



## Original Article

# Postischemic Stunned Myocardium Does Not Alter Cardiac Response to an Elevation in Contractile Frequency

Orlando Sant'Ana Jr, Roberto J. Nogueira, Neif Murad, Antonio Carlos Lopes, Paulo J. F. Tucci  
São Paulo, SP - Brazil

## Objective

To assess the influence of the postischemia/reperfusion stunned myocardium (PIRSM) on the inotropic and lusitropic effects of heart rate (HR).

## Methods

Nine preparations of isolated dog hearts in isovolumic contraction and nourished by the arterial blood of another dog underwent heart rate elevation from 60 bpm to 200 bpm, in 20-bpm stages. The following variables were assessed before (B) and after (A) ischemia (15 min) and reperfusion (30 min): the pressure developed during contraction (PD), its first positive (+dP/dt) and negative (-dP/dt) derivative, the time of maximum pressure (TMP), the pressure at rest (Pr), and the time necessary for the developed pressure to decrease by 90% of its maximum value (time for relaxation - TR90%).

## Results

The stimulating effects of HR elevation on inotropism and relaxation were similar before and after ischemia/reperfusion as follows: +dP/dt values increased, TMP decreased, -dP/dt values were intensified, and TR90% decreased. The values of PD did not change, and Pr increased.

## Conclusion

The results confirmed the positive inotropic effect of HR elevation (Bowditch effect) and the depressive action of ischemia/reperfusion. They also evidenced that PIRSM does not alter the stimulating action of the Bowditch effect, in accordance with the current concept that PIRSM does not impair calcium myocardial kinetics, favoring the prevalent hypothesis that the decrease in the contractile capacity after ischemia/reperfusion depends on the reduction of the myofilament responsiveness to calcium.

## Key words

postischemia/reperfusion stunned myocardium, Bowditch effect, heart rate, systolic function, diastolic function

Escola Paulista de Medicina, Universidade Federal de São Paulo  
Mailing address: Paulo J. F. Tucci - Rua Estado de Israel, 181/94  
Cep 04022-000 - São Paulo, SP, Brazil - E-mail: [tucci@fcr.epm.br](mailto:tucci@fcr.epm.br)  
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In 1975, Heyndrickx et al<sup>1</sup> reported for the first time the occurrence of myocardial depression following ischemia/reperfusion, which would be popularized in the English literature under the name postischemia/reperfusion stunned myocardium. Later studies<sup>2-6</sup> showed that postischemia/reperfusion stunned myocardium is generated by 2 factors: 1) the action of reactive oxygen species released during reperfusion, and 2) the increase in intracellular calcium concentration (calcium overload) that occurs during both ischemia and reperfusion. Calpain, a protease that promotes the lysis of troponin I, plays a relevant role in triggering myocardial depression after ischemia/reperfusion. The consequence is the decrease in the myofilament reactivity to calcium, the typical functional impairment of postischemia/reperfusion stunned myocardium. In this circumstance, the affinity of the ligation between troponin and calcium decreases, the quantity of the ion bound to troponin decreases, and, consequently, myocardial contractility decreases. Latency in the recovery of the inotropism lasts until troponin I integrity is reestablished.

Very convincing indications exist that calcium kinetics is not altered in myocardial depression following ischemia/reperfusion. Therefore, postischemia/reperfusion stunned myocardium has been proven not to reduce the intracellular content of the ion<sup>3,4,7</sup> and to preserve the physiological and pharmacological maneuvers that stimulate inotropism by increasing the intracellular content of calcium ([Ca]<sub>i</sub>): beta-adrenergic agonists<sup>8-11</sup>, milrinone<sup>8,12</sup>, calcium administration<sup>5,13</sup>, postpause potentiation<sup>14</sup>, and postextrasystolic potentiation<sup>9,13</sup>.

