Continuous fluorimetric assessment of the changes in cytoplasmic calcium concentration during exposure of rat isolated myocardium to conditions of simulated ischaemia

B.J. Northover

Department of Pharmacology, School of Pharmacy, Leicester Polytechnic, Leicester LE1 9BH

- 1 The cytoplasmic calcium concentration ($[Ca]_c$) of rat isolated atrial myocardium was assessed with the dye indo-1. Dye-loaded atria were superfused with physiological salt solution and excited with radiation at 360 nm, while epifluorescence emissions were collected simultaneously at 400 nm and 500 nm. The ratio of these emissions was used as a measure of $[Ca]_c$.
- 2 Dye-loaded atria showed a phasic rise and fall in $[Ca]_c$ with each applied electrical pacing stimulus. The amplitudes of systolic increments in tension and $[Ca]_c$ were augmented by the presence of isoprenaline.
- 3 Atria superfused with a solution the composition of which resembled that found extracellularly in regions of ischaemia rapidly lost systolic increments in tension and $[Ca]_c$, while end-diastolic $[Ca]_c$ and tension gradually rose.
- 4 The presence of lactate (20 mM) or flufenamate (5 μ M) in the superfusate during simulated ischaemia aggravated the rises in both end-diastolic tension and end-diastolic $[Ca]_c$. Inclusion in the superfusate of sulphapyrazole (50 μ M) or glucose (20 mM) protected against some of the deleterious effects of lactate seen during simulated ischaemia.

Introduction

A raised cytoplasmic concentration ($[Ca]_c$) has been demonstrated during periods of depletion of the myocardial stores of adenosine 5'-triphosphate (ATP), and this occurs during ischaemia, for example (Barry *et al.*, 1987; Lee *et al.*, 1987; 1988; Eisner *et al.*, 1989; Kohmoto & Barry, 1989; Morris *et al.*, 1989). These observations have been made with non-toxic fluorescent dyes, such as indo-1, that are sensitive to even the very low levels of $[Ca]_c$ that normally prevail during diastole. Indo-1 may be loaded into cardiac myocytes via an intact sarcolemma; but in order to take full advantage of the spectral properties of this dye, fluorescence needs to be measured at 2 emission wavelengths simultaneously. In that way a rise and a subsequent fall in $[Ca]_c$ may be demonstrated with each heart beat (Barry *et al.*, 1987; Lee *et al.*, 1987; 1988; Stern *et al.*, 1988; Eisner *et al.*, 1989; Kohmoto & Barry, 1989; Morris *et al.*, 1989).

The present experiments have used isolated atrial myocardium from the rat, superfused with a solution the composition of which resembles that found extracellularly in regions of ischaemia. This has permitted some previously described functional and biochemical abnormalities that occur during this type of injury (Northover, 1987; 1989; Northover & Northover, 1988) to be correlated with contemporary changes in $[Ca]_c$ measured with indo-1. It has also permitted a re-examination of the effects of drugs that were shown previously to be protective in this model of cardiac injury.

Methods

Male rats of the Sprague-Dawley strain weighing 340–480 g were killed by a blow to the head. The heart was excised quickly, the atria separated from the ventricles and the right atrium sutured via its epicardial surface to a flat rectangular plastic holder (11 mm $\times$ 50 mm) containing a pair of platinum wires that were insulated, except where the tips made contact with the epicardium. The atrium was stimulated throughout an experiment at 4 Hz with 2 ms square wave pulses, each of 10 V. Atria were bathed, unless otherwise stated, with normal superfusate (NS) of the following composition (mM): NaCl

138, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, NaH_2PO_4 0.5, $NaHCO_3$ 10, glucose 10 and gassed with 95% O_2 plus 5% CO_2 . A thread sutured to the left atrial appendage was connected to a force-displacement transducer (type SB-IT, Nihon Khoden) at a diastolic tension of 100 mg. Tension records were made on moving paper via a d.c. amplifier (type 3552, Cardiovascular Instruments) coupled to a heated stylus recorder (type 5041, LECTROMED). Atria were allowed to equilibrate in NS for 1 h before dye-loading.

