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Dissociation between myocardial relaxation and diastolic stiffness in the stunned heart: its prevention by ischemic preconditioning

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Abstract

The effects of myocardial stunning and ischemic preconditioning on left-ventricular developed pressure and end-diastolic pressure (diastolic stiffness) as well as on coronary-perfusion pressure were examined in isolated isovolumic rabbit hearts. The isovolumic relaxation was evaluated, and the time constant of pressure decay during the isovolumic period was calculated. Our experimental protocol comprised: 1) myocardial stunning-global ischemia (15 min) followed by reperfusion (30 min); 2) myocardial stunning-global ischemia (20 min) followed by reperfusion (30 min); and 3) ischemic preconditioning – a single cycle of brief global ischemia and reperfusion (5 min each), before a second ischemic period, of 20-min duration. There was no effect upon systolic and diastolic parameters when 15 and 20 minutes of ischemia were evaluated. In both stunned groups the left ventricular developed pressure first recovered to near control values, but then stabilized at only 60% of the control values. Whereas the isovolumic relaxation time constant was increased after 5 min of reperfusion, and return to control values at late reperfusion, the end diastolic pressure remained elevated during the entire period. Values of dP/dV calculated at common pressure levels, were used as a second index of diastolic stiffness. They were increased after stunning, as also was the coronary perfusion pressure. When the heart was preconditioned with a single episode of ischemia, the systolic and diastolic alterations were completely abolished. We thus concluded that diastolic abnormalities incurred by myocardial stunning consist in both an increase in diastolic stiffness and an early impairment of isovolumic relaxation. The increase in stiffness cannot result from incomplete relaxation since these two parameters become temporally dissociated during the reperfusion period. (Mol Cell Biochem **129:** 171–178, 1993).

Key words: myocardial stunning, ischemic preconditioning, myocardial relaxation, diastolic stiffness

Introduction

In recent years there has been increasing interest in diastolic alterations of myocardial stunning [1–4]. However, they still remain largely undefined. Przyklenk *et al.* [4] showed in open chest dogs with regional ischemia, slower relaxation at 30 min of reperfusion; and Charlat *et al.* [2] in a chronic canine model also of regional ischemia reported prolonged abnormalities of diastolic wall thinning after a brief episode of ischemia. However, these studies did not account for changes in diastolic stiffness. Asimakis *et al.* [5] have shown in a model of global ische-

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mia an increase in diastolic stiffness during the reperfusion period. No observations of velocity of relaxation were carried out on this study. Thus, although diastolic alterations have been reported during myocardial stunning, they have not been well characterized in the course of time and they examined only partially the diastolic phase.

Recently, it has been shown that several brief ischemic periods separated by short periods of reflow (ischemic preconditioning (IPC)) actually reduce necrosis after a subsequent longer period of ischemia [6]. This phenomenon has been shown also to protect the heart against arrhythmias [7], pathognomonic ultrastructural changes accompanying ischemia [8], and to preserve creatine phosphate levels and intracellular pH during ischemia [9]. From these observations it seemed logical that IPC might protect against myocardial stunning (MS) as well [10, 11]. However, this concept still remains controversial [12].

In the present study we are going to show experimental evidence that myocardial relaxation and diastolic stiffness are altered during stunning, but become time dissociated in the course of the myocardial stunning. In addition, experimental evidence will be reported indicating that in our model of global ischemia, preconditioning can protect the heart not only from the systolic but also from the diastolic abnormalities occurring during myocardial stunning.