However, the positive inotropic effect depending on heart rate elevation studied in myocardial segments of dogs undergoing regional ischemia has been reported to transform into negative inotropic action after myocardial ischemia/reperfusion<sup>12</sup>. If this inversion of the myocardial response to heart rate elevation is confirmed, the concept of the integrity of calcium kinetics after periods of ischemia/reperfusion should be revised, because, typically, the inotropic action linked to heart rate fluctuations is a physiological maneuver that depends exclusively on calcium kinetics.

In most mammals, heart rate elevations trigger the stimulus to myocardial inotropism. This stimulating effect of heart rate on myocardial contractility was given the name the Bowditch effect or treppe phenomenon. Two mechanisms fundamental to the positive inotropic action of the Bowditch effect are 1) an increase in intracellular sodium concentration followed by the reverse action of the sodium/calcium exchanger protein, and consequent elevation in intracellular calcium concentration ([Ca]<sub>i</sub>)<sup>15</sup> and 2) an increase

in calcium entrance through the slow channels during the second phase of the action potential<sup>16,17</sup>.

This study aimed at reassessing the influence of the transient period of ischemia followed by reperfusion in the response of isolated dog hearts to elevations in the frequency of the contractions. The preparation of isolated dog hearts nourished by the arterial blood of another dog and maintained in isovolumic contraction was used. The constant ventricular volume of this preparation allows the use of ventricular pressures as indicators of myocardial contractility and ventricular compliance with fair reliability. If the ventricular volume is constant, the pressure fluctuations occurring during the contractions point to variations in the inotropic state in the same direction, and alterations in the diastolic pressure point to modifications in ventricular compliance.

## Methods

This study was carried out with isolated dog hearts ( $n = 9$ ) nourished by the arterial blood of another animal (fig. 1), according to a method reported in other studies<sup>18,19</sup>.

Two dogs, anesthetized with a mixture of chloralose (60 mg/kg) and urethane (600 mg/kg) and heparinized (500 IU/kg), were used in each experiment as follows: one animal had its heart (2-16 kg) extracted, while the other was maintained as support (19-23 kg), providing arterial blood for the isolated heart. The

support animal was mechanically ventilated and received additional doses of chloralose (6 mg/kg), urethane (60 mg/kg), and heparin (50 IU/kg) every hour. The pH (7.35-7.45) and concentrations of blood gases ( $pO_2 > 100$  mmHg;  $pCO_2 = 35-45$  mmHg) were assessed every hour, and their deviations were corrected by changing the programming of the ventilation parameters or by administering an 8% sodium bicarbonate solution.

Blood in the femoral arteries of the support dog was pumped into a reservoir, where it was maintained under the constant pressure of 100 mmHg, to provide blood to the coronary arteries of the isolated heart through a pressure regulator with oxygen. Prior to perfusing the coronary arteries through a cannula placed in the ascending aorta of the isolated heart, blood was warmed to 37°C by use of a heat exchanger. The flow of the coronary sinus and of the thebesian circulation returned to the jugular veins of the support animal through catheters inserted in the main pulmonary artery and in the left ventricular apex, respectively.

Through an incision in the right atrial anterior wall, a 10% formol solution (0.6-2.0 mL) was injected in the region of the atrioventricular node to cause complete atrioventricular block. The heart rate was then commanded by an artificial stimulator whose electrodes were placed in the right ventricular anterior wall. The atriotomy was closed with a previously prepared pursestring suture. This procedure was performed during temporary occlusion of the coronary perfusion line, and the resulting cardiac ischemia lasted only 50-170 seconds. The mitral valvular apparatus was excised. A very flexible and delicate latex balloon mounted on a cannula (diameter of 18 mm) was placed inside the left ventricle, and a pursestring suture was performed around the base of the left atrium to maintain the balloon inside the ventricular cavity. The balloon was compliant and its dimensions did not exert pressure on the fluid used to distend the left ventricle. The distal extremity of the cannula of the balloon was occluded and transfixed by 2 catheters (length of 15 cm and internal diameter of 0.3 cm). One of the catheters was used for controlling the fluid volume inside the balloon, and the other was ligated to the P231D pressure transducer. The transducer was coupled to a Gold amplifier (13-6615-50 model) for determining the ventricular pressures, and the first temporal derivative of the ventricular pressure ( $dP/dt$ ) was obtained by using a Gold amplifier (13-4615-71 model). These variables were recorded on a Windograf polygraph.