Dye-loading

A 50 μ l aliquot of a 1 mM stock solution of the acetoxymethyl ester of indo-1 (Sigma) in dimethyl sulphoxide was first mixed with 0.2 ml of a 0.3% solution in dimethyl sulphoxide of the non-ionic detergent Pluronic F-127 (a polyoxyethylene/polyoxypropylene co-polymer, supplied by Mazer Chemicals). This mixture was then shaken with 10 ml of NS to give a 5 μ M dye solution. Atria were incubated for 1 h at 34°C in this dye solution. Ester solubility in an aqueous medium, and hence the ease of ester penetration of the sarcolemma and subsequent hydrolysis to indo-1 within the cell, have been shown to be increased in the presence of Pluronic (Poenie *et al.*, 1986; Schackman & Chock, 1986; Himpens & Somlyo, 1988; Owen, 1988).

Fluorescence

Dye-loaded atria, on the plastic holder, were positioned diagonally across a standard 1 cm square-section quartz cuvette and thermostated at 34°C in a specially designed fluorimeter equipped with one excitation monochromator and with two emission monochromators, each with a photon-counting photomultiplier tube (Alphascan System, Photon Technology International). Epifluorescence emissions were collected digitally at 60 data points s^{-1} from the front face and one side face of the cuvette. Fresh superfusate, bubbled continuously with 95% O_2 plus 5% CO_2 in an external reservoir, was pumped at 15 ml min^{-1} to the bottom of the cuvette via narrow-bore tubing. Superfusate was sucked off from the top of the cuvette. Inflow and outflow tubes and the wire electrodes were kept out of the light paths used by the fluorimeter.

Excitation was at 360 nm, and the two emission monochromators were set at 400 nm and 500 nm respectively. Grynkiewicz *et al.* (1985) showed that the intensity of fluorescence (F) emitted by indo-1 is increased by calcium in a concentration-dependent manner at 400 nm, but diminished at 500 nm, and that the ratio of emission intensities at these two wavelengths (F400/500) provides a measure of the free calcium concentration. Before dye-loading, the combined tissue and instrumental autofluorescence signals were recorded and filed in computer memory, and then routinely deducted from signals obtained later with atria in the dye-loaded state. However, tissue autofluorescence needed to be recorded during each experimental intervention, as it varied under different conditions of superfusion. Autofluorescence-corrected signals from dye-loaded atria were typically about 5 fold greater than tissue autofluorescence intensity.

Simulated ischaemia

In order to simulate ischaemic conditions the NS was replaced by a superfusate of abnormal composition (Ferrier *et al.*, 1985), containing KCl 7 mM, racemic sodium lactate 20 mM and no glucose. The pH was reduced to 6.4 by reducing the NaHCO₃ to 4 mM. Hypoxia was produced by replacing O₂ by N₂ (containing 0.0002% or less of O₂, British Oxygen Company).

Drugs

Sulphinpyrazone and flufenamate were gifts from Ciba-Geigy and Parke Davis & Company respectively. Both were dissolved in water with a small excess of Na₂CO₃ and the solution adjusted to neutrality with acetic acid.

Results

Effects of isoprenaline

Dye-loaded atria in NS displayed a rise in the F400 signal and a simultaneous fall in the F500 signal during each systole, causing marked positive changes to occur in the calculated F400/500 ratio, as shown in Figure 1.

Isoprenaline (1 μ M) caused positive inotropism when added to atria bathed with NS. Systolic developed tension became maximal within the first 2 min, then faded somewhat before reaching a plateau (Figure 2). Isoprenaline increased the rate of both systolic tension development and subsequent relaxation. The peak F400/500 ratio reached during systole and the rate of diastolic decline in this ratio were also augmented by isoprenaline, and both became maximal soon after starting exposure to this agent. End-diastolic values of both tension and the F400/500 ratio were unaltered by isoprenaline (Figure 2).

Exposure to simulated ischaemia

Atria that were exposed to conditions of simulated ischaemia (SI) showed a decline in systolic developed tension within 2 min, and after 6 min systolic developed tension was no longer detectable (Figure 3). Despite this, the systolic increments in the F400/500 ratio after 6 min of exposure to SI averaged 76% of the value in NS (Figure 3). Over the next few min, however, atrial contracture developed, accompanied by a slow rise in the end-diastolic F400/500 ratio, together with further diminution in the systolic increments in the F400/500 ratio (Figure 3). By the end of 14 min of exposure to SI the peak systolic and end-diastolic values of the F400/500 ratio were no longer different from each other. By the end of 20 min both had become similar to peak systolic values previously observed in NS (Figure 3), and by the end of 30 min both had become significantly higher than peak systolic values previously observed in NS.

