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Protection of rat atrial myocardium against electrical, mechanical and structural aspects of injury caused by exposure *in vitro* to conditions of simulated ischaemia

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1 Rat isolated and superfused atria were exposed for varying periods to a solution simulating the composition of extracellular fluid during myocardial ischaemia (SI).

2 Atria subjected to SI showed a loss of systolic contractile tension, a rise in diastolic tension, a shortening of electrical refractory periods, a slowing of action potential conduction velocity and disruption of the mitochondrial ultrastructure. All these features were reversible when the muscle was returned to normal superfusate.

3 Atria pretreated with a superfusate containing a calcium channel antagonist, a calmodulin inhibitor or an intracellular calcium antagonist showed fewer features of the response to SI than did controls.

4 Atria pretreated with a superfusate containing various non-steroidal anti-inflammatory agents did not show identical responses to SI. Sulphinpyrazone protected against all features of the response to SI but ibuprofen, flurbiprofen and GP25671 (a metabolite of sulphinpyrazone) had little effect. Flufenamate, phenylbutazone and salicylate enhanced the responses to SI.

Introduction

Simulated ischaemia (SI) is said to exist when a tissue is exposed to a solution whose composition resembles that found in regions of ischaemia. In SI of cardiac muscle *in vitro*, conduction velocity (CV) of action potentials is reduced, as are both action potential duration and refractory periods (Ferrier *et al.*, 1985; Northover, 1987). These changes probably constitute the electrical basis for at least some of the disturbances of rhythm that characterize myocardial ischaemia *in vivo* (Northover, 1986). Pretreatment with sulphinpyrazone protects the myocardium against these electrical changes (Northover, 1987). It was of interest to discover whether sulphinpyrazone also protects the myocardium against the structural and mechanical changes produced by SI and whether other non-steroidal anti-inflammatory agents share these properties. In view of the association between calcium overload and myocardial injury (Hearse *et al.*, 1977; Wojtczak, 1979; Koomen *et al.*, 1983; Cavero *et al.*, 1983; Fitzpatrick & Karmazyn, 1984; Karmazyn, 1986), the association between calcium ions and SI was also investigated.

Methods

Rats of the Sprague-Dawley strain weighing 340–480 g were killed by a blow to the head. The heart was removed quickly, the atria separated from the ventricles and the former attached with their endocardial surface upwards to the base of a superfusion trough maintained at 34°C. The muscle was exposed, unless specified otherwise, to a normal superfusate (NS) of the following composition (mM): NaCl 138, KCl 4.0, $CaCl_2$ 2.0, $MgCl_2$ 1.0, NaH_2PO_4 0.5, $NaHCO_3$ 10 and glucose 10, and gassed with a mixture of 95% O_2 plus 5% CO_2, giving a pH of 7.3. Atria were stimulated throughout the experiments at 4 Hz via a pair of platinum wire electrodes

placed on the right atrial appendage. Square wave pulses of current, each 2 ms in duration and isolated from earth, were used at a voltage of twice the prevailing diastolic threshold.

Electrical changes

Transmembrane potentials were recorded from subendocardial muscle fibres by means of glass microelectrodes filled with a 3 M solution of KCl and having resistances of $1-2 \times 10^7$ ohms. One microelectrode was inserted intracellularly in the right atrium. Voltages detected by this electrode were passed by a single-ended high input-impedance coupler with facilities for capacitance neutralisation (type 8124, C.F. Palmer) to both an oscilloscope and a transient store microprocessor (type 140, Bioscience). Stored signals were able to be replayed from the latter device at speeds of up to 2000 fold slower than those at which they were recorded. The effective refractory period (ERP) of the right atrial muscle was determined by use of paired stimuli, and was taken as the interval between the closest pair of stimuli both of which yielded action potentials. The interval separating the upstrokes of the pair of action potentials so obtained was taken as the functional refractory period (FRP). The CV of action potentials between right and left atria was measured with the aid of a second microelectrode inserted intracellularly in the left atrium. Voltage signals from both microelectrodes were displayed on a dual channel oscilloscope. Knowing the distance between the tips of the 2 microelectrodes and the time interval between the upstrokes of the action potentials recorded from them, it was possible to calculate the apparent CV. Since action potentials may not have been conducted via the most direct route between the 2 electrodes, however, the CV may have been underestimated.

Mechanical changes

For these experiments the right atrial appendage only was anchored to the base of the superfusion trough. A braided nylon thread was sutured to the left atrial appendage and the other end of the thread connected to a force-displacement transducer (type SB-IT, Nihon Khoden) at a diastolic tension of 100 mg. Tension records were made on a slowly moving paper via a d.c. amplifier (type 3552, Cardiovascular Instruments) coupled to a heated stylus recorder (type 5041, Lectromed).