Once the preparation was installed, the frequency of the pacemaker stimuli was regulated to 60 bpm, and the fluid volume inside the balloon was adjusted so that the systolic pressure observed in the polygraph monitor reached approximately 70 mmHg. The frequency of the stimulator was progressively elevated up to 200 bpm to guarantee that the following did not happen: 1) elevation of the developed pressure beyond the coronary perfusion pressure; 2) excessively high stimulation frequency capable of causing maladjustments in the contractile capacity, which would recommend interruption of the protocol. Then, the pacemaker was reprogrammed to emit the stimulation frequency considered baseline (60 bpm). A 10- to 20-minute interval was observed, so that the functional balance of the preparation could be reached.

Once stabilization was achieved, the variables were recorded at baseline. Then, the stimulation frequency of the pacemaker was successively increased at 20-bpm stages up to 200 bpm. At every increase, one minute was awaited so that the new functional

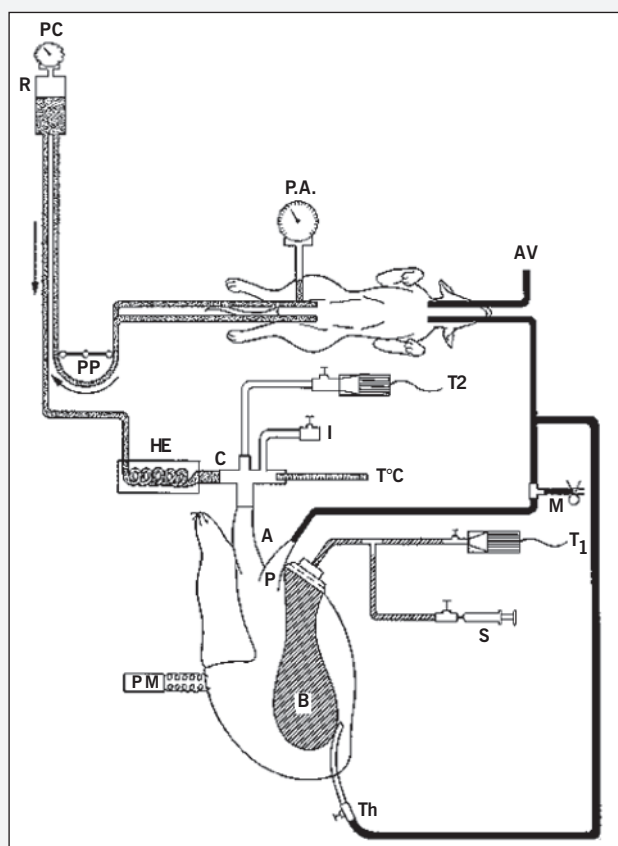


Fig. 1 - Preparation of isolated dog heart nourished by the arterial blood of another dog and maintained in isovolumic contractions. B: intraventricular balloon that allows control of ventricular volume and turning of left ventricular contraction into isovolumic; P and Th: catheters that return the blood of the pulmonary artery and of the thebesian circulation to the support dog, respectively; A: remaining portion of the aorta with the perfusion cannula (C); PM: artificial stimulator that acts like a pacemaker; HE: heat exchange; PP: perfusion pump; R: blood reservoir with pressure controller (PC).

balance was achieved, and the variables were recorded again. The maneuvers described comprised the control situation of the protocol identified as B (before ischemia/reperfusion).

Then, the postischemia/reperfusion contractile depression situation was promoted.