Atria that were exposed to conditions of lactate-free SI, like those exposed to full lactate-containing SI, lost all systolic developed tension within the first 6 min (Figure 4). However,

Figure 1 Changes in tension (a), in intensity of fluorescence emitted by indo-1 at 400 nm (F400) and 500 nm (F500) (b) and in the F400/500 ratio (c) during the same 7 consecutive systolic-diastolic cycles from a representative dye-loaded atrium while in normal superfusate and stimulated at the points marked in panel (a) and at the arrows in panels (b) and (c).

Figure 2 Mean tension (a) and contemporary mean ratios of fluorescence emissions at 400 nm and 500 nm (F400/500) (b) of 20 systolic-diastolic cycles from each of 10 dye-loaded atria exposed to normal superfusate which also contained isoprenaline (1 μ M) from time zero onwards. The upper edges of each panel represent peak systolic values and the lower edges end-diastolic values. Vertical bars represent s.e.mean.

Figure 3 Mean tension (a) and contemporary mean ratios of fluorescence emissions at 400 nm and 500 nm ($F_{400/500}$) (b) of 20 systolic-diastolic cycles from each of 10 dye-loaded atria exposed to normal superfusate until time zero and to conditions of simulated ischaemia (with 20 mM lactate) thereafter. The upper edges of each panel represent peak systolic values and the lower edges end-diastolic values. Vertical bars represent s.e.mean.

subsequent contracture development during lactate-free SI was milder than during lactate-containing SI. During exposure to the former condition, in contrast to the latter, the systolic increments in the $F_{400/500}$ ratio were preserved, even after contracture had become established (Figure 4). End-diastolic values of the $F_{400/500}$ ratio rose significantly during exposure to lactate-free SI (Figure 4), but the rise was significantly less than that seen during lactate-containing SI (Table 1).

Glucose was absent during conditions of full SI. Addition of glucose (20 mM) to the superfusate during SI protected atria against contracture development, almost abolished the accom-

Figure 4 Mean tension (a) and contemporary mean ratios of fluorescence emissions at 400 nm and 500 nm ($F_{400/500}$) (b) of 20 systolic-diastolic cycles from each of 10 dye-loaded atria exposed to normal superfusate until time zero and thereafter to lactate-free simulated ischaemia. The upper edges of each panel represent peak systolic values and the lower edges end-diastolic values. Vertical bars represent s.e.mean.

Figure 5 Mean tension (a) and contemporary mean ratios of fluorescence emissions at 400 nm and 500 nm ($F_{400/500}$) (b) of 20 systolic-diastolic cycles from each of 10 dye-loaded atria exposed to normal superfusate until time zero and thereafter to simulated ischaemia with glucose (20 mM) added. The upper edges of each panel represent peak systolic values and the lower edges end-diastolic values. Vertical bars represent s.e.mean.

panying rise in end-diastolic $F_{400/500}$ ratio, but did not protect against the diminution in systolic developed tension that was seen during SI (Figure 5 and Table 1). Moreover, the presence of this concentration of glucose only slightly delayed the disappearance of the systolic increments in the $F_{400/500}$ ratio usually produced during the first 14 min of SI. This can be seen by comparing the $F_{400/500}$ values 10–18 min after starting SI in Figures 3 and 5.

Effects of sulphinyprazole and flufenamate

Addition of sulphinyprazole (50 μ M) to the superfusate protected atria against contracture development during SI (Table 1). There was protection also against the SI-induced rise in the end-diastolic $F_{400/500}$ ratio (Table 1), although not against the early loss of systolic increments in atrial tension, and to only a slight extent against the loss of systolic increments in the $F_{400/500}$ ratio (Table 1). Sulphonyprazole failed to protect against any of the mechanical or optical changes induced by exposure to lactate-free SI (Table 1). Pretreatment of atria with flufenamate (5 μ M) aggravated the development of contracture and the associated rise in the end-diastolic $F_{400/500}$ ratio during exposure of SI. These effects of flufenamate were displayed equally prominently during lactate-free and lactate-containing SI (Table 1). Addition of sulphinyprazole or flufenamate to NS failed to modify systolic developed tension, end-diastolic tension, or the $F_{400/500}$ ratio measured at the peak of systole or at the end of diastole.

