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# Effects of Components of Myocardial Ischaemia on Cardiac Action Potentials In Vitro

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**Summary:** The aim of this study was to examine the effects of hypoxia ($PO_2$ = 34 mm Hg), acidosis (pH 6.8), lactate (10 m*M*), glucose removal, hyperkalaemia (8 m*M*), and lysophosphatidylcholine (5–50 μ*M*), alone and in combination, on paced sheep Purkinje fibre action potentials in an attempt to find a combination that simulated the electrophysiological changes associated with the delayed phase of ischaemia-induced arrhythmias. Modification of the physiological salt solution to produce a combination of acidosis, lactate, and lysophosphatidylcholine (5 μ*M*) reduced the resting membrane potential and the maximum rate of depolarisation and prolonged action potential duration, these effects being stable from 60 to 120 min of superfusion. Hypoxia, glucose removal, or elevation of the extracellular concentration of potassium alone or in combination with the above factors caused action potential shortening. Thus, the electrophysiological changes associated with the delayed phase of ischaemia-induced arrhythmias in vivo can be simulated in vitro by a combination of acidosis, lactate, and lysophosphatidylcholine. **Key Words:** Ischaemia—Action potentials—Purkinje fibres—Sheep.

Following coronary artery occlusion in experimental animals, arrhythmias occur in distinct phases. The early and the delayed phases occur within 1 h and between 6–48 h after occlusion, respectively (1). It has been shown that the early phase of arrhythmias is associated in vivo with a slowing in conduction velocity and an abbreviated action potential (2) whereas action potentials in Purkinje cells are prolonged during the delayed phase (3). Although it is thought that hyperkalaemia and hypoxia are two of the major causative factors underlying the characteristic action potential changes during the early phase of myocardial ischaemia (1), it is not known which metabolic and ionic changes are responsible for the later electrophysiological changes. In addition to hypoxia and hyperkalaemia, myocardial ischaemia results in acidosis, lack of substrate, and accumulation of lactate and lysophosphoglycerides (4). The aims of this study were, first, to determine how the action potential characteristics of sheep Purkinje fibres in vitro were modified by the factors thought to be of importance in the genesis of ischaemia-induced arrhythmias, i.e., elevated extracellular potassium, hypoxia, acidosis, lactate, lysophosphatidylcholine, and lack of substrate, and, second, to establish which combination of these factors mimicked the changes in action potential characteristics that occur in vivo during the delayed arrhythmic phase.

## METHODS

Sheep hearts were obtained from a local abattoir and delivered in physiological salt solution (PSS) to the laboratory within 30 min of excision. Purkinje fibres were pinned to the Silastic base of the recording chamber and superfused at a rate of 5 ml/min with a normal PSS equilibrated with 95% $O_2$/5% $CO_2$. An equilibration period of about 1 h in normal PSS was allowed before beginning the experimental protocol. The composition of the normal PSS was as follows (m*M*): NaCl, 125; $NaHCO_3$, 25; $NaH_2PO_4$, 1.2; $MgCl_2$, 1.0; KCl, 5.4; $CaCl_2$, 1.8; and glucose, 5.5. The $PO_2$ and pH of the PSS was 610 ± 25 mm Hg (measured with a $PO_2$ electrode immersed in the organ bath) and 7.4, respectively. The bath temperature was maintained at 36 ± 0.5°C.

The preparations were stimulated at a frequency of 1.5 Hz by rectangular pulses, 1 ms in duration and twice the threshold voltage, delivered through a bipolar silver electrode. Transmembrane action potentials were recorded

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using conventional microelectrode techniques. The variables measured were as follows: resting membrane potential (RMP); action potential amplitude (APA); the maximum rate of depolarization of phase 0 (MRD), which was determined by an electronic differentiating circuit; and the action potential duration at 50 and 90% repolarisation levels ($APD_{50}$ and $APD_{90}$, respectively).