The stimulation frequency was returned to the baseline values and the period of overall heart ischemia was initiated with occlusion of the coronary perfusion line. After 15 minutes of ischemia, coronary perfusion was reestablished; the reperfusion period lasted 30 minutes. During the period of ischemia, the artificial pacemaker was turned off right after the heart reached the condition of asystolia or ventricular fibrillation (approximately 10 minutes). At the beginning of the reperfusion period, blood originating from the heart was allowed to drain out (approximately 250 mL), so that the cardiac catabolites that accumulate during ischemia were not injected into the support dog. After 30 minutes of reperfusion, the variables of the stimulation frequency were recorded as were those in the B situation. These records represented the data of the depressed state, after ischemia/reperfusion (A). In some experiences, ventricular fibrillation occurred in the isolated heart during the period of ischemia or at the beginning of the reperfusion period, requiring electrical defibrillation with electrical discharge of 10 joules.

Each variable was analyzed according to 3 aspects: 1) whether the increase in heart rate caused a modification in the variables in situations B and A; 2) whether ischemia/reperfusion depressed the heart's mechanical action; 3) whether the behavior of the different variables was different within each control (B) and depressive (A) condition studied.

An analysis of variance (ANOVA) was initially performed for each variable with repeated measures within a single group of animals; in the statistical literature, this analysis is called block ANOVA<sup>20</sup>. After characterizing the difference between the various heart rates used ( $P < 0.0001$ ), 2 hierarchical partitions of the sum of the squares of the values of the different variables in the various levels of heart rate were performed. The first partition compared separately, for each condition B and A, the mean of each variable obtained in the various heart rates analyzed. This maneuver allowed assessment of whether the elevation in heart rate caused a modification in the variable within each condition studied. In the case of statistical significance, orthogonal contrasts were used<sup>21</sup> to test the existence of linear, quadratic, and cubic effects. The other partition tested for each variable, in each heart rate value, the hypothesis of equality of the means in conditions B and A. This analysis enabled characterization of whether the ischemia/reperfusion affected the value of the variables. In the cases with significant differences between the values in conditions B and A, we determined the 95% confidence intervals of the differences between the means of the variables at the heart rates of 60 and 200 bpm in the 2 experimental conditions. The results whose 95% confidence intervals did not overlap were admitted to indicate different behavior in the 2 conditions.

## Results

Data are presented as mean  $\pm$  standard error of the mean ( $x \pm \text{sem}$ ).

The following parameters were used to assess ventricular contractility: pressure developed during contraction (PD: systolic pres-

sure minus diastolic pressure); maximum positive value of  $dP/dt$  ( $+dP/dt$ ); and the time of maximum pressure (TMP: time period elapsed from the beginning of the systolic ascension of the ventricular pressure curve until its maximum value, indicating the duration of systole) (fig. 2). The values of these variables at the heart rate of 60 bpm in conditions B and A are shown in table I.

The elevation in heart rate exerted different influences on the PD and  $+dP/dt$  both before and after ischemia/reperfusion. In the entire heart rate range analyzed, no statistically different values of PD were identified before ( $P = 0.2986$ ) and after ( $P = 0.7027$ ) ischemia/reperfusion, indicating that PD did not vary in any of the conditions (fig. 3A). On the contrary,  $+dP/dt$  increased in both conditions (fig. 3B), and its values obeyed the distribution of an ascending line in B ( $349 + 3.108x$ ;  $P < 0.0001$ ) and in A ( $268 + 2.315x$ ;  $P = 0.0001$ ). On the other hand, the values of PD and  $+dP/dt$  in situation A were significantly lower when compared with those observed in B for the same levels of heart rate. In addition to these results that characterize the positive inotropic effect of the Bowditch phenomenon and the depressive action of ischemia/reperfusion on heart contraction, a clear overlapping was observed in the 95% confidence intervals and the differences between the  $dP/dt$  values observed at 60 bpm (29 mmHg/s; 249 mmHg/s) and at 200 bpm (147 mmHg/s; 366 mmHg/s). This indicates that the inotropic effects of the Bowditch phenomenon were comparable before and after ischemia/reperfusion.