Structural changes

At the end of a period of experimental treatment *in vitro* the right atrium was fixed for 3 h at 4°C in a 3% solution of glutaraldehyde in potassium oxalate buffer (90 mM, pH 7.4) and post-fixed at 4°C for 3 h in a 1% solution of osmium tetroxide in potassium acetate buffer (0.1 M, pH 7.4). Specimens were then dehydrated through graded concentrations of ethanol and embedded in Spurr medium. Both longitudinally and transversely cut fibres were examined with a Corinth 275 electron microscope. Fifty micrographs were taken at $\times 8000$ magnification from each of 3 or 4 atria per treatment group. To ensure adequate sampling one micrograph was taken routinely from each corner of each specimen-containing grid square. Each micrograph was scored for visible damage by two independent observers without knowledge of the treatment group from which the muscle had been derived. A score of 0, 1, 2 or 3 was awarded by each observer for the degree of mitochondrial injury in the manner described by Kloner *et al.* (1978, 1982), and as illustrated in Figure 1.

Simulated ischaemia

At the start of each experiment the atria were allowed to equilibrate in NS for 1 h, at which time control values for refractory periods and CV were determined. The superfusate was then changed to one of abnormal composition, simulating the extracellular fluid of ischaemic muscle, as suggested by Ferrier *et al.* (1985). This fluid contained KCl 7 mM and sodium lactate 20 mM, was made up without added glucose, and had a pH of 6.4 produced by reduction of the $NaHCO_3$ concentration to 4 mM. Hypoxia was produced by replacing O_2 by N_2. All gases used were supplied by British Oxygen Company. The O_2 was of Medishield purity. The other gases used contained 0.0002% or less of O_2.

Drugs and other agents

Non-steroidal anti-inflammatory agents, being acidic, were dissolved in water with the aid of a slight excess of Na_2CO_3 and the solution adjusted to neutrality with acetic acid. Sulphinpyrazone and its thioether metabolite GP25671 were gifts from Ciba-Geigy. Flurbiprofen was a gift from Boots Company and flufenamate was a gift from Parke Davis and Company. The other non-steroidal anti-inflammatory agents were purchased from Sigma Chemical Company. 8-(Diethylamino)octyl 3,4,5-trimethoxybenzoate hydrochloride (TMB8) was purchased from Aldrich and was dissolved in water for use. Verapamil hydrochloride was supplied by Abbott Laboratories as an aqueous solution. The remaining compounds were all purchased as powders from Sigma. The divalent ionophore A23187 was dissolved in dimethylsulphoxide before being added to the superfusate. The following sub-

