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The involvement of lactate and calcium as mediators of the electrical and mechanical responses of the myocardium to conditions of simulated ischaemia

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

2 Atria subjected to SI showed a decreased adenosine 5'-triphosphate (ATP) content, a rise in diastolic tension, a diminished conduction velocity of action potentials and shortened refractory periods. All these changes were less pronounced during lactate-free SI.

3 Atria preloaded with calcium displayed exaggerated responses measured electrically and mechanically during exposure to SI, whereas atria previously depleted of calcium displayed diminished electrical and mechanical responses to SI. Neither calcium loading nor calcium depletion modified the SI-induced depletion of the atrial stores of ATP.

4 Sulphinpyrazone protected atria against all aspects of the response to SI, but failed to protect the muscle under conditions of lactate-free SI. It is concluded that during SI, sulphinpyrazone protects against a lactate-mediated inhibition of the glycolytic synthesis of ATP.

5 Flufenamate exaggerated all responses of the atria to SI. These deleterious actions were still observed during lactate-free SI. It is concluded that flufenamate inhibits the synthesis of ATP in the mitochondria.

Introduction

Simulated ischaemia (SI) is said to exist when a tissue is exposed *in vitro* to a solution the composition of which resembles that found extracellularly in regions of ischaemia. During SI in rat atrial muscle the conduction velocity (CV) of action potentials is reduced, as are the durations of both action potentials and refractory periods. These responses are accompanied by progressive contracture development and a characteristic pattern of structural changes, notably in the mitochondria (Northover & Northover, 1988). Return of the myocardium to a solution of normal composition gradually reverses all of these changes. Pretreatment of the atria with sulphinpyrazone protects the myocardium during SI, but flufenamate, another non-steroidal anti-inflammatory agent, has a deleterious action (Northover & Northover, 1988).

A rise in the diastolic concentration of Ca in the myoplasm is believed to represent an important stage in the series of events responsible for the structural and functional changes observed during SI (Northover & Northover, 1988). In some of the present experiments the diastolic Ca concentration in the myoplasm has been deliberately elevated before exposure to SI. By noting which components of the response to SI were enhanced under these circumstances evidence was obtained concerning the ways in which Ca is involved as a mediator.

Lactate is another substance known to accumulate in the ischaemic myocardium (Neely & Grotyohann, 1984), and is an ingredient of the fluid used to produce SI. Moreover, lactate is known to potentiate some of the functional changes in the atria produced by the other ingredients of this fluid (Northover, 1987). Under certain circumstances lactate is known to inhibit glycolysis (Rovetto *et al.*, 1973, 1975; Mochizuki & Neely, 1979; Neely & Grotyohann, 1984), to exert a negative inotropic action (Tennant, 1935), and to interfere with both mitochondrial structure (Armiger *et al.*, 1974) and the oxidation of

fatty acids (Bielefeld *et al.*, 1983). The present experiments have sought to identify the circumstances under which lactate inhibits ATP production, and the involvement of such inhibition in the overall deterioration produced by SI.

Finally, attempts have been made to identify the extent to which sulphinpyrazone and flufenamate alter responses to SI by modifying the involvement of lactate and calcium in this process.

Methods

Rats of the Sprague-Dawley strain weighing 340–410 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. In experiments where the NaCl concentration was halved, sucrose (138 mM) was added to maintain osmolality. In experiments where lanthanum chloride was added to the superfusate, sodium phosphate and bicarbonate were replaced by 4 mM sodium *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulphonate as a pH buffer. Atria were stimulated throughout an experiment 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 3M 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 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 paired stimuli, and was taken as the interval between the closest pair of stimuli both of which yielded action potentials. The CV of action potentials between the 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 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 conducted via the most direct route between the 2 electrodes, however, the CV may have been underestimated.

Mechanical changes

The right atrial appendage was anchored to the base of the superfusion trough and the left atrial appendage was connected via a length of nylon suture to a Nihon Khoden strain gauge (Type SB-1T). Diastolic tension was adjusted at the start of each experiment to 100 mg. Tension records were made on slowly moving paper via a d.c. amplifier (Type 3552, Cardiovascular Instruments) coupled to a heated stylus recorder (Type 5041, Lectromed).