Concomitantly, as a consequence of the elevation in heart rate, shortening of the contraction occurred (fig. 3C). As heart rate ranged from 60 to 200 bpm, TMP decreased progressively following a descending straight line in B ( $252 - 0.618x$ ;  $P < 0.0001$ ) and in A ( $247 - 0.634x$ ;  $P < 0.0001$ ). Moreover, no significant difference was observed ( $P = 0.0782$ ) between the TMP values in conditions B and A. These results indicate that ischemia/reperfusion did not alter the duration of the contraction and did not affect shortening of the systole that accompanied the increase in heart rate.

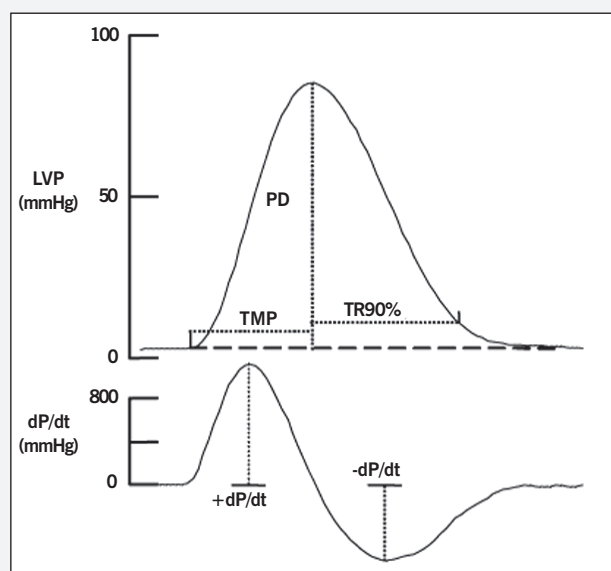


Fig. 2 - Tracings illustrating the left ventricular pressure curve (LVP) and its first temporal derivative ( $dP/dt$ ) with the variables analyzed: PD: pressure developed (systolic pressure minus diastolic pressure);  $+dP/dt$ : first positive maximum temporal derivative of ventricular pressure;  $-dP/dt$ : first negative maximum temporal derivative of ventricular pressure; TMP: time of maximum pressure; TR90%: time necessary for the pressure developed to decrease by 90% of its maximum value.

**Table I - Data obtained in 9 preparations of isolated hearts at the heart rate of 60 bpm before (B) and after (A) ischemia/reperfusion**

PD (mmHg)		+dP/dt (mmHg/s)		-dP/dt (mmHg/s)		TMP (ms)	
B	A	B	A	B	A	B	A
61±3	43±4*	547±81	408±76*	447±82	277±62*	217±5	208±8

PD - pressure developed; +dP/dt - positive maximum value of the first temporal derivative of the ventricular pressure; -dP/dt - negative maximum value of the first temporal derivative of the ventricular pressure; TMP - time of maximum pressure. \*: P < 0.05 in regard to B.