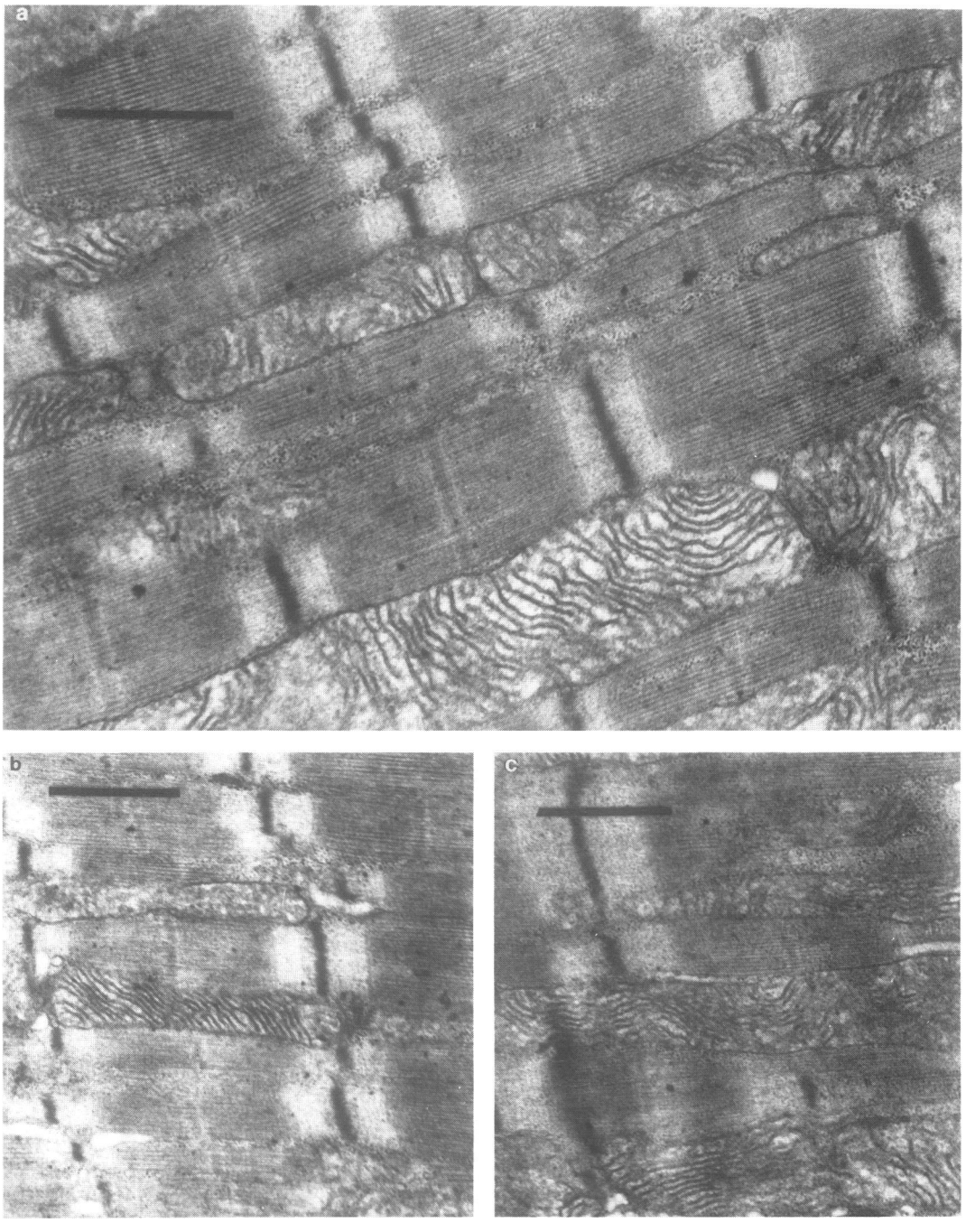
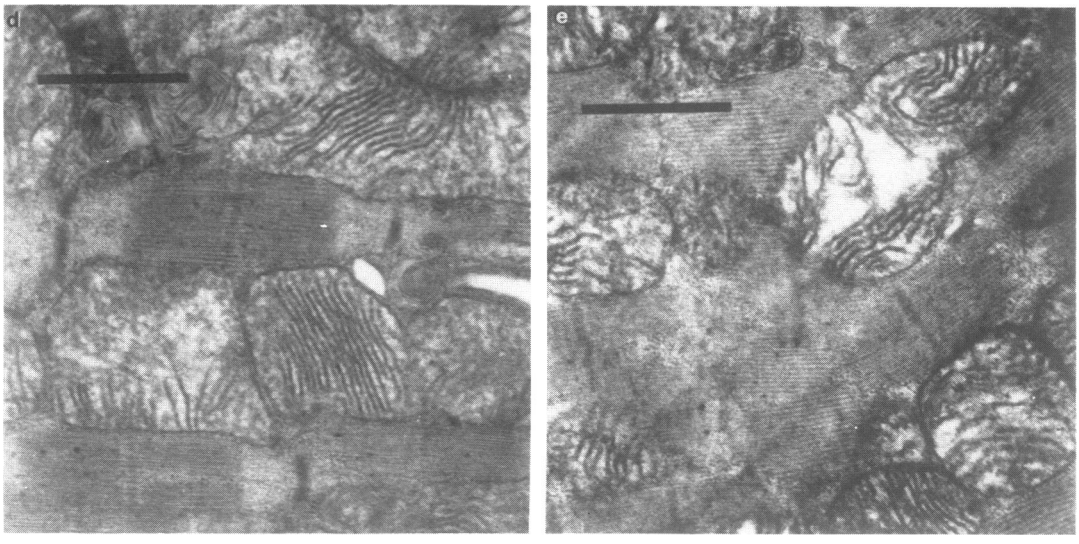


Figure 1 Electron micrographs of rat atrium: (**a**) shows the tight packing of the mitochondria between the myofibrils. Note that adjacent mitochondria show slightly varying degrees of injury. In (**b**) the mitochondria score = 0, with long and thin profiles and regularly arranged cristae. In (**c**) the mitochondrial score = 1, with some signs of swelling, and with cristae less well organised than in (**b**). In (**d**) the mitochondrial score = 2, with greater swelling and rounding-up than in (**b**) or (**c**), and with the cristae less organised than in (**c**). In (**e**) the mitochondrial score = 3, on account of gross swelling with signs of membrane disruption. The bar represents 1 μm.