Simulated ischaemia

At the start of each experiment the atria were allowed to equilibrate in normal superfusate 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). Unless specified otherwise, this fluid contained racemic sodium lactate (20 mM), was made up without added glucose, and had a pH of 6.4 produced by reduction of the $NaHCO_3$ content 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 contained 0.002% or less of O_2. Po_2 of the superfusate during SI was 16–25 mmHg. A tissue was allowed to remain in SI for 15 min and thereafter returned to normal superfusate. Measurements given in this paper, unless otherwise specified, were made after 15 min in SI. Each tissue was exposed to SI on 4 occasions, each of 15 min. Preliminary experiments showed that there was no statistically significant difference between the responses to 4 consecutive exposures to SI. In some of the experiments SI was superimposed upon another intervention that was intended to raise the myoplasmic Ca concentration. For this purpose the tissue was exposed to the Ca-raising intervention for 30 min, during the last 15 min of which conditions of SI also prevailed.

Assay of ATP

Atria to be assayed for ATP were clamped between the jaws of a pair of metal tongs that had been chilled in liquid N_2. The frozen tissue was powdered in a steel percussion mortar, also cooled in liquid N_2. The powdered muscle was rapidly transferred to a preweighed glass vial containing ice-cold 10% aqueous perchloric acid solution, and the vial reweighed. The contents of the vial were then homogenized in a cold room, with a small hand-operated Dounce tissue grinder surrounded by melting ice. The homogenate was centrifuged at 2000 *g* for 15 min at 2°C and the supernatant collected. The solid residue was then re-extracted with further perchloric acid solution. The two supernatants were combined, neutralised with 10 M KOH solution, and then centrifuged at 2000 *g* for 15 min at 2°C. The neutralised supernatant was assayed for ATP by first reacting it with glucose in the presence of hexokinase. The glucose-6-phosphate produced was oxidised in the presence of nicotinamide adenine dinucleotide and glucose-6-phosphate dehydrogenase. The reaction was monitored in a spectrophotometer at 340 nm and the glycogen content expressed as μmol glucose per g wet weight of muscle.

Drugs and other agents

Sulphinpyrazone was a gift from Ciba-Geigy and flufenamate was a gift from Parke Davis and Company. They were dissolved in water with a small excess of Na_2CO_3 and the solution adjusted to neutrality with acetic acid. All other drugs and reagents were purchased from Sigma.

Statistics

Variables are expressed throughout as means ± s.e. Means for different treatment groups were compared by means of Student's *t* tests.

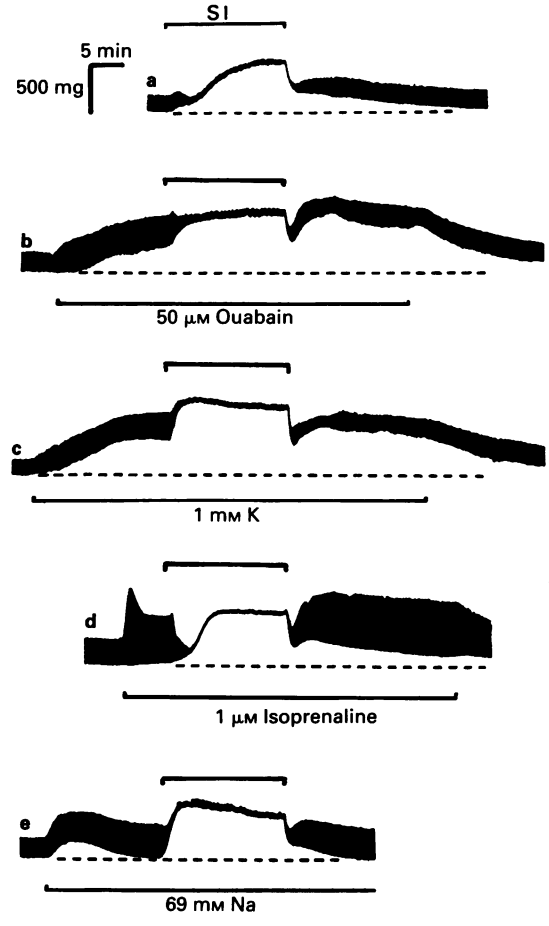

Figure 1 Atrial tension recorded during exposure to simulated ischaemia (SI) for the period indicated by the horizontal bar above each panel. In panels (b)–(e) there are also continuous horizontal bars beneath each record, and during the latter periods the superfusate contained 50 μM ouabain (b), 1 mM K (c), 1 μM isoprenaline (d), or 69 mM Na (e).