### Experimental protocol

To investigate the effects of the following factors, the normal PSS was modified as detailed below: (i) The extracellular concentration of potassium was increased to 8.0 m*M* by raising the concentration of KCl. The relatively small increase in $[Cl^-]$ that resulted (from 133.2 to 135.8 m*M*) was not compensated. As in all of the other experiments where only one factor was studied, the remainder of the modified solution was as described above for the normal PSS. (ii) Hypoxia was introduced by bubbling with a gas mixture of 95% $N_2$ and 5% $CO_2$ to yield a bath $PO_2$ of 34 ± 1 mm Hg. (iii) Acidosis was achieved by lowering the $NaHCO_3$ concentration to 8.5 m*M* (metabolic acidosis). This yielded a bath pH of 6.8 ± 0.1. The fall in $[Na^+]$ was compensated by an increase of NaCl to 141.5 m*M*. (iv) Zero substrate superfusion was performed by omission of the glucose from the PSS. (v) The effect of 10 m*M* lactate was investigated by adding an appropriate volume of 8.65 *M* stock solution of sodium lactate (Fisons, Loughborough, U.K.) to the normal PSS. NaCl was reduced to 115 m*M* to compensate for the change in $[Na^+]$ produced by the sodium lactate. (vi) The effects of various concentrations of α-lysophosphatidylcholine (α-LPC) (Sigma Chemical Company, St. Louis, MO, U.S.A.) were studied by preparing daily a stock solution dissolved in distilled water. Appropriate volumes of stock solution were then added to the normal PSS to yield the required test concentrations of α-LPC (5–50 μ*M*). The following combinations of factors were studied: (vii) hyperkalaemia, hypoxia, and acidosis; (viii) hypoxia and zero substrate; (ix) lactate (10 m*M*), acidosis, and α-LPC (5 μ*M*); (x) lactate (10 m*M*), acidosis, hypoxia, and zero substrate; and (xi) lactate (10 m*M*), acidosis, hypoxia, zero substrate, and α-LPC (5 μ*M*).

In all experiments, the Purkinje fibres were allowed to equilibrate for 1–2 h in normal PSS. Control action potentials (approximately 10) were recorded and superfusion with one of the modified physiological salt solutions (modified PSS) was commenced. Action potentials (six to ten) were recorded at 15, 30, 60, 90, and 120 min following the commencement of superfusion with modified PSS. In some experiments, action potentials were not recorded at the 15 min period.

### Analysis of data

The measurements from each set of six to ten multiple impalements were averaged and the mean values used to represent the data from each preparation. Data are reported either as mean values ± SEM or mean percentage change from control values ± SEM. Multiple treatment and control mean values were analysed using a one-way analysis of variance and, where the *F* value permitted further analysis, individual treatment means were compared with respective control values by a modified Student's *t* test. A value of $p < 0.05$ was considered statistically significant.

## RESULTS

### Effects of hypoxia, acidosis, zero substrate, and elevation of extracellular concentration of $K^+$ either alone or in combination

Figures 1 and 2 illustrate the effect of acidosis, hypoxia, and elevation of $K^+$ alone and in combination on the MRD and $APD_{90}$, respectively. The only significant effect of a fall in pH from 7.4 to 6.8 was a prolongation of the APD that was stable from 30–120 min of superfusion (Fig. 2). Similarly, lowering the $PO_2$ from 610 to 34 mm Hg only affected the APD, reducing both the $APD_{50}$ and $APD_{90}$ (Fig. 2). Removal of glucose had no significant effect on the variables measured and it did not exacerbate the effect of hypoxia. For example, $APD_{90}$ was reduced from 265 ± 13 to 205 ± 13 ms for hypoxia alone and 274 ± 11 to 222 ± 14 ms (after an exposure period of 2 h) for hypoxia plus glucose removal. Elevation of the extracellular concentration of $K^+$ to 8 m*M* significantly reduced the MRD (Fig. 1) and this was accompanied by a fall in the RMP (from −87.9 ± 0.8 to −73.5 ± 1.0 mV at 120 min of superfusion) and in APA (from 118.3 ± 1.7 to 89.3 ± 4.5 mV). Both $APD_{50}$ and $APD_{90}$ (Fig. 2) were significantly reduced by 8 m*M* $K^+$. The combination of acidosis, hypoxia, and elevation of $K^+$ significantly reduced all of the measured variables within 15 min of superfusion and, as illustrated in Figs. 1 and 2 for MRD and $APD_{90}$, these changes were stable between 30 and 120 min of superfusion. The changes induced by this combination of factors were similar to those observed by elevating the concentration of $K^+$ alone with the exception that the combination also decreased the excitability. In the presence of the modified PSS, the threshold voltage was increased by a factor of about 2 in 50% of the preparations.

### Effects of lactate and lysophosphatidylcholine alone and in combination with the above factors

Addition of lactate (10 m*M*) to the PSS caused a lengthening of the $APD_{90}$ (Fig. 3) that was stable from 60–120 min of superfusion. Lactate did not significantly modify any of the other measured variables, as shown in Fig. 4 for the MRD.