Figure 1 (*cont*)



stances were used as aqueous solutions of their hydrochloride salts:- diltiazem, N-(6-aminohexyl)-5-chloro-1-naphthalene sulphonamide (W7) and N-(6-aminohexyl)-1-naphthalene sulphonamide (W5).

Statistics

Continuous variables were expressed as means ± s.e. Means for different treatment groups were compared by Student's *t* test. Mitochondrial damage scores were expressed as medians. Median values for different treatment groups were compared using a χ^2 test.

Results

Simulated ischaemia

Exposure of the myocardium to SI produced a pattern of electrical changes similar to that described previously (Northover, 1987). There was a progressive slowing of CV and a shortening of both action potential duration and refractory periods. The shortened action potential duration contributes to, but does not fully account for, the shortened refractory period, as discussed more fully in a previous paper from this laboratory (Northover, 1987). Shortening of refractory periods was most marked 12–15 min after the beginning of SI. Thereafter refractory periods slowly increased again, although action potential duration showed no tendency to relengthen. Return of the myocardium to NS after 20–60 min in SI fully restored all measured electrical parameters to approximately their starting values within 30–40 min.

Exposure of the myocardium to SI also caused the disappearance of all systolic developed tension within 5 min. Diastolic tension rose to a maximum within 15–20 min (Figure 2). Continued exposure to SI for up to 60 min caused the contracture tension to fade slightly. Return of the myocardium to NS after 15–60 min of exposure to SI fully restored diastolic tension to its starting value of 100 mg within approximately 30 min, by which time systolic developed tension also had recovered to 85–120% of its value in NS immediately prior to SI (Figure 2).

A series of time-dependent structural changes resulted from exposure of the myocardium to SI, with the accumulation of increased amounts of what

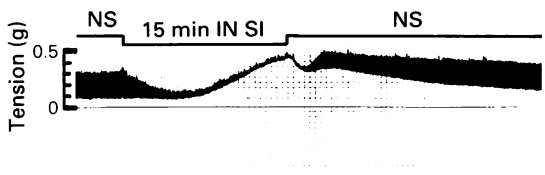


Figure 2 Effects on tension recording of exposing an atrium to simulated ischaemia (SI) for 15 min and then returning the tissue to normal perfusate (NS).

Table 1 Effects of various treatments on mitochondrial damage scores in rat atrial myocardium

Drug	*Treatment*	*Damage*
Absence of drugs	20 min in NS	0.069
Absence of drugs	20 min in SI	0.575*
Absence of drugs	60 min in SI	1.183*
Absence of drugs	140 min in NS	0.085
Absence of drugs	20 min in SI followed by 120 min in NS	0.138†
Absence of drugs	60 min in SI followed by 80 min in NS	0.068*
Sulphinpyrazone 50 μM	60 min in SI	0.783*
Verapamil 1 μM	60 min in SI	0.895*
Flufenamate 3 μM	20 min in SI	0.807†
W7 30 μM	60 min in SI	1.444*
TMB8 2 μM	60 min in SI	0.782*
Calcium 0.5 mM	60 min in SI	1.007

Tabulated values represent the results of 150–200 measurements.
* Denotes a significant difference from group exposed to normal solution (NS) for 20 min in the absence of drugs.
† Denotes a significant difference from group exposed to simulated ischaemia (SI) for 20 min in the absence of drugs.
* Denotes a significant difference from group exposed to SI for 60 min in the absence of drugs.
Significance was taken as $P < 0.05$.

appeared to be intracellular fluid. The mitochondria became distributed more randomly throughout the cell within 20 min of first exposure to SI, instead of being arranged in longitudinal rows tightly packed between the myofibrils, as in muscle bathed throughout in NS. Mitochondria in healthy muscle were cylindrical in shape, with a long axis parallel to that of myofibrils (Figure 1). After exposure to SI for 20 min or longer the mitochondria became more spherical and enlarged, with a progressive loss of the normally compact arrangement of cristae (Table 1). All these structural changes were more marked after 60 min of exposure to SI than after only 20 min (Table 1). Return of the myocardium to NS for 1–2 h, even after as long as 60 min in SI, permitted substantial recovery of the mitochondria (Table 1). They became cylindrical in shape once more, but the arrangement of cristae was not fully restored to normality.