Results

Effects of elevating the myoplasmic Ca concentration

The Ca concentration in the myoplasm was elevated in the present experiments by means of several different pharmacological interventions, namely by exposure to NS containing ouabain (10–50 μM), by exposure to NS containing a reduced K concentration (1 or 2 mM) or Na concentration (69 mM), or a raised Ca concentration (10 mM), or by means of NS containing isoprenaline (0.1–1.0 μM). Each of these interventions caused the expected increase in systolic developed tension (Figure 1). Positive inotropism was well sustained, except with isoprenaline. Some, but not all of these interventions also caused a rise in diastolic tension. Any rise in diastolic tension, however, only began after systolic tension had already begun to rise. Moreover, a higher concentration of ouabain (> 10 μM) or a lower concentration of K (< 2 mM) was needed to produce contracture than was needed to cause detectable positive inotropism. Where diastolic tension did rise during one of these interventions, it was progressive with time, except in the case of the response to a low Na concentration, where only a temporary contracture was produced (Figure 1). Unlike SI, none of the above interventions caused a significant decline in the ATP

Table 1 Effects of various calcium-raising interventions on responses of atria to simulated ischaemia (SI)

Drug (μM)	*Na* (mM)	*K* (mM)	*Ca* (mM)	*ERP* (ms)	*CV* ($m\ s^{-1}$)	*DT* (mg)	*ATP* ($\mu mol\ g^{-1}$)
Control	138	5	2	16 ± 2	0.18 ± 0.06	424 ± 52	1.82 ± 0.21
	69	5	2	17 ± 3	0.19 ± 0.07	419 ± 75	1.86 ± 0.35
	138	2	2	16 ± 3	0.14 ± 0.06	488 ± 60	1.74 ± 0.20
	138	1	2	10 ± 2*	0.07 ± 0.03*	654 ± 62*	1.61 ± 0.19
	138	5	10	11 ± 2*	0.18 ± 0.03	570 ± 48*	1.70 ± 0.18
Ouabain (10)	138	5	2	15 ± 3	0.16 ± 0.05	466 ± 37	1.99 ± 0.25
Ouabain (50)	138	5	2	9 ± 2*	0.08 ± 0.02*	685 ± 40*	1.70 ± 0.16
Isopren (1)	138	5	2	17 ± 3	0.19 ± 0.07	302 ± 44*	2.05 ± 0.23

Tabulated values represent means of 6–18 observations. A significant difference exists ($P < 0.05$) between a value marked * and the corresponding value for control atria exposed to SI.
Isopren = isoprenaline; ERP = effective refractory period; CV = conduction velocity; DT = diastolic tension; ATP = adenosine triphosphate content.

content of the muscle within the first 30 min (cf. Tables 1 and 2). When SI was superimposed upon another intervention that had already raised diastolic tension, the final contracture tension reached after 15 min in SI was greater than that reached in the presence of SI alone (Table 1). Contracture tension was not augmented, however, when SI was superimposed upon an intervention that failed to raise diastolic tension, as in the case of isoprenaline (Table 1), or upon an intervention that raised diastolic tension only temporarily, as in the case of a low Na concentration (Table 1). Indeed, contracture tension during SI in the presence of isoprenaline was significantly less than in the absence of this catecholamine (Table 1).

Refractory periods and CV of action potentials declined in atria subjected to NS containing ouabain at a concentration sufficient to raise diastolic tension (20 or 50 μM in Table 2). When the K concentration of NS was lowered to 1 mM there was also a decline in ERP and CV values (Table 2). During exposure of atria to SI for 15 min in the presence of 50 μM ouabain or a lower K concentration (1 mM), the measured values of ERP and CV became significantly smaller than during SI in the absence of these interventions (Table 1).

