fig. 1. Slow time base records of 1 experiment, representative of developed pressure (DP) changes after a sudden ventricular dilation (arrowhead) at 3 levels of heart rate (HR). HR elevation clearly intensified and shortened the slow response.
Table 1 summarizes the characteristics of ventricular performance heightening induced by sudden ventricular dilation at the three HR levels. The total ($\Delta_1DP + \Delta_2DP$) increase of DP ($\Delta DP$) was intensified by increased HR, as indicated by a significant difference ($P < 0.001$) observed in all comparisons among the values obtained for low, intermediate, and high HR: 50.1 ± 2.1, 54.3 ± 1.9, and 61.1 ± 1.3 mmHg, respectively.

A remarkable difference was detected with respect to the influence of HR rise on immediate and slow contraction increases: although the treppe phenomenon does not influence the immediate pressure increase evoked by ventricular expansion, a clear intensification of the slow response was promoted by HR elevation. The values for $\Delta_1DP$ at low, intermediate, and high HR (32.8 ± 1.6, 33.6 ± 1.5, and 34.3 ± 1.2 mmHg, respectively) were not significantly different (Fig. 3A), whereas $\Delta_2DP$ was augmented by HR elevation in every experiment, resulting in a significant difference ($P < 0.001$; Fig. 3A) for mean values at low, intermediate, and high HR (17.3 ± 0.9, 20.7 ± 1.0, and 26.8 ± 1.2 mmHg, respectively). In addition, $\Delta_2DP$ values were significantly correlated ($r = 0.7028$, $P < 0.001$) with the respective HR values (Fig. 3B). This kind of behavior for $\Delta_1DP$ and $\Delta_2DP$ resulted in a progressive increment of the $\Delta_2DP$ contribution to $\Delta DP$, as can be inferred by the increase in $\Delta_2DP/\Delta DP$ that accompanied HR elevations (0.34 ± 0.01, 0.38 ± 0.01, and 0.43 ± 0.02 for low, intermediate, and high HR, respectively, $P < 0.001$; Fig. 3C).

With all data sets taken into account, the time to reach half-maximal $\Delta_2DP$ ($T/2$) ranged from 45 to 96 s, and 58–126 heartbeats were needed to complete $T/2$. In every case, increments in stimulation frequency shortened the slow response time course (82 ± 3.6, 67 ± 2.6, and 53 ± 2.0 s for low, intermediate, and high HR, respectively, $P < 0.001$; Fig. 4A), even though they increased the number of heartbeats needed to complete the slow response $T/2$ (72 ± 2.9, 95 ± 2.9, and 111 ± 3.2 beats for low, intermediate, and high HR, respectively, $P < 0.001$; Fig. 4B). Lakatta and Jewell (26) described a rectangular hyperbolic correlation between $T/2$ and frequency stimulation in two of four cat papillary muscles in which the $xy$ product is a constant (i.e., $y = a/x$), suggesting that $T/2$ could be entirely determined by the number of heartbeats. In our data, a nonsignificant correlation ($r = 0.5469$, $P > 0.05$) was found for this fitting. For our results, we found a significant correlation ($r = 0.8756$, $P < 0.0001$; Fig. 5) for a rectangular hyperbola of the following type: $y = ab/(b + x)$, where the $xy$ product is not a constant. This result indicates that $T/2$ only partially depends on the number of heartbeats.

**DISCUSSION**

The left ventricular systolic response to a sudden dilation observed in the present study reproduced the pattern previously described for myocardial stretch of intact ventricle (44, 45) and unicellular (20, 47) or multicellular preparations (1, 4, 6, 10, 12, 14, 21, 24, 38).

The present results should be analyzed from the viewpoint of a confirmation of force-interval relationships described for similar experimental conditions. It has been said that inotropism shows a flat or only a modest response to HR increases in conscious animals (7, 15, 19, 34). However, in isolated preparations, the inotropic effect of force-frequency relationships is de-
scribed as a more prominent phenomenon (2). In our experiments, a positive inotropic effect of HR elevation consistently occurred in all experiments within the entire frequency range utilized, and, as previously established (41), systolic enhancement was more pronounced at shorter than at longer lengths.