Agents which alter intracellular calcium ion homeostasis

When tested in NS the ionophore A23187 (2 μM) shortened refractory periods and slowed CV (Table 2). The ionophore also potentiated the SI-induced shortening of refractory periods and slowing of CV. Dimethylsulphoxide, in which the ionophore was dissolved, was inactive in these respects at concentrations up to 0.5%. Systolic developed tension in NS was enhanced by A23187 but diastolic tension was not changed (Table 2). Under conditions of SI the presence of A23187 aggravated contracture (Table 2) and delayed subsequent recovery in NS. At a final concentration of 0.2 μM A23187 was inactive in all these respects.

The calmodulin inhibitor W7 (30 μM) prolonged refractory periods in NS (Table 2), but failed to alter CV. The SI-induced shortening of refractory periods was inhibited by W7, but the slowing of CV (Table 2) and the changes in mitochondrial structure (Table 1) were unaltered. W7 exerted a modest negative inotropic effect in NS at 30 μM, and inhibited SI-induced contracture (Table 2). However, at a concentration of 10 μM W7 was inactive in all these respects. Unlike all the other agents tested in the present experiments, the effects of W7 were slow to develop and very persistent during periods of wash-out in drug-free superfusate. It required more than 1 h, for example, to reverse the effects of 30 μM W7 on refractory periods and on systolic tension in NS. A non-chlorinated analogue of W7, with less potency as a calmodulin inhibitor, W5, was without significant effect at 30 μM on CV and refractory periods in both NS and SI (Table 2).

Exposure of atria for 15 min to NS made up with an abnormally low concentration of $CaCl_2$ (0.5 mM) caused a substantial reduction in systolic developed tension and a prolongation of refractory periods, but there was no significant change in CV (Table 2). This low concentration of calcium protected the muscle against SI-induced contracture, against the shortening of refractory periods and against the slowing of CV normally seen at 2.0 mM $CaCl_2$ (Table 2); however, it failed to prevent the mitochondrial structural changes seen at 2.0 mM $CaCl_2$ (Table 1).

Diltiazem (2 μM), a calcium channel antagonist, prolonged refractory periods in NS and inhibited the SI-induced shortening of atrial refractory periods (Table 2). Diltiazem also reduced the SI-induced slowing of CV, but without altering CV in NS (Table 2). At 2 μM diltiazem exerted a negative inotropic effect in NS, and inhibited SI-induced contracture (Table 2). At 0.4 μM diltiazem was inactive against all aspects of the response to SI and exerted no effect upon the systolic developed tension in NS. At 10 μM, however, diltiazem was almost completely protective against the effects of SI. Verapamil, another calcium channel antagonist, exerted similar electrical and mechanical effects to those shown by diltiazem (Table 2) and protected the mitochondria against SI-induced structural changes (Table 1).

Table 2 Effects of altering calcium homeostasis on responses to simulated ischaemia (SI) for 15 min in rat atrial myocardium

Drug	*Concentration* (μM)	*NS or SI*	*ERP* (ms)	*CV* × 10 ($m s^{-1}$)	*Tension* (mg) *Minimum diastolic*	*Tension* (mg) *Peak systolic*
Control	—	NS	32 ± 4	6.1 ± 1.0	100	318 ± 27
Control	—	SI	14 ± 2	1.9 ± 0.7	393 ± 37	—
A23187	2	NS	22 ± 3*	3.2 ± 0.6*	108 ± 10	630 ± 13*
A23187	2	SI	8 ± 2**	0.7 ± 0.2**	498 ± 61**	—
W7	30	NS	58 ± 6*	5.8 ± 1.4	96 ± 6	241 ± 19*
W7	30	SI	30 ± 9**	1.8 ± 0.9	230 ± 21**	—
W5	30	NS	34 ± 5	6.3 ± 1.5	96 ± 5	325 ± 36
W5	30	SI	18 ± 4	1.7 ± 0.8	416 ± 42	—
0.5 mM $CaCl_2$	—	NS	41 ± 5	5.2 ± 1.2	88 ± 6	122 ± 13*
0.5 mM $CaCl_2$	—	SI	30 ± 9**	4.0 ± 1.4**	246 ± 18**	—
Diltiazem	2	NS	43 ± 7	5.7 ± 1.2	90 ± 6	224 ± 19*
Diltiazem	2	SI	25 ± 6**	5.5 ± 1.4**	289 ± 16**	—
Verapamil	1	NS	38 ± 10	5.2 ± 0.6	97 ± 6	180 ± 13*
Verapamil	1	SI	28 ± 6**	4.1 ± 1.2**	225 ± 16**	—
TMB8	2	NS	39 ± 6	6.4 ± 1.1	110 ± 8	310 ± 32
TMB8	2	SI	30 ± 7**	3.8 ± 0.6**	285 ± 17**	—

Tabulated values represent means of 8–34 observations ± s.e. and were recorded after 15 min exposure to the stated conditions. A significant difference exists ($P < 0.05$) between control values and those marked with asterisks.
* Denotes a comparison with controls exposed to normal solution (NS).
** Denotes a comparison with controls exposed to SI.