Effects of non-steroidal anti-inflammatory agents

Sulphinpyrazone (5–50 μM) protected the myocardium against contracture development and the associated electrical and biochemical disturbances produced by exposure for 15 min to SI in the presence, but not in the absence of lactate. Flufenamate (1–5 μM), on the other hand, aggrevated all the disturbances produced by SI both in the presence of lactate and in its absence (Table 3). Neither sulphinpyrazone nor flufenamate in these ranges of con-

Table 2 Effects of composition of normal superfusate (NS) on responses of atria to various calcium-raising agents

Drug (μM)	*Na* (mM)	*K* (mM)	*Ca* (mM)	*ERP* (ms)	*CV* ($m\ s^{-1}$)	*DT* (mg)	*ATP* ($\mu mol\ g^{-1}$)
Control	138	5	2	33 ± 5	0.63 ± 0.11	100	3.71 ± 0.24
	69	5	2	32 ± 6	0.59 ± 0.16	98 ± 12	3.65 ± 0.27
	138	2	2	30 ± 5	0.55 ± 0.14	106 ± 15	3.70 ± 0.20
	138	1	2	20 ± 4*	0.26 ± 0.12*	419 ± 60*	3.39 ± 0.27
	138	5	10	25 ± 3*	0.45 ± 0.10*	148 ± 24	3.48 ± 0.30
Ouabain (10)	138	5	2	32 ± 6	0.57 ± 0.11	105 ± 23	3.66 ± 0.29
Ouabain (20)	138	5	2	30 ± 4*	0.48 ± 0.06*	125 ± 18	3.59 ± 0.25
Ouabain (50)	138	5	2	23 ± 3*	0.24 ± 0.10*	394 ± 50*	3.37 ± 0.22
Isopren (1)	138	5	2	20 ± 4*	0.55 ± 0.09	106 ± 9	3.54 ± 0.23

Tabulated values represent means of 6–36 observations. A significant difference exists ($P < 0.05$) between a value marked * and the corresponding value for control atria exposed to NS.
Isopren = isoprenaline; ERP = effective refractory period; CV = conduction velocity; DT = diastolic tension; ATP = adenosine triphosphate content.

Table 3 Influence of lactate on the effects of various drugs on the responses of atria to simulated ischaemia (SI)

Drug (μM)	*Lactate* (mM)	*ERP* (ms)	*CV* ($m\ s^{-1}$)	*DT* (mg)	*ATP* ($\mu mol\ g^{-1}$)
Control	20	16 ± 2	0.18 ± 0.06	424 ± 52	1.82 ± 0.21
Control	0	21 ± 3*	0.36 ± 0.07*	305 ± 29*	2.50 ± 0.27*
Sulphinpyrazone (50)	20	29 ± 4*	0.40 ± 0.10*	232 ± 25*	2.98 ± 0.22*
Sulphinpyrazone (50)	0	26 ± 4	0.41 ± 0.09	316 ± 41	2.94 ± 0.20
Flufenamate (5)	20	8 ± 2*	0.06 ± 0.05*	510 ± 57*	1.02 ± 0.13*
Flufenamate (5)	0	14 ± 2**	0.21 ± 0.07**	462 ± 43**	1.10 ± 0.11**
Dinitrophenol (3)	20	7 ± 2*	0.05 ± 0.04*	596 ± 60*	1.05 ± 0.17*
Dinitrophenol (3)	0	13 ± 2**	0.22 ± 0.04**	477 ± 36**	1.59 ± 0.26**
Lanthanum (1000)	20	26 ± 3*	0.38 ± 0.04*	211 ± 22*	1.94 ± 0.26
Lanthanum (1000)	0	29 ± 4*	0.50 ± 0.05**	155 ± 25**	2.58 ± 0.20**
Pyruvate (10,000)	20	17 ± 3	0.20 ± 0.08	394 ± 46	2.05 ± 0.23
Glucose (20,000)	20	30 ± 4*	0.50 ± 0.09*	123 ± 18*	3.57 ± 0.26*
Zero calcium	20	22 ± 3*	0.45 ± 0.08*	196 ± 25*	1.98 ± 0.19*
Zero calcium	0	29 ± 3**	0.57 ± 0.10**	181 ± 22**	2.62 ± 0.23**

Tabulated values represent means of 6–24 observations. A significant difference exists ($P < 0.05$) between a value marked * and the corresponding value for control atria exposed to lactate-containing SI, and between a value marked ** and the corresponding value for control atria exposed to lactate-free SI.
ERP = effective refractory period; CV = conduction velocity; DT = diastolic tension; ATP = adenosine triphosphate content.

centrations significantly altered diastolic tension or the systolic developed tension in NS, nor did either drug alter the loss of systolic developed tension during SI. Sulphinpyrazone failed to protect atria against the mechanical and electrical changes produced by exposure to NS containing ouabain (50 μM) or a low K concentration (1 mM). Flufenamate, however, enhanced all of the measured responses of the myocardium to both interventions.

