# Force-frequency Relations in Hypertrophic Heart Muscle: A Mathematical Model for Excitation-contraction Coupling

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**Abstract.** Static and dynamic chrono-inotropic responses were recorded from both normal and hypertrophic rat auricular myocardium The slope of the static force-frequency relation for hypertrophic hearts was steeper than that for control hearts Computer experiments were designed to study the cellular mechanisms underlying the changes in the force-frequency response associated with heart hypertrophy, with the aid of a mathematical model for excitation-contraction coupling in rat heart A set of equations was derived which permitted to study the effects on the chronoinotropic relations of both the geometrical dimensions of cardiomyocytes and the sarcoplasmic reticulum, and of the variation in activity of mechanisms for Ca movements through the sarcolemma and the sarcoreticular membrane A comparison of data obtained from simulated and real experiments suggested that the features characteristic of force-frequency relations for hypertrophic heart are a result of an enhanced volume of intracellular Ca-stores rather than of the total volume of the cardiomyocyte

**Key words:** Heart — Hypertrophy — Force-frequency relations

## Introduction

Regardless of numerous investigations, the question of cell mechanisms underlying peculiarities of the functional state of hypertrophic myocardium still remains open Moreover, the available experimental data on the contractility of hypertrophic myocardium are rather controversial, the impact of myocardial hypertrophy on cardiac contractility being strongly dependent both upon the stages of the compensatory hypertrophy (Meerson 1969) and upon the experimental model used to induce hypertrophy (TerKeurs et al 1983) In particular, the dependence of parameters of excitation-contraction coupling in the heart muscle on the size of cardiomyocytes is not clear Simulations seem expedient to investigate the problem, they permit to obtain data on the dynamics of cellular processes which are not accessible with direct measurements

The aim of the present investigation has been to elucidate cellular mechanisms responsible for chronoinotropic relationships in hypertrophic cardiac muscle With this aim in view, the dependence of static and dynamic chronoinotropic responses of myocardium (Izakov and Markhasin 1980) on geometrical parameters of cardiomyocytes and of subcellular Ca-compartments was studied with the aid of a mathematical model for excitation-contraction coupling in rat heart (Pratusevich et al 1987, see also Mukumov et al 1986) in computer experiments The results were compared to data obtained from experiments with normal and hypertrophic rat myocardia

## Materials and Methods

Experiments were carried out on myocardial preparations from spontaneously hypertensive rats (SHR) with Wistar rat (WKY) myocardium used as control Left auricular strips, 4 5 × 1 5 mm were used Nearly isometric contractions were recorded by a 6MX1C movable-electrode tube Square-wave stimulating current pulses were used, twice the threshold amplitude and 5 ms in duration The muscle was bathed in Tyrode solution of the following composition (in mmol/l) NaCl, 132, KCl, 4 5, $NaHCO_3$, 11, $NaH_2PO_4$ $2H_2O$, 0 6, $MgCl_2$ $6H_2O$, 0 25, $CaCl_2$ $2H_2O$, 2 16, glucose, 11 The bathing solution was aerated with a mixture of 95% $O_2$ and 5% $CO_2$, $pO_2$ = 500 mm Hg, pH 7 2–7 4, and the temperature was kept at 28 °C The average relation of animal mass to heart mass for SHR and WKY rats was 4 9±0 1 and 3 9±0 2, respectively The difference is significant at $p < 0\ 05$

Prior to the experiment, the preparation was subjected to rhythmic pacing at a frequency of 0 5 Hz for 40 min The following parameters were estimated to quantify the time course of single twitches amplitude ($A$, mg), contraction time ($tc$, ms) and relaxation time ($tr$, ms) (both measured with respect to the half amplitude level of the contractile force), and maximal rate of rise ($V+$, mg/s) and decline ($V-$, mg/s) of contractile force Then, the static force-frequency response was derived from the amplitudes of the steady-state twitch tension at different stimulation frequencies in the range of 0 1 to 1 0 Hz The mechanical restitution curve was taken for the dynamic force-frequency response which included the twitch tension of the first beat after a 15 to 600 s pause in periodic stimulation of the preparation at 0 5 Hz related to the steady-state twitch tension at 0 5 Hz (as measured just before the pause) Exactly the same protocol of data acquisition and processing was implemented in computer experiments