In 10 preliminary experiments, the effects of several concentrations of LPC ranging from 10–50 μ*M* were examined. Concentrations of 20 μ*M* and above caused a marked depolarisation and concomitant reduction in MRD in all preparations. Of the six fibres exposed to 10 μ*M* LPC, three exhibited a marked depolarisation and formation of slow response-type action potentials in common with the effects of higher doses of LPC. In the remaining three preparations, this dose of LPC reduced the MRD by 24 ± 6% and shortened the $APD_{50}$ by 17 ± 5% without modifying the RMP. As illustrated in Figs. 3 (for $APD_{90}$) and 4 (for MRD), 5 μ*M* LPC had

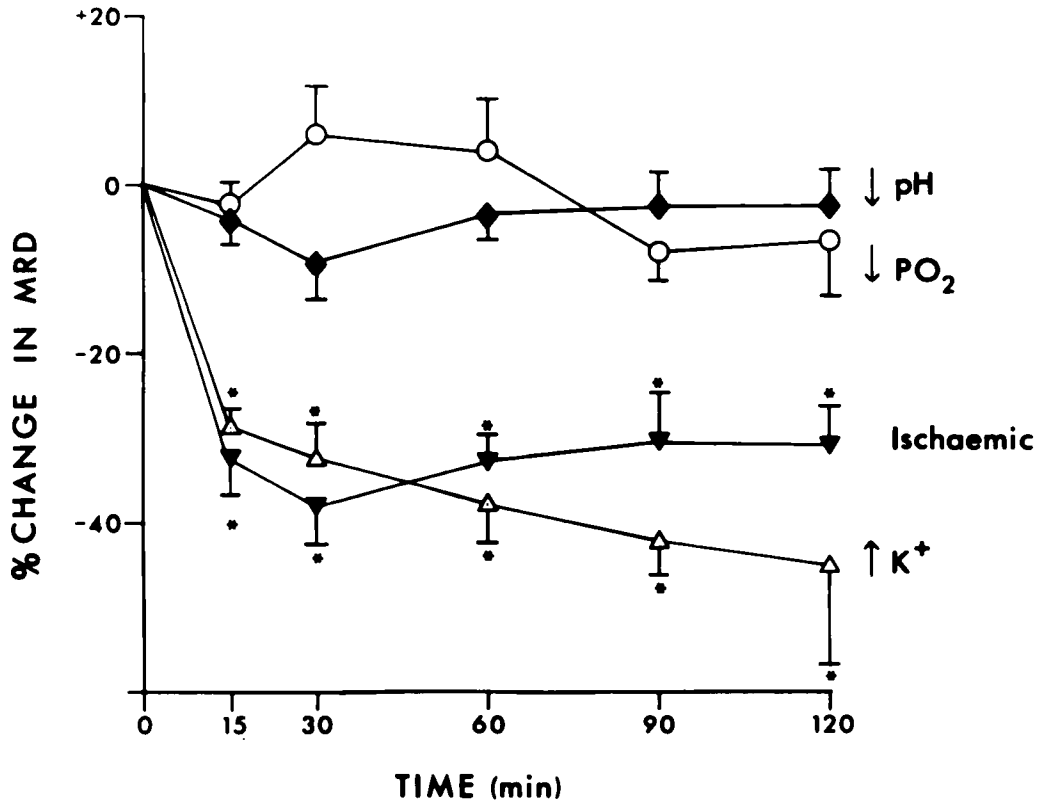


**FIG. 1.** The effect of acidosis, hypoxia, and elevation of $K^+$ alone and in combination (ischaemic) on MRD of sheep Purkinje fibres. n = 5 or 6; *significantly different from zero. Control values of MRD for these experimental groups ranged from 486 ± 27 to 604 ± 25 V/s.

no significant effect on any of the variables measured. However, the combination of LPC (5 μ*M*) with acidosis and lactate (10 m*M*) significantly reduced the MRD (Fig. 4) and this was accompanied by a reduction in RMP and a slight prolongation of $APD_{50}$ (of 6 ± 2 and 7 ± 5%, respectively, at 120 min of superfusion). As shown in Fig. 3, the $APD_{90}$ was also slightly increased between 30 and 120 min of superfusion with this modified PSS. This depressant effect on MRD did not reach statistical significance until 1 h of superfusion.