Effects of glucose

Atria continued to behave quite normally, both mechanically and electrically, for 2–3 h in NS made up without glucose, despite a reduction in glycogen content from 12 $\mu mol\ g^{-1}$ in glucose-containing NS to only 2.6 $\mu mol\ g^{-1}$ after 1 h in glucose-free NS ($P < 0.05$). The ATP content of atria exposed to glucose-free NS for 1 h was almost identical to that

Table 4 Influence of lactate on the effects of various drugs on the responses of glycogen-depleted atria to simulated ischaemia (SI)

Drug (μM)	*Lactate* (mM)	*ERP* (ms)	*CV* ($m\ s^{-1}$)	*DT* (mg)	*ATP* ($\mu mol\ g^{-1}$)
Control	20	11 ± 2	0.12 ± 0.04	530 ± 49	1.02 ± 0.10
Control	0	12 ± 3	0.17 ± 0.06	541 ± 60	1.16 ± 0.14
Sulphinpyrazone (50)	20	12 ± 2	0.20 ± 0.07	496 ± 38	1.20 ± 0.15
Sulphinpyrazone (50)	0	14 ± 4	0.18 ± 0.05	490 ± 42	1.24 ± 0.16
Flufenamate (5)	20	6 ± 1*	0.07 ± 0.03	639 ± 57	0.39 ± 0.06*
Flufenamate (5)	0	7 ± 1**	0.05 ± 0.02**	615 ± 46	0.55 ± 0.09**
Dinitrophenol (3)	20	6 ± 2*	0.08 ± 0.04	701 ± 58*	0.36 ± 0.10*
Dinitrophenol (3)	0	6 ± 2**	0.06 ± 0.03**	690 ± 51**	0.67 ± 0.14**
Lanthanum (1000)	20	21 ± 4*	0.38 ± 0.12*	206 ± 29*	1.22 ± 0.19
Lanthanum (1000)	0	23 ± 5**	0.31 ± 0.10**	194 ± 16**	1.25 ± 0.22

Tabulated values represent means of 6–24 observations. A significant difference exists ($P < 0.05$) between a value marked * and the corresponding value for control atria exposed to lactate-containing SI, and between a value marked ** and the corresponding value for control atria exposed to lactate-free SI.
ERP = effective refractory period; CV = conduction velocity; DT = diastolic tension; ATP = adenosine triphosphate content.

of atria bathed with glucose-containing NS. Nevertheless, atrial fibrillation did eventually supervene in glycogen-depleted muscle, but this was not until the tissue had been exposed to glucose-free NS for at least 3 h. To avoid this rhythm disturbance all subsequent experiments of this type were performed after exposure to glucose-free NS for just 1 h. Responses to SI after prior glycogen-depletion were significantly greater than in atria which began a period of 15 min exposure to SI with a normal glycogen store (cf. Tables 1 and 4). More significantly, responses of the glycogen-depleted muscle to SI were no longer inhibited by sulphinpyrazone (Table 4), although still potentiated by flufenamate or dinitrophenol (Table 4). Glucose (20 mM) was protective against all aspects of the response to SI, an action not shared by pyruvate (Table 3). A supraphysiological concentration of glucose failed to protect atria, however, against any of the responses to ouabain or a low K concentration in NS. Unlike the situation during SI, the ATP content of the myocardium was not significantly depleted during the latter 2 interventions (Table 2).

Effects of lactate

Conditions of SI, in the absence of added lactate, elicited smaller electrical, mechanical and biochemical responses than in the presence of lactate (Table 3). The deleterious effects of lactate on responses to SI were no longer seen, however, in atria that had already been depleted of glycogen by prior treatment with glucose-free NS for 1 h (Table 4).