A remarkable feature of our results was that the HR increase did not affect immediate heightening of contraction due to ventricular dilation, but the systolic influence of HR was limited to the slow response. These data agree with the present concept that immediate contractile strength after stretching is due to changes in physical factors and in the myofibrillar force-Ca\textsuperscript{2+} relationship (4, 13, 18, 20, 24, 25, 28) and with the fact that there is no report that the treppe phenomenon alters these factors. In contrast, force-frequency relationships and slow response to a sudden stretch are thought to be entirely linked to changes in Ca\textsuperscript{2+} kinetics. On this basis, the concept of the isolated influence of HR on the slow response fully agrees with present concepts about the changes of length-tension relationships elicited by a sudden myocardial stretch.

On the other hand, a greater slow force response elicited by the inotropic effect of HR calls close attention to the reason why this action contrasts with the influence on the time-dependent response reported for other inotropic interventions acting on the degree of myocell activation before stretch. Indeed, the more marked slow increase obtained by stimulation frequency was a singular action compared with results reported in studies with muscles that were spontaneously close to full activation (4) and in studies on the

Fig. 3. A: immediate (Δ\textsubscript{1}DP) and slow (Δ\textsubscript{2}DP) increases of DP after sudden ventricular dilation at L, M, and H. Values are means ± SE. *P < 0.05 vs. L; #P < 0.05 vs. M. HR elevations did not affect the immediate increase but potentiated the slow increase. B: slow increase (Δ\textsubscript{2}DP) plotted as a function of HR. Intensity of the slow response is linearly correlated with the stimulation frequency. C: relative contribution of slow increase of contraction to total pressure elevation (Δ\textsubscript{2}DP/ΔDP) after sudden ventricular dilation at L, M, and H. Symbols, individual values; solid horizontal lines, means; dotted horizontal lines, SE. *P < 0.05 vs. L; #P < 0.05 vs. M.

Fig. 4. A: time for half of the slow response (T/2) at L, M, and H. *P < 0.05 vs. L; #P < 0.05 vs. M. B: number of heartbeats needed to complete T/2 at L, M, and H. *P < 0.05 vs. L; #P < 0.05 vs. M. Symbols, individual values; solid horizontal lines, means; dotted horizontal lines, SE.
We found a significant correlation for a rectangular Lakatta and Jewell (26) for \( T/2 \) and HR is of interest. Therefore, in interpreting our results, it is necessary to take into account an inotropic action of HR elevation that not only augments \([\text{Ca}^2+]_i\) in the basal (deflated) condition but also renders the myocyte prone to intensifying the \([\text{Ca}^2+]_i\) increase due to stretch proportionally to HR increments.

The hyperbolic relation (\( y = a/x \)) suggested by Lakatta and Jewell (26) for \( T/2 \) and HR is of interest. We found a significant correlation for a rectangular hyperbola in which the \( xy \) product is not a constant \( y = ab/(b + x) \), indicating that \( T/2 \) and HR are inversely correlated, but the phenomenon depends only partially of the number of heartbeats. The possible heartbeat dependence of the slow response seems to be a consequential finding, because this kind of association reinforces the concept that excitation-contraction events are a relevant mechanism for intracellular \( \text{Ca}^{2+} \) gain after a stretch.

Our results can be understood if we take into consideration two previously described mechanisms that may possibly affect \( \text{Ca}^{2+} \) kinetics in our experiments: 1) upregulation of \( \text{Ca}^{2+} \) channel currents (\( I_{\text{CaL}} \)) by frequency of stimulation and 2) \( \text{Na}^+/	ext{Ca}^{2+} \) exchanger as the basis for a slow force response.

It has been shown (8, 9, 29, 31, 39, 40, 49) that elevation of stimulation rate in frog and mammalian myocardium induces an \( I_{\text{CaL}} \) potentiation consisting of a moderate increase of peak current amplitude and a marked slowing of the inactivation kinetics by changing gating properties. Moreover, \( I_{\text{CaL}} \) were potentiated by frequency stimulation in a manner quite interesting for the understanding of our results: there is a striking proportionality between frequency stimulation and \( I_{\text{CaL}} \) potentiation. In human atrial myocytes, \( I_{\text{CaL}} \) was potentiated in a gradually progressive manner with increasing heart stimulation rates in a range as wide as 0.3–5 Hz (39).