The simulation procedure of excitation-contraction coupling (ECC) in normal and hypertrophic heart is discussed in "Results and Discussion" Computer experiments were performed using an EC-1045 computer and the VM/SP system, chronoinotropic effect simulation programs were written in FORTRAN

## Results and Discussion

The parameters of individual twitches recorded for normal and hypertrophic auricular strips under periodic stimulation at a frequency of 0 5 Hz are shown in Table 1 It can be seen that, in our model, cardiac hypertrophy causes no significant changes of these parameters

Fig. 1 shows the static chronoinotropic responses observed in our experiments with auricular strips from control (1) and hypertrophic (2) rat hearts. The interval-force relationship in both cases was negative (contraction amplitude drop with the increasing stimulation frequency); there were no significant changes in contraction/relaxation time intervals ($tc$ and $tr$) *vs* heart rate while the positive and negative slopes of the individual twitches ($V+, V-$) varied in accordance with the contraction amplitude. It can be seen that hypertrophy results in a certain increase in the slope of the static force-frequency relationship due to an upward shift of the low-frequencies limb of the curve.

**Table 1.** Effect of cardiac hypertrophy on the time course of nearly isometrical twitch of rat auricular strips at a basal frequency of 0.5 Hz.

| Twitch parameter | Normal (WKY) myocardium | Hypertrophic (SHR) myocardium |
|---|---|---|
| $A$ (mg) | 212 ± 41 | 351 ± 109 |
| $tc$ (ms) | 31.7 ± 1.3 | 33.7 ± 1.7 |
| $tr$ (ms) | 29.3 ± 1.4 | 31.0 ± 1.1 |
| $V+$ (g/s) | 5.39 ± 0.96 | 8.6 ± 2.7 |
| $V-$ (g/s) | 5.6 ± 1.1 | 8.7 ± 2.6 |

$A$ is the twitch amplitude; $tc$ is the time of contraction and $tr$ is the time of relaxation (both measured with respect to the half amplitude level of the contractile force); and $V+$ is the maximal rate of rise and $V-$ is the maximal rate of decline of the contractile force.

Fig. 2 shows the mechanical restitution curves obtained in experiments with WKY (1) and SHR (2) myocardial preparations. In both cases, the mechanical restitution curve was ascending (the longer the pause in stimulation the higher the contraction amplitude); there were no significant changes in contraction/relaxation time intervals ($tc$ and $tr$) *vs* pause duration while the positive and negative slopes of the individual twitches ($V+, V-$) varied in accordance with the contraction amplitude. As compared to controls, the hypertrophic heart is characterized by an upward shift of the mechanical restitution curve (Fig. 2).

The simplified general scheme for calcium balance in the cardiomyocyte (Pratusevich et al. 1987) implies that the regulation of myocardial cell contractile activity is based on Ca ion exchange between the extracellular medium and the two basic intracellular compartments: the myoplasm and the intracellular Ca stores. Isometric force developed by isolated myocardial strips is supposed to be proportional to the Ca content of the myoplasm. In order to make allowance for geometric parameters of the myocardial cell in the explicit form, let us rewrite the equations of Ca balance in the compartments with variables $\tilde{c}$ and $\tilde{r}$ standing for calcium