The intracellular calcium antagonist TMB8, at 2 μM in NS, caused prolongation of atrial refractory periods without changing CV or systolic developed tension and substantially protected against SI-induced electrical, mechanical and structural changes (Tables 1 and 2). However, at a concentration of 10 μM TMB8 caused a negative inotropic effect in NS and even more protection against the deleterious effects of SI.

Non-steroidal anti-inflammatory agents

The presence of sulphinpyrazone during SI substantially protected the atria against shortening of refractory periods and slowing of CV (Figure 3). In contrast, flufenamate, salicylate and phenylbutazone all potentiated the electrical effects of SI (Figure 3), whereas ibuprofen, flurbiprofen and GP25671, the thioether metabolite of sulphinpyrazone, had no statistically significant effect (Figure 3). Sulphinpyrazone (70 μM), ibuprofen (500 μM), GP25671 (70 μM) and flurbiprofen (200 μM) exerted no significant effect upon refractory periods or upon CV during 15 min exposure in NS, whereas phenylbutazone (200 μM), flufenamate (5 μM) and salicylate (600 μM) all shortened refractory periods under these conditions, without altering CV. During SI the loss of systolic developed tension was unaltered by sulphinpyrazone, or indeed by any of the other drugs tested in the present experiments. Contracture force after 15 min in SI, however, was lessened by sulphinpyrazone and subsequent recovery in NS rendered more complete by the drug (Figure 3). In contrast, salicylate, flufenamate and phenylbutazone all potentiated SI-induced contracture and impeded subsequent recovery in NS (Figure 3). Ibuprofen, flurbiprofen and GP25671 were virtually inactive in these respects (Figure 3). None of these non-steroidal anti-inflammatory drugs exerted a significant effect on systolic or diastolic tension during exposure to NS for 15 min in the range of concentrations tested. The SI-induced changes in mitochondrial structure were significantly alleviated by pretreatment with sulphinpyrazone (Table 1). Pretreatment with flufenamate, on the other hand, significantly aggravated the structural changes produced by exposure to SI for 20 min (Table 1).

Discussion

The deleterious changes in the electrical, mechanical and structural features of atrial muscle observed in the present study in response to SI agree with many of the features observed by previous workers during ischaemia or hypoxia *in vivo*. Most previous studies of this subject, however, have used ventricular muscle, where the deleterious changes are much less fully reversible (Penn, 1970; Karmazyn *et al.*, 1981; Rahamathulla *et al.*, 1983; Jennings *et al.*, 1985b;