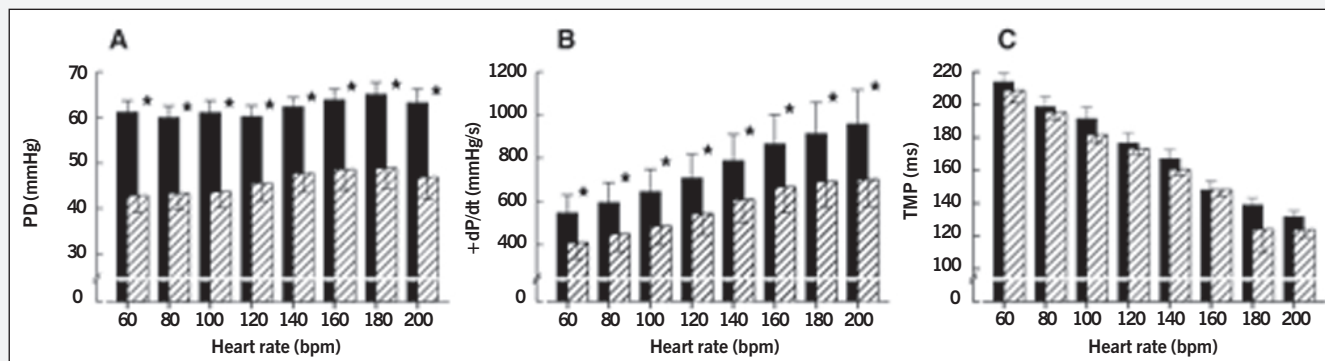


Fig. 3 - Means (columns) ± standard error of the mean (vertical lines) of the maximum pressure developed (panel A), of the first positive maximum temporal derivative of the ventricular pressure (panel B), and of the time of maximum pressure (panel C) at the various heart rate levels, before (B: full columns) and after (A: striped columns) ischemia/reperfusion. \*: P < 0.05 in the comparison between B and A.

Together, these results indicate that myocardial depression following ischemia/reperfusion had no quantitative or qualitative effect on systolic function modifications due to the Bowditch effect. The latter stimulated contraction, while postischemia/reperfusion depression diminished that function. It is worth noting that ischemia/reperfusion had no influence on the effect of heart rate elevation on ventricular contraction.

The diastolic ventricular function was analyzed by assessing ventricular compliance and myocardial relaxation. The values of the pressure at rest (Pr), when the ventricular volume is kept constant, were used as indicators of the fluctuations of ventricular compliance. The analysis of myocardial relaxation was performed by assessing the behavior of the negative maximum values of dP/dt (-dP/dt), and the time required for a 90% decrease in the maximum values of the developed pressure (time of relaxation at 90%: TR90%).

The values of the pressures at rest (Pr) for the various heart rates before and after ischemia/reperfusion are shown in figure 4A.

Statistically significant differences between the measures of the pressures at rest were observed before (P<0.0001) and after (P<0.0001) ischemia/reperfusion, indicating that Pr varied when heart rate increased. The behavior of Pr was that of a quadratic function of heart rate both before ( $y = 5.641 - 0.049x + 0.00024x^2$ ; P=0.0002) and after ( $y = 5.0 - 0.032x + 0.00018x^2$ ; P=0.0036) ischemia/reperfusion. Comparing the mean values of Pr before and after ischemia/reperfusion in each heart rate, the means of the diastolic pressure in both conditions were equal in the heart rates of 60, 80, 100, 120, and 140 bpm. For the heart rate of 160 bpm, we identified the significance suggestive of the Pr tendency to be greater after ischemia/reperfusion than before ischemia/reperfusion (P=0.0532), and, for the heart rate of 180 bpm, a statistically significant difference was detected between the means of the pressures at rest both before and after ischemia/reperfusion (B: 4.1±0.8 mmHg; A: 5.3±0.7 mmHg; P=0.0071).

For the heart rate of 200 bpm, no statistically significant difference (P=0.4239) was observed between Pr values before and after ischemia/reperfusion. All these results indicate that Pr varies with heart rate increases according to increasing parabolic patterns of upper concavities not coinciding before and after ischemia/reperfusion, as long as the equivalence of the B and A conditions only occurs until the heart rate of 140 bpm.

The increases in heart rate cause progressive increases in the absolute values of -dP/dt before ( $363 + 1.438x$ ; P<0.0001) and after ( $200 + 1.394x$ ; P<0.0001) ischemia/reperfusion (fig. 4B). In addition, a clear overlapping of the confidence intervals of the differences between the -dP/dt values before and after ischemia/reperfusion was observed at the heart rates of 60 bpm (97 bpm; 244 mmHg/s) and 200 bpm (91 mmHg; 239 mmHg/s), indicating that the stimulation of the lusitropic properties caused by the increase in heart rate was comparable before and after ischemia/reperfusion. On the other hand, all -dP/dt values after ischemia/reperfusion were lower than their respective values before ischemia/reperfusion, characterizing the slower relaxation after ischemia/reperfusion.