Material and methods

Isolated heart preparation

Adult rabbits (2 to 3 kg) were killed by a blow on the head. The thorax was rapidly opened and the heart excised. After removal of fat and connective tissue the heart was perfused with Ringer's solution (118 mM NaCl, 2 mM $CaCl_2$, 5.9 mM KCl, 1.2 mM $MgSO_4$, 20 mM $NaHCO_3$, and 11.1 mM dextrose), and equilibrated with 95% O_2 – 5% CO_2 (pH = 7.38, 37° C) by the non-recirculating Langendorff technique. A steady state was reached in 10 min and the preparation was kept stable for two hours. Care was taken not to use any preparation in which the hearts were ischemic for more than 60 sec, before perfusing with Ringer's solution. In our experience, longer periods of ischemia gave some protection to the hearts against postischemic dysfunction. Hearts were paced at 180 ± 9 beats/minute by atrial pacing, and were maintained at 37° C during the ischemic period by immersion in a water-jacketed beaker containing perfusate. A latex balloon tied to the end of a polyethylene tube was passed into the left ventricle through the mitral valve; the opposite end of the tube was then connected to a Statham P23xl pressure transducer. The fluid filled system for the evaluation of systolic and diastolic function using ventricular pressure in isolated hearts has been previously validated [13]. The balloon was filled with aqueous solution to an end diastolic pressure (LVEDP) of 8–12 mmHg, and then kept isovolumic throughout the experiment. Coronary perfusion pressure (CPP) was monitored at the cannulation point of the aorta. Left ventricular (LV) pressure and CPP were recorded with a direct writing recorder. After 10 min of stabilization, the coronary flow rate, controlled with a peristaltic pump, was 41 ± 1 ml/min and CPP equalled 80 ± 6 mmHg. By substracting LVEDP from the peak systolic pressure, the left ventricular developed pressure (LVDP) was obtained.

Indexes of diastolic function

a) Isovolumic relaxation

The time constant of relaxation (T) was calculated by using a monoexponential model with asymptotic offset:

$$P = P_0 \cdot e^{-t/T} + P_b$$

Where P is the left ventricular pressure measured during the isovolumic phase of relaxation from the peak of negative dP/dt to a pressure equal to end diastolic pressure + 5 mmHg. Pb is the asymptotic value, P_0 is the LV pressure at time t = 0 during relaxation, t is elapsed time during the isovolumic relaxation period, and T is the relaxation time constant.

b) Diastolic stiffness

Left ventricular diastolic stiffness curves were generated before and after myocardial stunning was induced. These curves were performed increasing the volume of saline in the balloon by 0.2 ml increments. The data from each curve were fitted to the form $P = A\, e^{K\,V}$, and the values of the volumes for a given LVEDP in each experiment obtained by interpolation. In each pressure-volume relationship values of dP/dV were calculated at common pressure levels [14]. Once the Starling curves were performed before the induction of stunning, the balloon was again filled to an LVEDP of 8–12 mmHg, and kept isovolumic throughout the experiment until

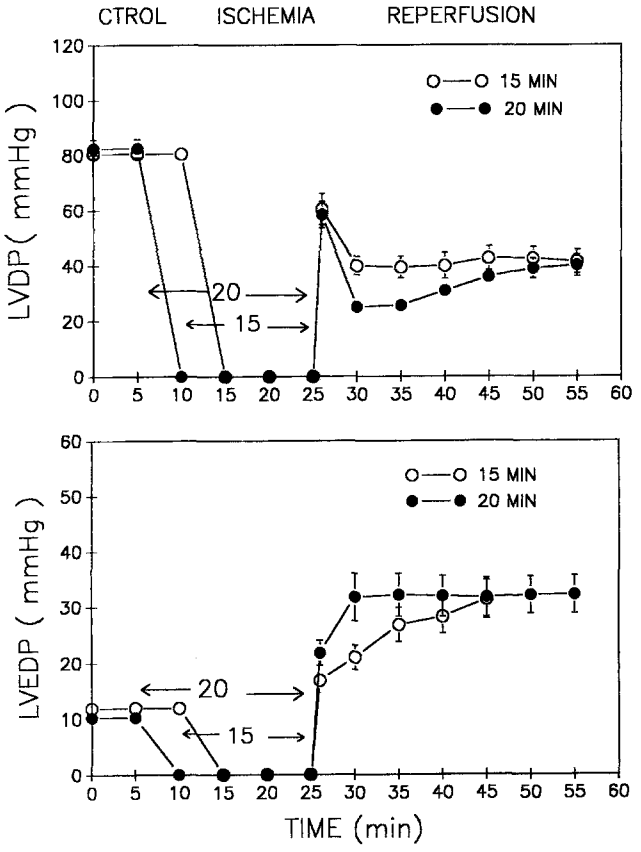

Fig. 1. Time course of left-ventricular developed pressure (upper panel) and left-ventricular end-diastolic pressure (lower panel), in the stunned group, after 15 and 20 min of ischemia, followed by 30 min of reperfusion.