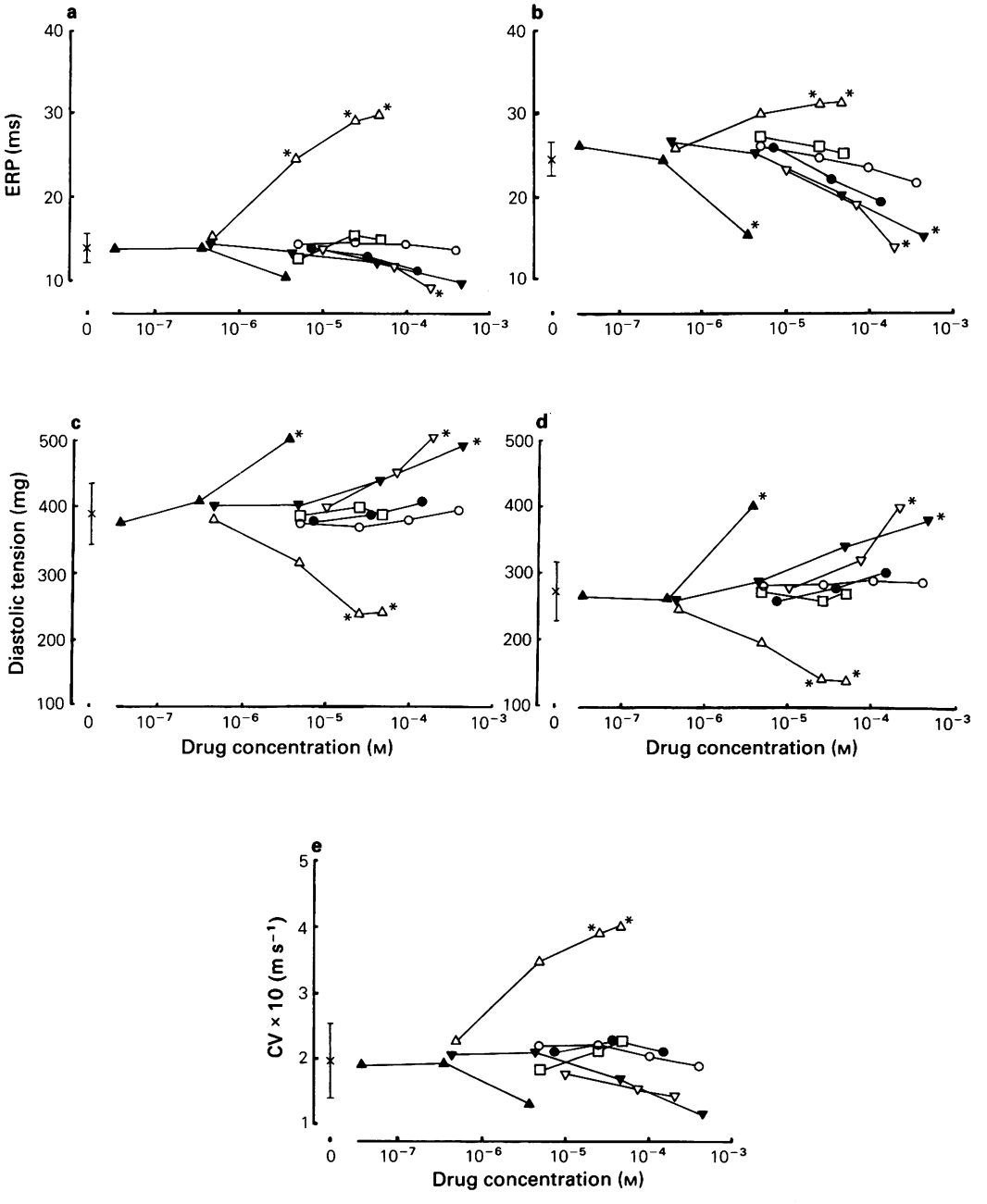


Figure 3 Effects of varying concentrations of sulphinpyrazone (△), flufenamate (▲), phenylbutazone (▽), salicylate (▼), ibuprofen (○), flurbiprofen (●) and GP25671 (□) on ERP, CV and diastolic tension of atria exposed to SI for 15 min (panels a, c and e), or to SI for 15 min followed by return to NS for 10 min (panels b and d). Vertical bars on control values represent s.e., but have been omitted from other data points for visual clarity. Each data point represents the mean of 9–25 observations. An asterisk denotes that there is a significant difference ($P < 0.05$) between a value and the drug-free control.

Lipasti *et al.*, 1985; Singal *et al.*, 1986). Further elucidation of the basis for the atypically reversible features of this form of injury in the rat atrium would be valuable.

The association between calcium overload and myocardial injury (see Introduction) was explored in the present experiments with drugs known to alter intracellular calcium homeostasis. Diltiazem and verapamil were shown to protect against all aspects of the response to SI. Previous workers have shown that these agents protect ventricular muscle against ischaemia-induced and hypoxia-induced shortening of refractory periods and slowing of CV (Elharrar *et al.*, 1977; Nakaya *et al.*, 1980; 1981; 1982; Fujimoto *et al.*, 1981; Kimura *et al.*, 1982; 1983; Peter *et al.*, 1982; 1983; Gettes *et al.*, 1985; Fleet *et al.*, 1986), against the associated contracture (Henry *et al.*, 1977; Poole-Wilson *et al.*, 1982; 1984; Cavero *et al.*, 1983; Fitzpatrick & Karmazyn, 1984; Henry, 1984; Ferrari *et al.*, 1986; Piacenza *et al.*, 1986), and against the mitochondrial structural alterations (Rahamathulla *et al.*, 1983). In the present experiments, verapamil and diltiazem protected the atria only at concentrations which exerted a negative inotropic effect in NS. Protection may have been due, therefore, solely to conservation of the stores of high energy phosphate compounds. A similar uncertainty was faced by most previous workers. Henry (1984), however, demonstrated that 1 μM diltiazem protected non-contracting papillary muscles against hypoxic contracture. It seems likely, therefore, that in addition to energy-sparing actions, these drugs also directly protect the injured myocyte. Whether this is by reducing the availability of intracellular calcium or by some other action remains to be determined.