The ventricular relaxation period, assessed through the time for relaxation at 90% of the PD, was reduced before ( $238 - 0.557x$ ; P<0.0001) and after ( $249 - 0.589x$ ; P<0.0001) ischemia/reperfusion, and no differences were identified between the pairs of TR90% values at all heart rate levels analyzed (fig. 4C).

The -dP/dt and TR90% data set indicated that myocardial relaxation was activated through an increase in heart rate. The data also showed that postischemia/reperfusion myocardial depression reduced relaxation velocity, although no alteration occurred in TR90%. In addition, the ischemia/reperfusion set proved not to alter the influence exerted by the Bowditch effect on the lusitropic properties, because the modifications in -dP/dt and TR90% caused by the increase in heart rate were not different before and after ischemia/reperfusion.

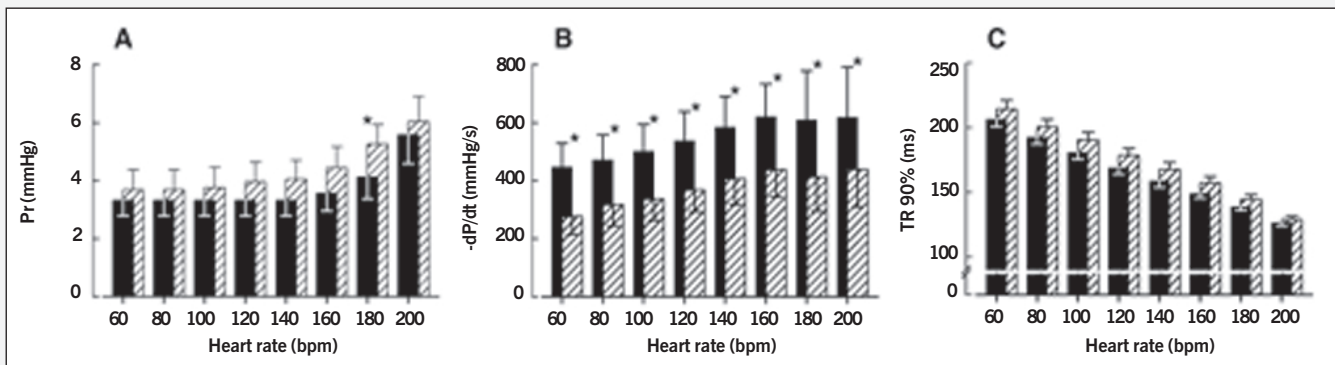


Fig. 4 - Means (columns)  $\pm$  standard error of the mean (vertical lines) of the pressure at rest (panel A), of the first negative maximum temporal derivative of the ventricular pressure (panel B), and of the time necessary for the pressure developed to decrease by 90% of its maximum value (panel C) at various heart rate levels, before (B: full columns) and after (A: striped columns) ischemia/reperfusion. \*:  $P < 0.05$  in the comparison between B and A.

## Discussion

Our results clearly characterized the positive inotropic effect of heart rate elevation and the depressive action of the contraction caused by ischemia/reperfusion. The stimulating action of contractility was very well manifested in the progressive increase in  $+dP/dt$  that accompanied the increase in heart rate. One may argue that the lack of increase in PD, simultaneously with the increase in heart rate, is contrary to the idea of the positive inotropic action of the Bowditch effect. This possible controversy is refuted by information in the literature. Other authors<sup>22-24</sup> have already reported that the force generated during systole (indicated by the maximum value of PD) depends on the intensity with which the contraction is generated (better reflected by the maximum value of  $+dP/dt$ ) and on the duration of the contraction (indicated by the TMP). Characteristically, as an integrating part of the Bowditch effect, these 2 variables that regulate the force generated undergo modifications in opposite directions, exactly as occurred in our experiments: the contraction gets more intense (increasing  $+dP/dt$ ) and shorter (reducing TMP). The final result about the value of the force will depend on the predominance of one of these factors, and its reduction may even occur.