post-stunning Starling determinations were obtained. Because our experimental model is an isovolumic preparation, LVEDP is also an index of ventricular diastolic stiffness [13].

Protocol

The experiments were divided into three groups. Postischemic dysfunction was induced by 15 min (group a) or 20 min (group b) of global ischemia followed by 30 min of reperfusion. IPC (group c) was induced by one cycle of 5-min global ischemia and 5-min reperfusion, before the 20-min of ischemia period. Only one period of 5-min ischemia has been expected to be enough to precondition the isolated rabbit heart [16]. Temperature was kept constant at 37° C during ischemia and reperfusion. The heart was allowed to stabilize for at least 10 min before the experiment was begun.

Results are given as mean ± standard error. Data were analyzed by means of the Student's t test or analysis of variance. A P value smaller than 0.05 was considered to be of significant difference.

Results

The effects of 15 and 20 min of global ischemia followed by 30 min of reperfusion were examined upon LVDP (upper panel) and LVEDP (lower panel) (Fig. 1). The absence of flow resulted in the rapid onset of contractile insufficiency. After 1.18 ± 0.18 min of reperfusion, contractile activity returned to 76 ± 6% of the preocclusion level in the group with 15 min of ischemia. However, LVDP quickly waned and stabilized at 58 ± 5% of preocclusion pressure at 19 ± 3 min of reperfusion in the same group. No difference was found on systolic and diastolic parameters when groups with 15 and 20 min of global ischemia were compared. Contractility then showed a biphasic behaviour during reperfusion: an initial phase showing a partial recovery toward control levels, followed by a decrease in contractile function to about 60% of the control values. This condition did not improve during the 30 min of reperfusion, suggesting that a stunned myocardium had been induced. Thus 15 and 20 min of global ischemia caused a comparable and severe postischemic mechanical dysfunction.

Figure 2 shows LVP and dP/dT digitized records from one typical experiment during control, and at 5 and 30 min of reperfusion, after 15 min of ischemia. In addition to the changes in systolic function, diastolic alter-

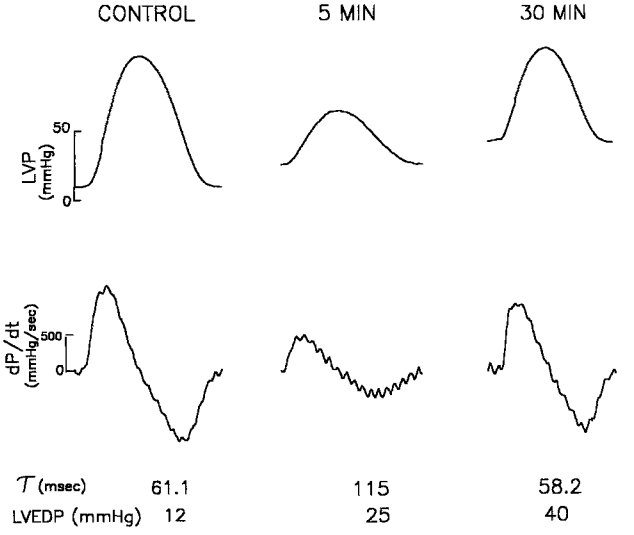

Fig. 2. Response of left-ventricular pressure of dP/dt to control, 5 min and 30 min of reperfusion in the stunned group in a representative experiment. The numbers at the bottom indicate the values of the time constant of relaxation (T) and LVEDP.