The calmodulin antagonist W7 (Hidaka & Tanaka, 1982) inhibited some of the SI-induced changes in the atrial myocardium in the present experiments, suggesting an involvement of calcium and calmodulin in this form of injury. Unfortunately, protection against the electrical and mechanical consequences of SI was observed here only at negatively inotropic concentrations. An unchlorinated analogue of W7 called W5, with much less potency as a calmodulin inhibitor (Hidaka & Tanaka, 1982), was inactive in the present experiments. Previous workers have obtained evidence for calmodulin involvement in some of the electrical responses to myocardial ischaemia and hypoxia (Anno *et al.*, 1986; Barron *et al.*, 1986), although no previous report has been found for an action of W7 on ischaemic or hypoxic contracture. The failure of W7 to protect mitochondria in the present experiments suggests that calmodulin is not involved in that particular aspect of the response to SI. Phenothiazine inhibitors of calmodulin also protect the sarcolemma of tissue-cultured hypoxic cardiac myocytes without visible protection of the mitochondria (Scott *et al.*, 1986).

Further evidence for involvement of calcium in the response of atrial muscle to SI was obtained by lowering the calcium concentration of the superfusate to 0.5 mM. This decreased systolic developed tension and prolonged refractory periods in NS, without altering CV. Exposure of atria to SI at a calcium concentration of 0.5 mM caused less contracture, and less shortening of refractory periods and slowing of CV than at 2 mM $CaCl_2$. This confirms previous findings in ventricular muscle (Hearse *et al.*, 1977; Wojtczak, 1979; Cavero *et al.*, 1983; Koomen *et al.*, 1983; Fitzpatrick & Karmazyn, 1984; Anno *et al.*, 1986; Karmazyn, 1986), although contrary findings have also been published (Nayler & Williams, 1978; Lewis *et al.*, 1979). Why mitochondria were not protected structurally against SI at 0.5 mM calcium in the present experiments is unknown, but has been reported previously for ventricular muscle *in vivo* (Jennings *et al.*, 1985a; Pilati & Paradise, 1984).

Addition of the ionophore A23187 to NS in the present experiments shortened refractory periods, slowed CV and increased systolic developed tension. In the presence of the ionophore there was greater shortening of refractory periods during SI, greater slowing of CV, and greater contracture development. The positive inotropic effect of A23187 has been reported previously (Holland *et al.*, 1975; 1978) and probably reflects a raised concentration of intracellular calcium, which would be expected to augment that due to SI.

TMB8 not only inhibits the release of intracellular stored calcium (Chiou & Malagodi, 1975; Mix *et al.*, 1984), but also seems to inhibit some of the intracellular actions of calcium (Hassid & Oudinet, 1986). Although TMB8 exerts negative inotropic effects on the rat atrium, it protected against all three aspects of the response to SI at 2 μM, which was below the concentration required for a significant negative inotropic action. This provides further evidence that intracellular calcium is involved in a direct way with the response to SI.

Non-steroidal anti-inflammatory drugs are a group of pharmacologically heterogeneous agents. Sulphinpyrazone, unlike some other members of the group that was tested, protected the myocardium against SI in terms of electrical, mechanical and structural changes. The protective effect of this drug against disturbances of cardiac rhythm is now well established (Raeder *et al.*, 1982; Northover, 1986; 1987; Eller *et al.*, 1987) and Karmazyn *et al.* (1981) have demonstrated its ability to protect rat ventricular myocardium against hypoxic contracture. Flufenamate, salicylate and phenylbutazone potentiated the electrical, mechanical and structural injury

caused by SI in the present experiments. The reason why they exerted opposite effects to those of sulphinpyrazone is unknown at present, although previous workers have noticed variable effects on hypoxic contractures among different members of this group of drugs (Karmazyn *et al.*, 1981; Karmazyn, 1986).

The thioether metabolite of sulphinpyrazone, GP25671, was inactive in the present experiments at concentrations where the parent drug was protective. This metabolite is 10–20 times more potent than sulphinpyrazone as a cyclo-oxygenase inhibitor (Del Maschio *et al.*, 1984) and possesses the additional property of antagonizing the action of thromboxane-mimetics, at least in platelets (Hatmi *et al.*, 1987). This suggests that arachidonic acid metabolites are not involved in the protective effects of sulphinpyrazone observed under the present experimental conditions, nor under those used by Eller *et al.* (1987), where again the thioether metabolite was inactive against ischaemia-induced decline in the ventricular fibrillation threshold, although sulphinpyrazone was effective.

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