Lactate (20 mM) exerted a negative inotropic effect when added to NS. Despite this, no significant change in ATP content or in ERP and CV values was seen under these conditions. When lactate-containing NS was suddenly replaced by lactate-free NS, however, the muscle developed a brief but intense contracture (Figure 2) associated with depletion of ATP content, shortened ERP values and a diminished CV (Table 5). In some tissues there were also short self-terminating paroxysms of atrial tachycardia provoked by the paired stimuli used for measuring refractory periods. All these features were maximally developed 4–6 min after lactate was discontinued and had fully subsided within a further 15–20 min. Similar responses were observed when the lactate-containing NS was made up with the NaCl content reduced to preserve isotonicity throughout. A reciprocal relationship was found between the concentration of glucose in the NS and the severity of the responses to sudden discontinuation of lactate (Table 5). Sulphinpyrazone and

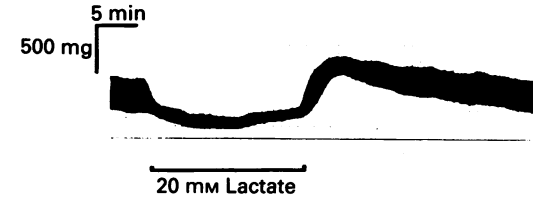

Figure 2 Atrial tension during exposure to normal superfusate. The superfusate contained sodium lactate (20 mM) during the 20 min period marked by the horizontal bar at the foot of the figure. Note that contracture tension was maximal about 5 min after the discontinuation of lactate.

Table 5 Effects of various drugs on the responses of atria to the sudden discontinuation of the presence of lactate in normal superfusate (NS)

Drug (μM)	*Glucose* (mM)	*Ca* (mM)	*ERP* (ms)	*CV* ($m\ s^{-1}$)	*DT* (mg)	*ATP* ($\mu mol\ g^{-1}$)
Control	10	2	18 ± 3	0.20 ± 0.07	488 ± 35	1.96 ± 0.29
	0	2	9 ± 2*	0.11 ± 0.02	562 ± 50	1.04 ± 0.12*
	20	2	29 ± 4*	0.45 ± 0.12*	210 ± 14*	3.24 ± 0.35*
	10	0	28 ± 4*	0.40 ± 0.11*	207 ± 22*	2.02 ± 0.13
Sulphinpyrazone (50)	10	2	30 ± 5*	0.39 ± 0.08*	182 ± 35*	2.57 ± 0.14*
Pyruvate (10,000)	10	2	33 ± 5*	0.50 ± 0.12*	151 ± 29*	2.61 ± 0.16*
Flufenamate (5)	10	2	10 ± 2*	0.08 ± 0.02*	582 ± 46*	0.96 ± 0.17*
Dinitrophenol (3)	10	2	8 ± 2*	0.09 ± 0.03*	576 ± 55*	0.89 ± 0.10*
Lanthanum (1000)	10	2	26 ± 3*	0.62 ± 0.13*	132 ± 18*	2.08 ± 0.14

Atria were exposed for 20 min to NS containing 20 mM lactate. Tabulated values represent means of 6–18 observations and were recorded 5 min after the sudden discontinuation of the presence of lactate in the NS. A significant difference exists ($P < 0.05$) between a value marked * and the corresponding value for control atria.
ERP = effective refractory period; CV = conduction velocity; DT = diastolic tension; ATP = adenosine triphosphate content.

pyruvate both inhibited the responses (Table 5), whereas flufenamate and dinitrophenol both potentiated them (Table 5).

Effects of lanthanum

In order to explore further the involvement of calcium in the reponses to SI, experiments were conducted in the presence of added lanthanum chloride (1 mM), a classical calcium antagonist. Systolic developed tension in NS was approximately zero in the presence of this concentration of lanthanum. Lanthanum protected against the mechanical and electrical responses normally produced by SI (Table 3), by ouabain in NS and by a low K concentration in NS. Lanthanum failed to alter the depletion of ATP, however, that was normally observed during SI (Table 3). The protective effects of lanthanum during SI were observed both in the presence and in the absence of added lactate (Table 3), and so differ from the protective effects of sulphinpyrazone. Contractures due to discontinuation of lactate were inhibited by lanthanum but the depletion of ATP was unaltered (Table 5). Again, this is different from the protective effect of sulphinpyrazone (Table 5).