On the other hand, the depressive effect on contraction caused by ischemia/reperfusion was clear. After the 30-minute period of restarting the coronary flow, the PD and  $dP/dt$  values were clearly inferior to those observed in the control situation. In the literature, it is worth noting the tendency to consider the alterations in the responsiveness of the myofilaments as the most relevant pathogenetic mechanism of myocardial depression following ischemia/reperfusion<sup>3,6,7,25,26</sup>. This interpretation is gaining importance mainly because of convincing demonstrations that the calcium offer to the myoplasm is not altered a few minutes after myocardial reperfusion is started. This hypothesis is yet supported by the fact that the functional capacity of the depressed myocardium may be stimulated by inotropic maneuvers that act on calcium cellular kinetics, such as sympathomimetic amines<sup>8-11,27</sup>, milrinone<sup>12</sup>, calcium administration<sup>5,13</sup>, postextrasystolic stimulation<sup>9,13</sup>, and postpause stimulation<sup>14</sup>.

The reduction in the  $-dP/dt$  values after ischemia/reperfusion contradicts the idea that the calcium kinetics is not affected by the ischemia/reperfusion binomial. This result cannot be understood as alterations that have occurred in the responsiveness of the myofilaments to calcium, and would initially indicate the possibility

that calcium reuptake by the sarcoplasmic reticulum may have been impaired. However, other authors<sup>7,28</sup> have reported that, when myocardial relaxation of the intact ventricle is analyzed, factors other than responsiveness of the myofilaments and calcium kinetics need to be considered. The results of those authors indicate that myocardial edema and coronary turgor, present in the intact ventricle, represent characteristics of impairment of the muscular relaxation that are not identified in isolated samples of the myocardium. This allows the assumption that the depression of relaxation observed after ischemia/reperfusion should not be associated with impairment of the calcium intracellular kinetics caused by the ischemia/reperfusion binomial.

Our results indicated that global ischemia of the isolated hearts followed by reperfusion results in myocardial depression that does not alter the influence of the Bowditch effect on contraction and relaxation. Moreover, the equivalence of the response to the increase in the frequency of contractions allows one to conclude that not even the intensity of the myocardial responses to *trappe* is altered in the state of post-ischemia/reperfusion depression.

We have heard from only one single study by Schad et al<sup>12</sup> that aimed at assessing the influence of heart rate increase on the depressed myocardium due to ischemia/reperfusion. Those authors caused regional ischemia in the territory of the anterior descending coronary artery and compared the systolic performance of the myocardium irrigated by that vessel with that exteriorized by the muscle supplied by the circumflex artery. After causing ischemia/reperfusion of the region irrigated by the anterior descending coronary artery, those authors reported that, in that territory, the increase in heart rate was accompanied by a reduction in myocardial shortening. In the territory of the circumflex artery, used as a reference for normal myocardial behavior, the muscular contraction was activated by an increase in the frequency of contractions. When interpreting their results, these authors did not consider that the assessment of systolic performance in the experimental conditions used is hindered by the interaction established between the depressed and the healthy myocardium. The method used has the depressed myocardium contracting in series with the healthy myocardium. In this condition, normal myocardial contraction, exacerbated by the inotropic stimulus, tends to stretch the depressed myocardium, concealing the activation of the contractile capacity that may have occurred in the latter.

Therefore, our data indicate that, in the canine myocardium,

myocardial depression after ischemia of 15 minutes and reperfusion of 30 minutes does not prevent the responses of contraction and relaxation to heart rate increase from appearing with characteristics equivalent to those of the healthy myocardium. This inference confirms the proposition that the functionality of the regulating mechanisms of calcium intracellular kinetics remains close to normality after ischemia/reperfusion myocardial depression.

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