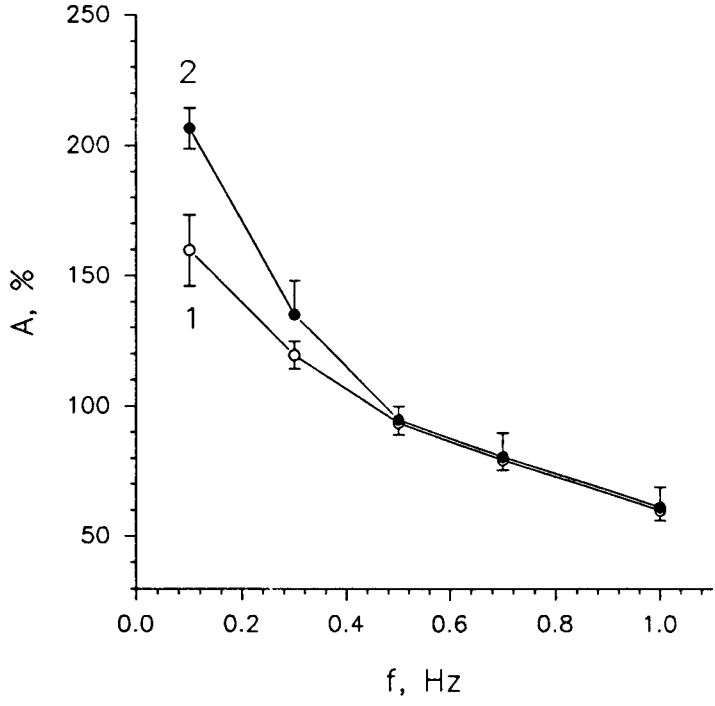


**Figure 1.** Static chronoinotropic responses of auricular strips from normal (1) and hypertrophic (2) rat heart. Conditioning rhythmic pacing at 0.5 Hz for 5 min was followed by stepwise variation of the stimulation frequency to the test value in a range between 0.1 and 1.0 Hz. Each point on the curve represents peak steady-state contractile force at a given stimulation frequency, related to the steady-state peak contractile force at 0.5 Hz as measured during conditioning pacing. $A$ is the normalized peak contractile force, $f$ is stimulation frequency in Hz.

concentrations in the myoplasm and the Ca-stores, $Vc$ and $Vr$ standing for the corresponding compartment volumes, and $Sc$ and $Sr$ being the compartment surface areas:

$$\mathrm{d}(\tilde{c}Vc)/\mathrm{d}t = \tilde{a}_0 Sc + \alpha a_1 Sc - \tilde{a}_2 Sr\tilde{c} + \alpha\tilde{a}_3 Sr\tilde{c}\tilde{r}$$

$$\mathrm{d}(\tilde{r}Vr)/\mathrm{d}t = \tilde{a}2Sr\tilde{c} - \alpha\tilde{a}_3 Sr\tilde{c}\tilde{r} - \alpha\tilde{a}_4 Sre\tilde{r} - \tilde{a}_5\tilde{r}Sre,$$

where $\tilde{a}_0, \tilde{a}_1$ are the densities of the stationary and potential-dependent Ca influx, respectively; $\tilde{a}_2$ is the density of Ca flux from the myoplasm into the stores, $\tilde{a}_3$ is the density of Ca release from the intracellular stores to the myoplasm; $\tilde{a}_4, \tilde{a}_5$ are the densities of the potential-dependent and stationary components of Ca efflux from the stores to the extracellular medium; $Sre$ is the effective surface area for Ca efflux to the extracellular medium.; $\alpha$ is a stepwise time function describing the excitation of myocardial cell: $\alpha = 1$ during AP, and $\alpha = 0$ during the rest of time.