Discussion

Involvement of calcium

Two lines of evidence from the present experiments suggest that during the various conditions chosen to create Ca-loading an increased diastolic Ca concentration in the myoplasm was responsible for contracture and the associated changes in ERP and CV. Firstly, when a contracture was produced by exposure to NS containing ouabain or a low K concentration the changes in ERP and CV occurred in parallel with contracture development (Table 2). Milder forms of these same interventions, sufficient to produce positive inotropism but insufficient to cause contracture, caused no significant change in ERP or CV (Table 2). Secondly, those treatments that protected atria against contracture development, such as a lack of extracellular Ca, also protected against the associated reductions in ERP and CV (Table 2).

Conditions of SI, in common with several others that are known to be associated with rapid depletion of the myocardial stores of ATP, are associated with a progressive rise in the myoplasmic concentration of Na (Murphy *et al.*, 1987a) and Ca (Murphy *et al.*, 1985; Haworth *et al.*, 1987; Steenbergen *et al.*, 1987, Barry *et al.*, 1987; Smith & Allen, 1988). The myoplasmic Ca concentration during diastole has been suggested by several of these investigators to be partly responsible for the observed contracture. Other workers have suggested that there is a similar basis for the slowing of CV (Pressler *et al.*, 1982) and for the shortening of ERP (Northover, 1987; Northover & Northover, 1988). The total cellular Ca content has been reported to rise during these and related circumstances (Murphy *et al.*, 1987b). A rapidly exchanging pool of intracellular Ca also increases under these circumstances (Murphy *et al.*, 1987a), although the sarcoplasmic reticular store of Ca may actually decline (Hasin *et al.*, 1984; Haworth *et al.*, 1987). It seems probable that ATP depletion is the primary disturbance during SI. Since the extrusion of Na and Ca from the myoplasm into the extracellular compartment, and the uptake of Ca from the myoplasm into the sarcoplasmic reticulum are ATP-dependent processes, it follows that ATP-depletion will cause a rise in the myoplasmic concentrations of Ca and Na, particularly during diastole.

In the present experiments, when SI was superimposed upon a situation that had already produced Ca-overload and hence contracture, the electrical and mechanical responses during the period of SI were enhanced (Table 1), although the depletion of ATP stores was not significantly altered (Table 1). Pretreatment of the atria with isoprenaline significantly inhibited the development of contracture during SI (Table 1). The ability of isoprenaline and other β-adrenoceptor agonists to inhibit contractures due to Ca-overload has been observed previously (Kavaler & Morad, 1966; Graham & Lamb, 1968; Lakatta & Lappe, 1981). A possible explanation is stimulation of glycolysis (MacLoed & Prasad, 1969), but this is unlikely to have contributed in the present experiments since ATP-depletion during SI was unaltered (Table 1). Alternatively, a raised concentration of adenosine 3′:5′-cyclic monophosphate (cyclic AMP) may have reduced the sensitivity of the contractile proteins to Ca, as suggested by Ray & England (1976) and by Endoh & Blinks (1988). This would also account for the early fading of the positive inotropic response to isoprenaline shown in Figure 1. Other works have concluded, however, that the early fading of inotropic responses to isoprenaline was due to a partial failure of systolic release of Ca from the sarcoplasmic reticulum (Erne & Hermsmeyer, 1988).

All of the measured electrical and mechanical responses to SI in the present experiments were inhibited by the absence of Ca from the superfusate or by the presence of lanthanum (Table 3). The myoplasmic Ca concentration is known to be reduced by lanthanum during ATP-depletion (Hasin *et al.*, 1984; Haworth *et al.*, 1987; Murphy *et al.*, 1988) as well as under more normal conditions (Barry & Smith,

1982). In the present experiments the absence of Ca from the superfusate or the presence of lanthanum failed to alter the SI-induced depletion of ATP significantly, yet both reduced all the other measured components of the response to SI (Table 3). The electrical and mechanical responses to SI, therefore, seem to be secondary to changes in the myoplasmic Ca concentration, which in turn are the result of the ATP depletion.