Discussion

The present work has revealed that atrial muscle loaded with indo-1 and superfused with NS displays a phasic rise and fall in the $F_{400/500}$ ratio in response to each applied electrical stimulus. Previous workers have obtained similar findings

Table 1 Effects of different superfusates on the mechanical and optical responses of dye-loaded atria

	Glucose (mM)	Superfusate		Drug (μ M)	Tension (mg)		$F_{400/500}$ ratio	
		Lactate (mM)			End-diastolic	Systolic increment	End-diastolic	Systolic increment
NS	10	0	—	—	102 $\pm$ 11**	218 $\pm$ 29*	1.030 $\pm$ 0.012**	0.104 $\pm$ 0.015**
SI	0	20	—	—	386 $\pm$ 40*	12 $\pm$ 17**	1.097 $\pm$ 0.016*	0.013 $\pm$ 0.018*
SI	0	0	—	—	204 $\pm$ 35**†	15 $\pm$ 18**	1.057 $\pm$ 0.013**†	0.085 $\pm$ 0.020**†
SI	20	20	—	—	115 $\pm$ 18**	-1 $\pm$ 12**	1.036 $\pm$ 0.014**	0.025 $\pm$ 0.013
SI	0	20	Sulphonyprazole (50)	—	238 $\pm$ 30**	2 $\pm$ 8**	1.048 $\pm$ 0.017**	0.054 $\pm$ 0.012**
SI	0	20	Flufenamate (5)	—	465 $\pm$ 46**	-3 $\pm$ 5**	1.131 $\pm$ 0.015**	-0.003 $\pm$ 0.019
SI	0	0	Sulphonyprazole (50)	—	227 $\pm$ 25**	16 $\pm$ 14**	1.042 $\pm$ 0.012	0.051 $\pm$ 0.014
SI	0	0	Flufenamate (5)	—	449 $\pm$ 51†	11 $\pm$ 20**	1.132 $\pm$ 0.018†	0.008 $\pm$ 0.011†

Tabulated values represent means $\pm$ s.e.mean of 20 systolic-diastolic cycles from each of 10 atria after exposure to the stated conditions for between 14 and 15 min. A significant difference exists ($P < 0.05$) between a value marked * and corresponding values marked **, and also between members of a corresponding pair of values marked †. NS: normal superfusate; SI: conditions of simulated ischaemia.

with both intact ventricular myocardium and isolated ventricular myocytes, and have concluded that this optical phenomenon represents the systolic rise and diastolic fall in $[Ca]_i$, upon which contraction and relaxation depend (Dubell & Houser, 1987a; Lee *et al.*, 1987; 1988). Both the systolic rise in the F400 signal and simultaneous fall in the F500 signal also reflect the rise in $[Ca]_i$. However, calculated changes in the ratio of emission intensities at 400 nm and 500 nm respectively were larger than the changes observed in either the F400 or the F500 signal separately, and the ratio has been shown to be less subject to interference and motion artefact (Lee *et al.*, 1987; 1988; Eisner *et al.*, 1989). For these reasons the F400/500 ratio, rather than its components was used routinely in the present work. Nevertheless, when changes in this ratio resulted from an experimental intervention, concomitant, albeit smaller, changes were detected in both the F400 and F500 signals. Grynkiewicz *et al.* (1985) showed that aqueous solutions of indo-1 gave F400/500 ratios that accurately reflected the ambient free calcium concentration. In the myoplasm, however, the likely presence of residual unhydrolysed indo-1 ester (Dubell & Houser, 1987b; Lee *et al.*, 1987; 1988; Peeters *et al.*, 1987; Tamura *et al.*, 1989), plus the probable binding of some of the hydrolysed indo-1 to structural and other cytoplasmic components (Owen & Shuler, 1989), make the calculation of absolute $[Ca]_i$ values from the F400/500 ratios rather questionable (Dubell & Houser, 1987b; Cork *et al.*, 1989; Tamura *et al.*, 1989). Nevertheless, changes that appear in the F400/500 ratio of indo-1 loaded myocardium are probably directly proportional to the accompanying changes in $[Ca]_i$ (Barry *et al.*, 1987; Dubell & Houser, 1987a; Lee *et al.*, 1987; 1988). Actual F400/500 ratios rather than derived $[Ca]_i$ values are presented in this study. However, it seems safe to assume that changes in the former faithfully monitored any changes that occurred in the latter.