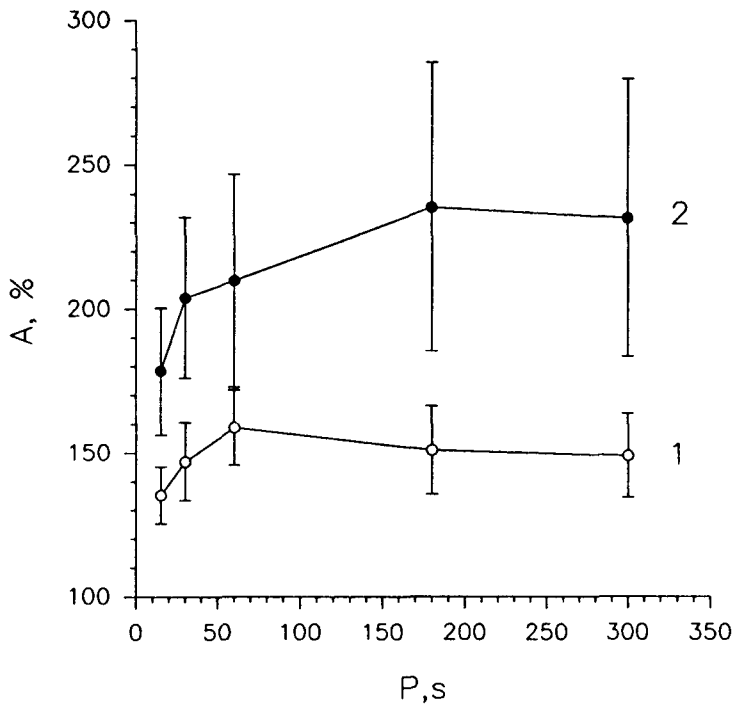


**Figure 2.** Mechanical restitution curves of auricular strips from normal (1) and hypertrophic (2) rat heart. Each point on the curve represents peak force of the first contraction after a break in the rhythmical pacing of the preparation at 0.5 Hz, related to the steady-state peak contractile force (as measured just before the pause). $A$ is the normalized peak force of the post-pause contraction, $P$ is the pause duration (in seconds).

Proceeding from Ca concentrations to Ca content in compartments by introducing new variables $\tilde{c}Vc = c$ and $\tilde{r}Vr = r$, a set of equations is obtained for $c$ and $r$ which is formally identical to that proposed by Pratusevich et al. (1987). Provided the coefficients describing the flow rates are related to the coefficients describing the flow densities and geometrical parameters of the cardiomyocyte:

$$\tilde{a}_0 Sc = a_0; \quad \tilde{a}_1 Sc = a_1; \quad \tilde{a}_2 Sr/Vc = a_2;$$
$$\tilde{a}_3 Sr/(VcVr) = a_3; \quad \tilde{a}_4 Sre/Vr = a_4; \quad \tilde{a}_5 Sre/Vr = a_5.$$

The resultant set of equations allows to study the effect on the chronoinotropic relations of both the geometrical dimensions of cardiomyocytes and the sarcoplasmic reticulum (SR), which is the main operative intracellular Ca store (Carafoli 1985), and of the variation in activity of mechanisms of Ca movements through the

sarcolemma and SR membrane.

First, let us consider the dependence of the cardiac muscle chrono-inotropy on SR and cell volumes, as well as on the surface area through which Ca passes from SR to extracellular medium. The respective results of computer experiments are presented in Fig. 3 and Table 2. The static force-frequency relations are quantified in Table 2 by the slope, an index proven to closely correlate with the functional state of the cardiac muscle (Liakhovich et al.1987). The slope was calculated as the ratio of the peak steady-state contractile force at 0.1 Hz stimulation frequency to that at 1.0 Hz. Computer experiments involved the testing of readjustments of three basic model parameter:

i) (twofold) increase in $Vc$ value with constant Ca stores volume and $Vr$; ii)