Table 1. Effect of myocardial stunning on dP/dV values (mmHg/ml) at common levels of pressure

Pressure	Control	Stunning
5 mmHg	22.5 ± 4.4	59.6 ± 8.9*
10 mmHg	27.1 ± 3.0	62.9 ± 8.0*
15 mmHg	31.5 ± 2.3	68.4 ± 7.8*
20 mmHg	36.0 ± 2.6	73.8 ± 8.3*

*: $P < 0.05$ compared to control.

Table 2. Values of LVEDP and CPP during myocardial stunning and ischemic preconditioning

	LVEDP		CPP	
	MS	IPC	MS	IPC
Control	11.9 ± 0.7	9.2 ± 1.7	72 ± 3	65 ± 7
5 min	21.0 ± 2.2*	10.1 ± 1.6	83 ± 4*	69 ± 4
30 min	38.0 ± 5.6*	12.3 ± 2.6	95 ± 7*	73 ± 8

Values are mean ± SEM; LVEDP: left ventricular end diastolic pressure, CPP: coronary perfusion pressure. *: $P < 0.05$ compared to control.

ations are here reflected in the abnormal LVEDP and T values. In spite of the normalization of T values after 30 min of reperfusion, the increase in LVEDP still persisted (Table 2).

Figure 3 shows the values for LVDP and LVEDP as a function of the time after the preconditioning of the heart by a single episode of 5 min of occlusion. During the reperfusion period, LVDP returned to control values, reflecting the expected protection of the heart from systolic dysfunction by IPC. Diastolic function, assessed here only by means of LVEDP, also returned to initial values thus providing evidence that IPC, provides protection not only for systolic, but also for diastolic function.

Figure 4 shows digitized LVP and dP/dT records from a representative experiment during control, and early and late reperfusion after 20 min of ischemia, preceded by a single episode of ischemia. The values of T indicate that not only systolic function and LVEDP (diastolic stiffness) but also myocardial relaxation were normal when the heart was preconditioned.

Figure 5 shows the values of T and LVEDP in control and 5 and 30 min of reperfusion, during myocardial stunning and ischemic preconditioning. T increases temporarily at 5 min of reperfusion in the stunning group (from 62.4 ± 5.6 to 126.4 ± 9.2 msec at 5 min of reperfusion) but returned to the preoclusion values during late reperfusion. When the heart was preconditioned, T did not show any significant change with respect to control. LVEDP, an index of diastolic stiffness in an isovolumic heart, increased during the reperfusion period, indicating that the diastolic distensibility of the ventricle was also altered in the stunned group, a finding in agreement with a previous report [17]. However, the increase in LVEDP was prevented by ischemic preconditioning.

Figure 6 shows the pressure-volume relationship in the stunned group, before and after stunning. Stunning was accompanied by a significant leftward shift of the relationship. dP/dV values compared at common pressure levels were also higher after stunning (Table 1). Accordingly, both parameters: dP/dV and LVEDP, showed an increase in diastolic stiffness after the induction of stunning.

Thus, three pieces of evidence: LVEDP at a given volume, the shift in the pressure-volume relationship, and the dP/dV values at common levels of pressure exhibit the increase in diastolic stiffness during stunning. On the other hand velocity of relaxation assessed by the time constant, T showed a transitory impairment followed by normalization. This time dissociation allows us to conclude that the increase in diastolic stiffness is not the consequence of an incomplete relaxation. IPC prevented both the systolic and the diastolic dysfunctions, and also prevented the increase in CPP detected during stunning.