Isoprenaline produced a positive inotropic response when applied to atria superfused with NS in the present experiment. Fading of the early inotropic response, despite the continued presence of isoprenaline, was paralleled by a similar change in systolic increments in the F400/500 ratio (Figure 2). This suggests that fading of the response was likely to have been due, in part at least, to diminishing systolic rises in $[Ca]_i$. Previous workers have demonstrated that fading of the inotropic response to isoprenaline was associated with diminishing sensitivity of the contractile proteins to $[Ca]_i$ (Ray & England, 1976; Endoh & Blinks, 1988), but the extent to which this latter process operated in the present experiments is unknown.

Exposure of the rat atrium to conditions of SI previously was shown to elicit a wide range of mechanical, electrical, biochemical and structural abnormalities (Northover, 1987; 1989; Northover & Northover, 1988). The tension changes shown in Figure 3 confirm these earlier findings and permit comparisons to be made with contemporary changes in $[Ca]_i$. Clearly, the major loss of systolic developed tension preceded comparable diminution in the systolic increments in $[Ca]_i$. This suggests that early loss of systolic developed tension during SI is attributable, in the main, to reduced sensitivity of the contractile proteins to $[Ca]_i$. Others have reached similar conclusions using other myocardial preparations (Allen & Orchard, 1983; Allen *et al.*, 1989), but doubts remain about which of the changes in the chemical composition of the myoplasm during SI is responsible for this loss of sensitivity to $[Ca]_i$ (Godt & Nosek, 1989). Stern *et al.* (1988), on the other hand, concluded that failure of systolic shortening during SI is accounted for mainly by a loss of the systolic increments in $[Ca]_i$. The latter workers, however, used single isolated cardiomyocytes at zero tension, which may explain these atypical findings.

Exposure to SI in the absence of lactate was associated with a loss of systolic developed tension similar to that seen in the presence of lactate (Figures 3 and 4). During the former, as opposed to the latter condition, little diminution in the systolic increments in the F400/500 ratio occurred. This is further

evidence that the loss of systolic developed tension during SI primarily reflects an insensitivity of the contractile proteins to $[Ca]_i$. Why the presence of lactate during SI was necessary for the loss of systolic increments in $[Ca]_i$ is not known. Depletion of the myocardial store of ATP is known to be less severe when rat atria are subjected to SI in the absence rather than the presence of lactate, and this difference has been attributed to the ability of lactate to inhibit glycolysis (Northover, 1989). The ATP derivable from glycolysis in the absence of added lactate, therefore, may have been sufficient to preserve systolic rises in $[Ca]_i$ under anaerobic conditions. Allen & Orchard (1983) also noted that small amounts of ATP derived from glycolysis were able to preserve systolic rises in $[Ca]_i$ under conditions of hypoxia, or under the influence of respiratory poisons such as cyanide, even when systolic increments in myocardial tension had already failed.

The importance of a rising end-diastolic $[Ca]_i$ value in the production of an ischaemic contracture is still debated. Clearly, however, the technique used to study $[Ca]_i$ needs to be sensitive to Ca at normal end-diastolic levels. This may not have been true with some aequorin luminescence measurements, and is in contrast to measurements based upon indo-1, as discussed by Eisner *et al.* (1989).