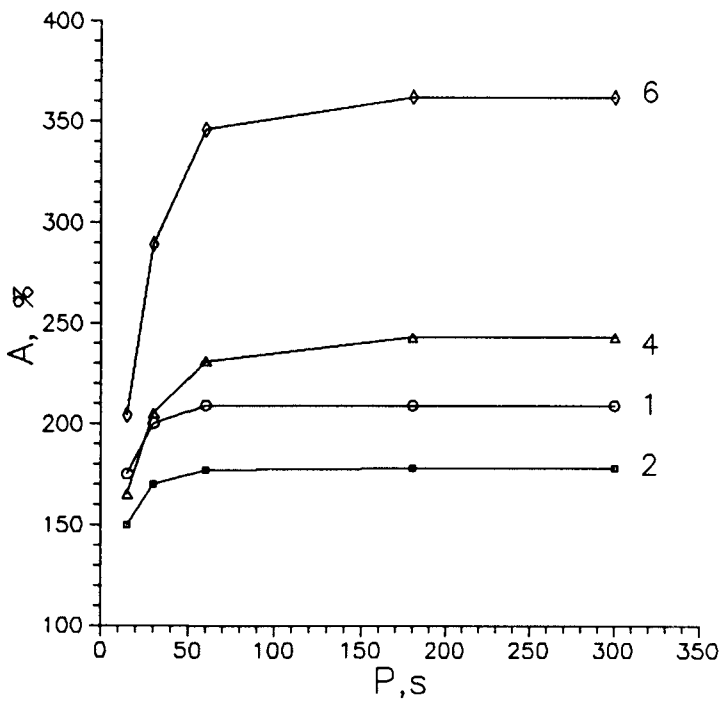


**Figure 3.** Simulated curves of mechanical restitution: The effects of the geometrical parameters. Each point on the curve represents peak force of the first contraction after a break in the rhythmical pacing of the preparation, related to the steady-state peak contractile force. The stimulation frequency was 0.5 Hz. For geometrical parameters used in calculations see Table 2 (the respective parameter setting is indicated by the figure at the corresponding curve). $A$ is the normalized peak force of the post-pause contraction. $P$ is the pause duration (in seconds).

synchronous increase in $Vc$ and $Vr$; iii) increase in the Ca stores volume ($Vr$) with the volume of the myoplasmatic compartment ($Vc$) held constant. Two alternatives were considered for each of these cases: 1) volume variations do not affect the area of the surface ($Sre$) through which Ca ions are extruded from the stores to the extracellular fluid; 2) surface area increases. The calculations used a factor of 2 for all the increases in volumes, and compartment surfaces were increased by a factor of $2^{2/3}$.

When the value of $Sre$ was left unaltered, an increase in the cell volume with the volume of the intracellular stores remaining constant (Table 2, row 2) lead to a decrease in the slope of the static force-frequency relationship. In this case, the mechanical restitution curve (curve 2 in Fig. 3) shifts downwards with respect to the control one (curve 1 in Fig. 3): the force of contractions decreases at every point within the entire range of resting intervals. On the contrary, an increase in the Ca stores volume with the cell volume held constant lead to a dramatic increase in the slope of the static chronoinotropic response (Table 2, row 6) while the mechanical restitution curve (curve 6 in Fig. 3) shifts upwards with respect to the control one (curve 1); this indicates strengthening of the first beat after a pause in rhythmical stimulation. Simultaneous twofold increases in $Vc$ and $Vr$ volumes resulted in a rela-

**Table** 2. Simulated effect of geometrical parameters of rat cardiomyocytes and their subcellular compartments on the slope of the static force-frequency response.

| Parameter setting | Geometrical parameters (arbitrary units) | | | | | x $\tilde{a}_2$ | Slope |
|---|---|---|---|---|---|---|---|
| | $Vc$ | $Sc$ | $Vr$ | $Sr$ | $Sre$ | | |
| 1 (control) | 1 | 1 | 1 | 1 | 1 | 20 | 2.046 |
| 2 | 2 | $2^{2/3}$ | 1 | 1 | 1 | 20 | 1.77 |
| 3 | 2 | $2^{2/3}$ | 1 | 1 | $2^{2/3}$ | 20 | 1.35 |
| 4 | 2 | $2^{2/3}$ | 2 | $2^{2/3}$ | 1 | 20 | 2.63 |
| 5 | 2 | $2^{2/3}$ | 2 | $2^{2/3}$ | $2^{2/3}$ | 20 | 2.17 |
| 6 | 1 | 1 | 2 | $2^{2/3}$ | 1 | 20 | 3.43* |
| 7 | 1 | 1 | 2 | $2^{2/3}$ | $2^{2/3}$ | 20 | 2.59 |
| 8 | 1 | 1 | 1 | 2 | 1 | 20 | 2.31 |

*The values of other model coefficients fitted for the rat myocardium (Pratusevich et al. 1987) were in all cases (No.1 – No.9): $\tilde{a}_0 = 0.1$; $\tilde{a}_1 = 10$; $\tilde{a}_3 = 100$; $\tilde{a}_4 = 10$; $\tilde{a}_5 = 0.1$.