Discussion

We have demonstrated in this study that in the isolated rabbit heart the diastolic alterations during global stunning vary depending on which phase of diastole has been

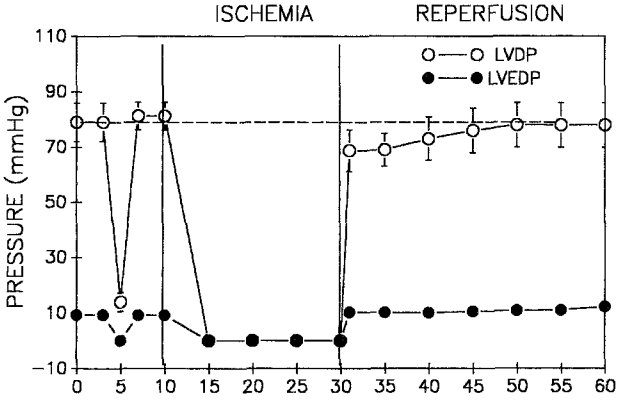

Fig. 3. Time course of left-ventricular developed pressure and left-ventricular end-diastolic pressure, in the preconditioned group. Twenty minutes of ischemia were preceeded by a single episode of 5 min of ischemia.

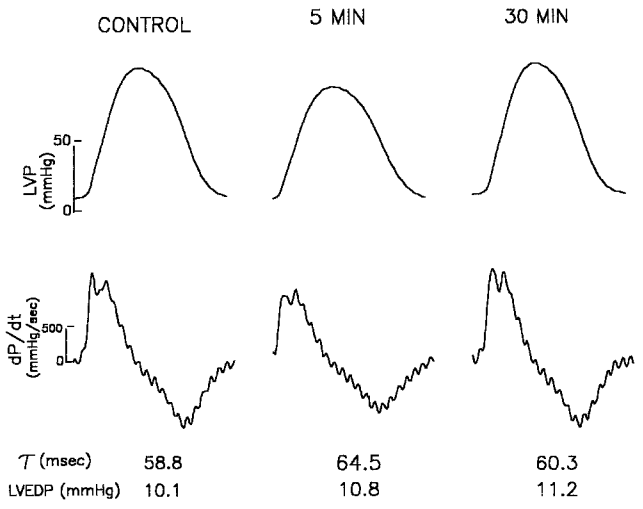

Fig. 4. Response of left-ventricular pressure and dP/dt to control, 5 min and 30 min of reperfusion in the preconditioned group in a representative experiment. The numbers at the bottom indicate the values of the time constant of relaxation (T) and LVEDP. Ischemic preconditioning prevented both systolic and diastolic alterations.

evaluated. If the early diastolic phase is analyzed, i.e., myocardial relaxation, we can see that the relaxation rate has a biphasic behaviour. The time constant increases after 5 min of reperfusion, but returns to baseline values in late reperfusion when stunning has been established. When diastolic stiffness is considered through an increase in LVEDP, it is observed that the myocardium during the reperfusion period is stiffer from the first minute of reperfusion and remains so during the entire reperfusion period. It must be kept in mind when comparing the results of the present to prior studies that: 1) a model of global, instead of regional ischemia was used; and 2) both early diastolic (relaxation) and late diastolic (stiffness) indices of left ventricular function were evaluated by us. The advantage of global ischemia over other models is that it is not necessary to consider changes in coronary collateral circulation, different contractile patterns due to ventricular asynchrony, or discrepancy between regional and global ventricular function after reperfusion [18, 19]. In our study the protocol of 15 min of global ischemia was selected after comparing the mechanical effects of twenty and fifteen minutes of global ischemia, in which no differences were found when developed and end diastolic pressures were compared. Periods of global ischemia of 15 [20] and 20 min [21] were previously used for the induction of myocardial stunning in isolated rabbit hearts with the assumption of no irreversible damage. A recent paper showed histopathologic evidence that 20 min of global ischemia in rats did not produce irreversible injury [22].