Both end-diastolic tension and end-diastolic $[Ca]_i$ gradually rose during exposure to SI in the present experiments, confirming previous findings based upon other models of ATP-depletion (Barry *et al.*, 1987; Eisner *et al.*, 1989; Kohmoto & Barry, 1989; Morris *et al.*, 1989). Both rises had a similar time course, suggesting a causative connection between them. This was reinforced by the fact that during lactate-free SI, during glucose-containing SI, as well as during SI conducted in the presence of sulphinyprazole, the rises in end-diastolic tension and the F400/500 ratio were less than those seen during the usual conditions of glucose-free SI in the presence of lactate. The presence of glucose, however, only slightly delayed the SI-induced decline in the systolic increments in the F400/500 ratio, and this may be seen by comparing Figures 3 and 5. This finding was unexpected since previous work showed that the presence of glucose (20 mM) almost fully protects atria against the depletion of ATP normally found at the end of 15 min of SI (Northover, 1989). Systolic increments of $[Ca]_i$ may decline during some types of SI, therefore, even without depletion of the ATP stores. During hypoxia the free energy change resulting from the hydrolysis of ATP has been calculated to decline due to the accumulation of catabolites of ATP (Kammermeier *et al.*, 1982; Allen & Orchard, 1983). Kentish & Allen (1986) were unable to account for the extent of the decline in systolic developed tension during ischaemia solely in terms of the decrement in the calculated free energy change of ATP hydrolysis. Under certain circumstances, however, it is possible that even normal stores of ATP may be insufficient to preserve normal systolic rises and falls in $[Ca]_i$. Sulphonyprazole is another agent that is known to protect atria against ATP-depletion during the first 15 min of SI (Northover, 1989). Sulphonyprazole, like glucose, only slightly delayed the SI-induced reduction in systolic increments in the F400/500 ratio in the present experiments (Table 1). Stern *et al.* (1988) suggested that the failure of systolic increments in $[Ca]_i$ during SI was due to shortening of action potential duration. Atrial action potentials certainly shorten during the first 15 min of SI (Northover, 1987). Since sulphonyprazole has been shown to protect against this shortening (Northover, 1987) but failed to protect significantly against the loss of the systolic increments in $[Ca]_i$, seen after 15 min of SI in the present experiments, the loss of the systolic increments in $[Ca]_i$ would seem to depend mainly upon factors other than action potential shortening, at least in rat atria.

Previous work with this model of myocardial injury has shown that the presence of lactate during SI enhances the observed shortening of atrial refractory periods and slowing of conduction velocity (Northover, 1989). The fact that the presence of lactate during SI enhanced the rise in end-diastolic $[Ca]_i$ in the present experiments is consistent with a raised

[Ca]_i being responsible for these electrical disturbances. This suggestion gains additional support from the fact that the presence of glucose during SI protected the myocardium not only against the rise in end-diastolic [Ca]_i in the present experiments (Table 1), but also against the shortening of atrial refractory periods and the slowing of conduction velocity in previous work (Northover, 1989). Possible mechanisms whereby the changes in [Ca]_i that are seen during SI may provoke these electrical disturbances have been discussed previously (Northover, 1987; 1989; Northover & Northover, 1988).

The ability of sulphinpyrazone in the present experiments to protect atria against the SI-induced rises in both end-diastolic tension and end-diastolic [Ca]_i constitutes further evidence that the rising end-diastolic [Ca]_i contributes to contracture development. This is reinforced by the fact that under conditions of lactate-free SI sulphinpyrazone no longer was able to prevent either of these changes, whereas flufenamate was still able to potentiate both changes. The protective effects of sulphinpyrazone and the deleterious effects of flufenamate previously were shown to include the SI-induced shortening of atrial refractory periods and slowing of conduction velocity (Northover, 1987; 1989; Northover & Northover, 1988). This earlier work indicated that sulphinpyrazone protects the ischaemic myocardium by preventing the inhibition of anaerobic glycolysis by lactate. Anaerobic ATP production from endogenous stores of glycogen effectively slows the decline in ATP at the start of ischaemia. Glycolytic ATP

production fails, however, if lactate is plentiful. Sulphinpyrazone under these circumstances can preserve this route of ATP production. Flufenamate, on the other hand, aggravates the decline in ATP stores during ischaemia by disrupting the net synthesis of ATP. The fact that sulphinpyrazone in the present experiments protected against the SI-induced rise in end-diastolic [Ca]_i, and that flufenamate had an opposite effect, is consistent with the rising end-diastolic [Ca]_i during SI being responsible for the electrical disturbances noted in previous studies.

In conclusion therefore, the present experiments provide evidence that during SI the early losses of atrial systolic tension development do not reflect the contemporary changes in [Ca]_i, whereas the more slowly developing contracture reflects the progressive rise in end-diastolic [Ca]_i. The alterations in end-diastolic [Ca]_i show a time-course that also suggests that they are responsible, in part at least, for the accompanying slowing of action potential conduction velocity and shortening of refractory periods. Sulphinpyrazone has been shown to protect against all of the changes produced by SI with the exception of the early losses of systolic developed tension. Evidence both from this and previous studies suggests the ability of sulphinpyrazone to protect against the disturbances of calcium metabolism during SI is partly explained by an ability to protect against early ATP depletion.

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