$Vc$ and $Vr$ are volumes of the myoplasmic compartment and of the intracellular Ca stores, respectively; $Sc$ and $Sr$ are the compartments respective surface areas; $Sre$ is the area of the surface through which Ca ions are extruded from the stores to the extracellular fluid; $\tilde{a}_2$ is the density of Ca flux from the myoplasm into the stores.

tively less significant variation both in the dynamic and the static chrono-inotropic response of the myocardium. In this case, the mechanical restitution curve changes in the following way: the force of the first contraction upon stimulation restoration is below that in control preparations for shorter pauses (up to 25 s), and exceeds them for longer pauses. Thus, the above mentioned alternative hypotheses concerning plausible mechanisms underlying features characteristic of hypertrophic heart chrono-inotropic response lead to qualitatively different variations in the pattern of static and dynamic chrono-inotropic responses of the myocardium according to computer simulation data. Increasing parameter $Sre$ in each case only lowered the slope of the static force-frequency response (see Table 2, rows 3, 5, 7).

It can be concluded from the comparison of experimental and simulation data that, from the above hypotheses concerning mechanisms of myocardial hypertrophy in terms of alterations in cardiomyocyte or subcellular compartments size related to contractile proteins activation, the most probable is that according to which hypertrophy causes a substantial increase in the volume of intracellular Ca stores and a less significant increase in the total myoplasmic volume.

The set of hypotheses considered is far from being exhaustive. Additionally, the following modes of alterations in the parameter setting of the model for excitation-contraction coupling were tested: 1) Decrease (twofold in our calculations) in Ca-ATPase activity or, more exactly, in the density of Ca flux from the myoplasm to the intracellular stores. The data suggesting a decreased activity of the sarcoplasmic reticulum Ca-ATPase have been presented elsewhere (Kimura et al. 1989); 2) Increase in the interface area between the intracellular Ca stores and the myoplasm ($Sr$) with no significant increase in compartmental volumes. According to the working formulae, the first possibility of those mentioned above implies, in terms of the mathematical model, a decrease in the value of coefficient $\tilde{a}_2$ with all other coefficient values remaining unchanged as compared to the control values (Table 3, row 2); the second possibility implies a simultaneous increase (twofold in our calculations) in coefficients $a_2$ and $a_3$ (Table 2, row 8).

Moreover, we also simulated the effects of an increase in the rates of Ca uptake and release from intracellular stores upon the shape of the force-frequency response in rat heart muscle. The calculated slopes of the static force-frequency response are presented in Table 3, and the corresponding simulated mechanical restitution curves are illustrated in Fig. 4.

The calculations showed that upon reducing the Ca uptake rate (Table 3, row 2) or upon elevating the Ca release rate (Table 3, row 4) the slope of the static force-frequency response is reduced. An increase in the interface area (Table 2, row 8) or in the Ca uptake rate (Table 3, row 3) only produced a rather moderate increase in the slope (compare Table 2, row 6). From Fig. 4 it can be seen that the changes in the simulated mechanical restitution curve were consistent with the changes in the calculated slope of the static force-frequency curve: an augmentation of the slope

**Table 3.** Simulated effect of densities of Ca release and uptake by the intracellular stores on the slope of the static force-frequency response of rat cardiomyocytes

| Parameter setting | $\tilde{a}_2$ | x $\tilde{a}_3$ | Slope |
|---|---|---|---|
| 1 (control) | 20 | 100 | 2.046 |
| 2 | 10 | 100 | 1.69 |
| 3 | 40 | 100 | 2.25 |
| 4 | 20 | 200 | 1.43 |

*The values of other model coefficients fitted for the rat myocardium (Pratusevich et al. 1987) were in all cases (No.1 – No.4): $\tilde{a}_0 = 0.1$; $\tilde{a}_1 = 2$; $\tilde{a}_3 = 100$; $\tilde{a}_4 = 10$; $\tilde{a}_5 = 0.1$

$\tilde{a}_2$ is the density of Ca flux from the myoplasm into the intercellular Ca stores, $\tilde{a}_3$ is the density of Ca release from the stores. All the geometrical parameters (cf. Table 2) were kept equal to unity.