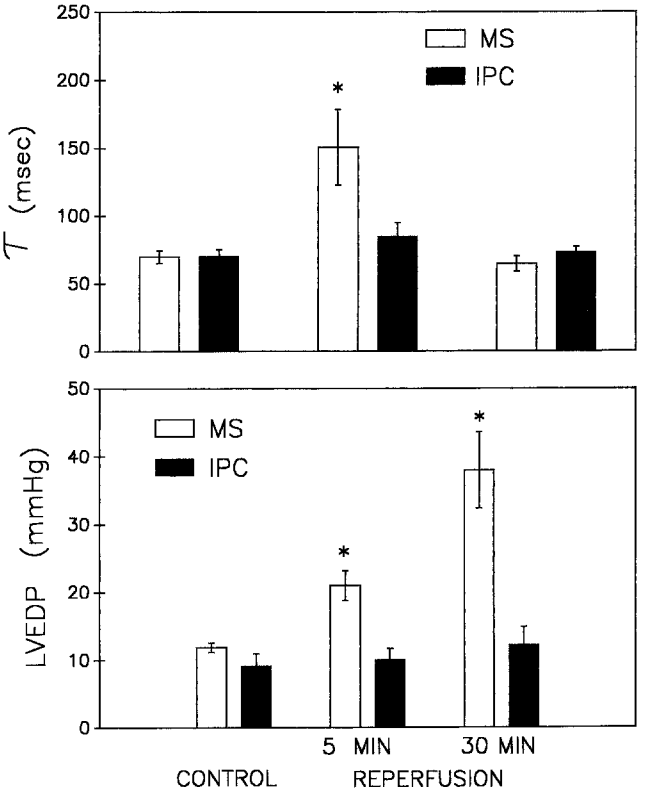

Fig. 5. Shows the values of the time constant of relaxation (T) (upper panel) and LVEDP (lower panel) in control and 5 and 30 min of reperfusion in both groups: stunned and preconditioned hearts. T increased at 5 min of reperfusion but returned to control values in the late reperfusion. *: $P < 0.05$.

In our study we can not rule out the possibility of small areas of necrotic tissue behave in such way that the abnormal contractile state may be a mixture of necrotic (irreversible injury) and stunned (reversible injury) myocardium. Although the fact that impaired relaxation is

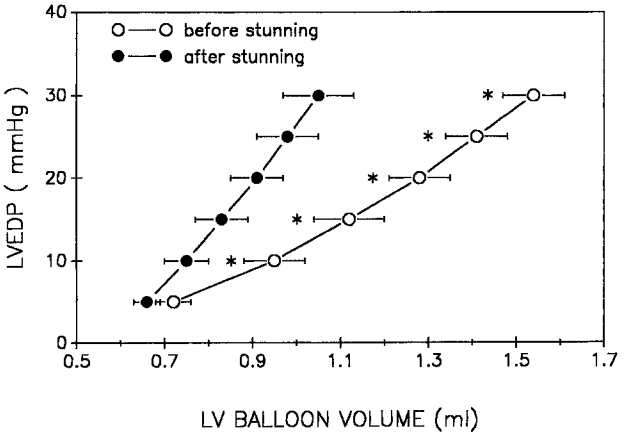

Fig. 6. Shows pressure-volume relationship before and after postischemic dysfunction. The increase in diastolic stiffness is reflected by the shift to the left of the relationship during postischemic dysfunction after 30 min of reperfusion. *: $P < 0.05$.

not always accompanied by increased stiffness is not a new observation [13], we are not aware of previous reports showing this dissociation between these two diastolic parameters during stunning.

Myocardial stunning appears to be the result of calcium overload and/or the formation of oxygen-derived free radicals *in situ* [23–26]. Experimental interventions aimed at decreasing calcium entrance [27, 28], however, yield only contradictory results [29, 30].

Heusch [31] refers to the paradox whereby cytosolic calcium may be increased during stunning, but contractility may be improved through various inotropic interventions that themselves act through an increase in cytosolic calcium. The ostensible contradiction in these observations was reconciled by Opie [30] in what he called the 'two-stage' model for the participation of calcium in reperfusion injury. His proposal is that a first period characterized by excessive cytosolic calcium, either alone or in conjunction with free radicals, damages the contractile machinery so that the myocytes enter into a second hypocontractile stage. This hypothesis is compatible with our results here reported in which an early impairment of relaxation occurred after reperfusion (at around 5 min after). Myocardial relaxation can become compromised when the calcium overload exceeds the functional capacity of the sarcoplasmic reticulum.