Involvement of lactate

Although lactate is a fuel for the aerobic heart (Takenaka & Watanabe, 1976) it potentiates the SI-induced reduction in ERP (Northover, 1987), contracture development, slowing of CV and ATP depletion (Table 3). Lactate is known to inhibit the glycolytic synthesis of ATP in ventricular muscle (Rovetto *et al.*, 1973; 1975; Mochizuki & Neely, 1979; Neely & Grotyohann, 1984). The present experiments provide two lines of evidence that a similar effect is produced in atrial muscle. In the first place, the SI-potentiating effects of lactate were absent in previously glycogen-depeleted muscle (Table 4). During SI there would be no fuel for glycolysis so inhibitors of glycolysis would not be expected to be deleterious. In the second place, muscle exposed to a supra-physiological concentration of glucose was protected against SI (Table 3). One might expect that the more exogenous glucose was available during SI the less vulnerable would the glycolytic synthesis of ATP become to partial inhibition of any of the enzymes in this pathway. Indeed, Table 3 shows that muscle exposed to SI in the presence of 20 mM glucose had an ATP content almost as high as that of muscle in NS, and significantly higher than that of muscle exposed to SI in the absence of glucose. In contrast, the presence of 20 mM glucose did not protect against contractures caused by ouabain or a low K concentration. Presumably this was due to the normally preserved ATP content in the latter 2 situations (Table 2). Pyruvate is a fuel for ATP synthesis, but only under aerobic conditions, and would not be expected to protect under the anaerobic conditions of SI (Table 3).

Abrupt discontinuation of a supply of lactate in NS produced a brief contracture, a depletion of ATP and a reduction in both ERP and CV (Table 5). Probably, therefore, for a time after the supply of lactate ended the glycolytic pathway was still inhibited. Oxygenated muscle, however, would be expected to obtain adequate amounts of ATP from triglyceride-derived fatty acids. Lactate, however, also inhibits the oxidation of fatty acids in the mitochondria (Bielefeld *et al.*, 1983). For a short period after discontinuation of lactate, therefore, ATP synthesis from both glucose and fatty acids would be impaired, accounting for the ATP depletion observed under these circumstances (Table 5). This explanation is also consistent with the fact that exogenous glucose at high concentration protected against all components of the response to discontinuation of lactate (Table 5), and this protection was shared by pyruvate (Table 5).

Non-steroidal anti-inflammatory drugs

The present experiments furnish four lines of evidence that sulphinpyrazone protects muscle against SI by preventing the inhibition of glycolysis by lactate. In the first place, sulphinpyrazone protected against the depletion of ATP during SI (Table 3). Secondly, it failed to protect previously glycogen-depleted muscle against SI (Table 4) and failed to protect in the absence of lactate (Table 3). Thirdly, the drug failed to modify responses to interventions which, unlike SI, elevate the diastolic Ca concentration in the myoplasm without causing ATP depletion (Table 2). Finally, sulphinpyrazone protected against responses to discontinuation of lactate (Table 5). A possible alternative site of action would be the mitochondrial oxidation of fatty acids, but this is unlikely for 2 reasons. Firstly, fatty acid oxidation would be inoperative during the anaerobiosis of SI, yet sulphinpyrazone is still protective (Table 3). Secondly, it fails to explain why previously glycogen-depleted muscle is not protected by sulphinpyrazone (Table 4).

Flufenamate potentiated all the measured responses to SI (Table 3) and to discontinuation of lactate (Table 5). Evidence was obtained from the present experiments which suggests that flufenamate exerted actions that were not just the opposite, however, of those shown by sulphinpyrazone. Thus, unlike sulphinpyrazone, the effects of flufenamate were independent of the presence of lactate (Table 3), and exerted in circumstances of overload with Ca unaccompanied by ATP depletion, such as in the presence of ouabain or a low K concentration in NS. The action of flufenamate seemed to resemble that of dinitrophenol, both of which agents are known to interfere with Ca storage by the mitochondria (Burch *et al.*, 1983; McDougall *et al.*, 1988). Flufenamate and dinitrophenol, therefore, may have enhanced the responses to SI, to ouabain and to lactate discontinuation by raising the myoplasmic Ca concentration by interfering with the storage of Ca in the mitochondria. At the concentrations used in the present experiments, however, both flufenamate and dinitrophenol are known to uncouple oxidative phosphorylation (McDougall *et al.*, 1988). Both are likely, therefore, to have interfered with

ATP production. This probably explains their ability to potentiate the SI-induced depletion of the stores of ATP (Table 3), an action likely to have been due in part at least to the activation of a mitochondrial ATP-ase enzyme (Saeki *et al.*, 1972; McDougall *et al.*, 1983).

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