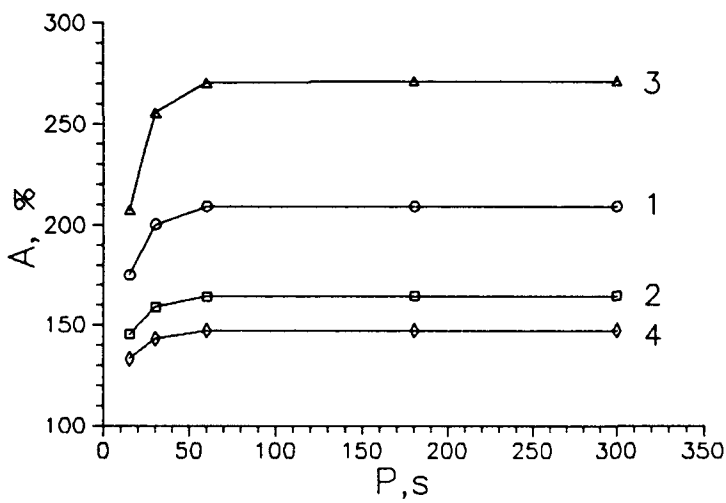


**Figure 4.** Simulated curves of mechanical restitution: The effects of changes in the rate of Ca uptake by, and Ca release from, the intracellular Ca stores. Each point on the curve represents peak force of the first contraction after a break in the rhythmical pacing of the preparation, related to the steady-state peak contractile force. The stimulation frequency was 0.5 Hz. For values of model parameters used, see Table 3 (the respective parameter setting is indicated by the figure at the corresponding curve). $A$ is the normalized peak force of the post-pause contraction, $P$ is the pause duration (in seconds).

was always accompanied by an upward shift of the mechanical restitution curve, and *vice versa*. A comparison of the data obtained during computer experiments

(Fig 3, 4) with those obtained during experiments on rat myocardium (Fig 1, 2) suggests that an increase in the SR surface or inhibition of the Ca transport mechanisms from the myoplasm to SR ( as well as increase in densities of Ca release and Ca uptake by the intracellular stores) are unlikely to be fundamental to the mechanism underlying the characteristic properties of the chronoinotropic response of hypertrophic heart muscle the experimentally observed hypertrophy-induced changes in both static and dynamic chrono-inotropic responses are most readily simulated by increasing the model parameter representing the volume of intracellular Ca stores

The negative force-frequency relationship, negative "staircase" response on rhythmical pacing after long rest intervals, and the ascending curve of mechanical restitution in normal rat myocardium may be attributed mainly to the participation of the intracellular Ca stores in providing the contractile machinery with the activator Ca ions The amount of $Ca^{2+}$ stored in SR at the beginning of each beat is critically dependent upon the beat to-beat interval, thus, under rhythmic stimulation the Ca stores are partly exhausted and the twitch amplitude is accordingly diminished with respect to the contractile force of a beat induced after longer pauses in rhythmical pacing The lowering of the contraction amplitude with the increasing stimulation frequency indicates a lesser extent of recovery of the $Ca^{2+}$ level in the stores during shorter beat-to-beat intervals at higher frequencies Similarly, as to the mechanical restitution curve, the stronger beats after longer pauses in rhythmical pacing are associated with an increase in the amount of $Ca^{2+}$ accumulated in the stores (which were partly exhausted during the conditioning rhythmical pacing) during longer intervals between the interruption of conditioning rhythmical pacing and the test beat The observed effects of hypertrophy on static force-frequency relations and mechanical restitution curve imply that these mechanisms are operative in hypertrophic rat myocardium as well as in normal rat heart muscle, moreover, in hypertrophic rat heart these SR-associated effects seem more pronounced The simulation studies with the aid of the mathematical model of excitation-contraction coupling support the suggestion that the features of the force-frequency phenomena observed in hypertrophic rat myocardium are consistent with the hypothesis that hypertrophy strengthens the functional role of the intracellular stores in regulating the contractile activity of the myocardium, this is probably due to the relatively more pronounced enhancement of the store volume as compared to that of the myoplasmic compartment

Thus, it could be shown that the characteristic properties of hypertrophic myocardium are due to an increased volume of the intracellular Ca stores rather than of the entire cardiomyocyte

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