Diastolic stiffness, nevertheless, seems to increase during stunning in spite of the progressive improvement in relaxation. We can speculate that functional activity of the sarcoplasmic reticulum is affected after five minutes and then improves because either the calcium overload decreases or ATP becomes available. This improvement in SR activity however, is enough to normalize relaxation but not diastolic stiffness.

The role of sarcoplasmic-reticular function in inducing ischemic and postischemic mechanical alterations has been recently studied [17]. These results indicate the occurrence of a failure of calcium uptake by the sarcopasmic reticulum resulting from an increased ionic efflux through the release channels. This latter response seems to be an early manifestation of sarcoplasmic reticulum dysfunction during reperfusion, which abnormality becomes partially restored after reperfusion. On the basis of these reports, we would speculate that early in reperfusion two factors are contributing to an impaired relaxation: maximal calcium overload and poor sarcoplasmic reticulum reuptake. A partial recovery of sarcoplasmic reticulum function together with a decrease in the calcium overload could accordingly restore myocardial relaxation back to normal levels. An important consideration should be mentioned here with respect to the increase in diastolic stiffness: this change cannot result from an impairment in relaxation since, in our experiments, the normalization of relaxation was not accompanied by an improvement in stiffness.

Murry *et al.* [6] reported that brief episodes of ischemia can protect the heart against a sustained ischemic period ('IPC'). Recently the IPC phenomenon has also been shown to prevent arrhythmias [7] and ischemia [8]. However, at present it is not known whether IPC observed in different models of ischemic injury, different animal species, or based on different end points share the same mechanisms that account for the reduction in infarct size. In general we can say that IPC against lethal cell injury in large animal models with regional ischemia has been clearly demonstrated [6]; and against arrhythmias has been described only in rats [7]. Recently, it has been reported that IPC may attenuate both postischemic systolic dysfunction and the increase in LVEDP in rats with global ischemia [5], or in rabbits with regional ischemia [11], but it failed to protect the heart of large animals [12].

In our study IPC completely abolished the postischemic alterations upon ventricular function, including not only systolic but also diastolic impairment. In addition to the prevention of increased diastolic stiffness, IPC also prevented impairment of the isovolumic relaxation. While Asimakis *et al.* [5] reported an attenuation of the postischemic dysfunction with IPC, we completely abolished both the systolic and diastolic alterations. However, there are two major differences between the present and Asimakis' study. The first difference relates to animal specie (rats vs. rabbits), a second difference relates to the results. While they found an increase in LVEDP during the ischemic period, in our experiments LVEDP did not change when the coronary flow was stopped. It has been previously demonstrated that ischemic contracture may play a role in the development of vascular damage during ischemia and reperfusion [32]. If this is the case, the absence of increased LVEDP in our study may have contributed to the complete recovery of both systolic and diastolic dysfunction. The analysis of the mechanisms involved in this protection could help in elucidating the mechanism involved in the diastolic alterations. It does, however, seem that through activation of its A_1 receptors in cardiac muscle, adenosine might be mechanically involved in the protection conferred by IPC: indeed the A_1 receptor [16] seems to be coupled with a Gi protein; and this Gi protein, in turn can act to open up ATP-activated K channels,

thereby inducing hyperpolarization and decreasing calcium influx through L channels during reperfusion. Such a mechanism would both prevent excessive early calcium overload and forestall the triggering of processes leading to cellular damage. Nevertheless, a decrease in calcium influx through L channels could still alleviate such a calcium overload, and ameliorate the mechanical changes. Such an effect has, in fact, been recently reported [29].

Finally, we can not disregard the fact that most of these diastolic alterations were induced during the ischemia rather than during the reperfusion period. There are several mechanisms that have been postulated to explain the diastolic alterations during ischemia, including altered elastic properties [33] of the myocardium and changes in coronary vessels due to the non-reflow phenomenon [34].

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