Interestingly, it is recognized that \( I_{\text{CaL}} \) amplification promoted by stimulus frequency is favored by stimulation of cAMP production (8, 9, 31, 39, 40, 49). In effect, it has been shown that cAMP elevations secondary to \( \beta \)-adrenergic agonists (8, 9, 31, 39, 40, 49) or nonspecific maneuvers (31) are capable of amplifying the \( I_{\text{CaL}} \) upregulation due to stimulation rate. According to the reports of Todaka et al. (44) and Calaghan et al. (12) that cAMP and \([\text{Ca}^2+]_i\) rise at the same time after myocardial stretch, we are tempted to suggest that \( I_{\text{CaL}} \) upregulation by the treppe phenomenon and its amplification by a cAMP increase possibly acted concurrently in our experiments, promoting intensification of the slow response by an increase in HR.

The potentiation of \( I_{\text{CaL}} \) by frequency stimulation can hardly explain the amplification of the HR slow response observed in our experiments if we admit that the time-dependent intact ventricle heightening of contraction elicited by sudden stretch is linked to accentuation of L-type \( \text{Ca}^{2+} \) channel inflow, as previously suggested in some reports (1, 27, 45). Some authors observed no influence of the \( \text{Ca}^{2+} \) channel blocker verapamil on the slow response. Chuck and Parmley (14), studying cat papillary muscles, reported that verapamil does not alter the slow response to a sudden stretch. Lew (33) studied the effect of verapamil on the slow force response in the in situ dog heart and reported that verapamil did not affect the volume load length-pressure shift verified before the drug administration. In contrast, in the blood-perfused intact isovolumic canine heart, transsarcolemmal L-type \( \text{Ca}^{2+} \) channel influx plays a relevant role in the stretch-induced slow heightening of contraction of the canine myocardium.

Recently, Alvarez et al. (6) reported that the slow force response to stretch can be inhibited by the blocker of the AT1 angiotensin receptor losartan, the endothelin receptor BQ-123, and the Na+/H+ exchanger amiloride. These authors proposed that the slow force response results from a cascade of events initiated by the release of ANG II elicited by stretch that successively stimulates endothelin and the Na+/H+ exchanger. The increase in [\( \text{Na}^+ \)]; thus promoted will allow that reversal action of Na+/Ca2+ exchanger terminates to increase force by elevating \([\text{Ca}^2+]_i\). It seems conceivable that the slow force enhancement due to the Bowditch effect observed by us could be understood if this mech-
anism were at the basis of the slow force response to stretch. It is well recognized that, during the upstroke of the action potential, the transmembrane potential transiently becomes more positive than the equilibrium potential of the Na+/Ca2+ exchanger, and its reversal action predominates. It seems likely that, as a consequence of the increase in action potential frequency promoted in our protocol, the transmembrane potential should continue to be positive in relation to the equilibrium potential for a longer period of time, allowing a more prominent reversal action of the Na+/Ca2+ exchanger.

The actual physiological significance of the slow response after a ventricular distension in the intact organism is still unknown. Data obtained in studies on anesthetized thoracotomized dogs (32, 33) have indicated that stronger contraction follows a volume load-induced ventricular expansion. It has been suggested that the time-dependent response can participate in the slow inotropic change related to the Anrep effect (6, 23, 24, 37), a phenomenon reported to occur in awake dogs (46). Interestingly, the Anrep effect was shown to be intensified by HR elevations (35, 46). On the other hand, the slow response has been recently described as a transient phenomenon that disappears within a few minutes after the peaks (44). In our experiments, it was not possible to analyze the vanishing characteristic of the slow response, since in our protocol the data after ventricular dilation were monitored up to the time that maximal DP values remained stable by −10 s.

Although the mechanisms underlying the changes in Ca2+ kinetics involved in the myocardial response to stretch are not fully understood, our results have clearly shown that, in the canine myocardium, HR can modulate the intensity and time course of the slow response. In contrast to other positive inotropic interventions that tend to blunt or abolish the time-dependent contraction enhancement after a stretch, the treppe phenomenon accentuates the slow contractile change. When frequency stimulation is increased, the time course of the slow response is reduced and the number of heartbeats included in T/2 increases in a fashion suggesting that excitation-contraction coupling may play a decisive role in the genesis of the slow response. It is unknown to what extent the data obtained with isolated blood-perfused dog heart can be extrapolated to a more physiological condition, since the treppe phenomenon is known to be less active in awake unrestrained resting animals. Likewise, it seems advisable to evaluate the applicability of these concepts to other species with consideration of peculiarities in the force-frequency relations.

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REFERENCES

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42. Steele DS and Smith GL. Effects of muscle length on diastolic [Ca\(^{2+}\)]\(_i\) in isolated guinea-pig ventricular trabeculae (Abstract). *J Physiol (Lond)* 467: 328P, 1993.