Figure 5 illustrates the effect on RMP, MRD, and $APD_{90}$ of a combination of acidosis (pH 6.8), lactate (10 m*M*), lysophosphatidylcholine (5 μ*M*), zero glucose, and hypoxia. Statistically significant reductions in RMP and MRD were observed by 30 min of superfusion and these changes were stable between 60 and 120 min of exposure. The $APD_{50}$ tended to shorten (by 11 ± 7% at 120 min) and $APD_{90}$ to lengthen (Fig. 5), but these differences did not reach statistical significance. If LPC was not included in the above combination, there was a marked shortening of both $APD_{50}$ and $APD_{90}$ (by 40 ± 15 and 16 ± 11%, respectively, at 120 min). Elevation of the $K^+$ concentration to 6 or 8 m*M*, in addition to the above combinations, resulted in a marked shortening of the action potential.

## DISCUSSION

Raising the extracellular concentration of potassium caused a fall in the RMP and MRD and shortened the action potential of sheep Purkinje fibres. These effects are similar to those reported in other species (5). Increasing the extracellular concentration of $K^+$ reduces the potassium gradient across the cell membrane, thereby lowering the RMP according to the Nernst equation. This fall in RMP causes partial inactivation of the sodium channels and a consequent fall in MRD and APA. The $K^+$-induced shortening of the action potential is caused mainly by an increased conductance of the delayed rectifier (6) and the background $I_{K1}$ channels (7).

Lowering the $PO_2$ to around 30 mm Hg also abbreviated the sheep Purkinje fibre action potential without modifying the other action potential characteristics. This result agrees with earlier work that showed that hypoxia, if severe or prolonged, can reduce the Purkinje APD (8). The hypoxic reduction in the Purkinje action potential was to about 80% of control and this change is much less marked than that observed in ventricular muscle (9). In ventricular tissue, it is thought that hypoxia may cause action potential shortening by opening of ATP-

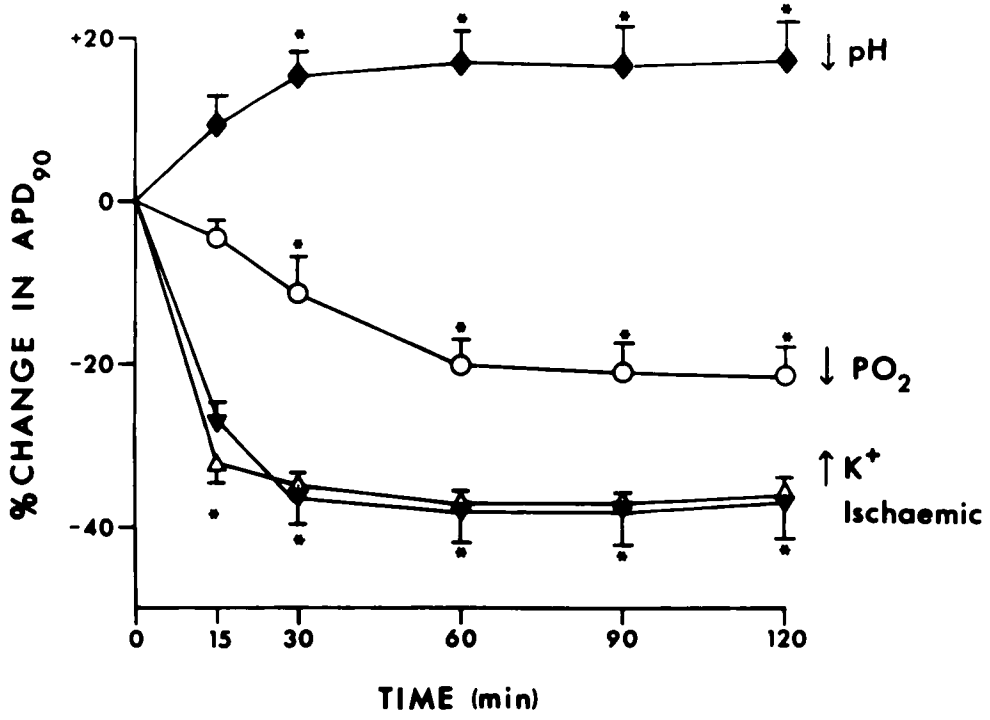


**FIG. 2.** The effect of acidosis, hypoxia, and elevation of $K^+$ alone and in combination (ischaemic) on $APD_{90}$ of sheep Purkinje fibres. n = 5 or 6; *significantly different from zero. Control values of $APD_{90}$ for these experimental groups ranged from 253 ± 13 to 265 ± 13 ms.

dependent $K^+$ channels (10) although a reduction in $Ca^{2+}$ current has not been ruled out (11). It is not known which, if either, of these two mechanisms is responsible for the hypoxic shortening observed in Purkinje tissue.

The only significant effect of an acidosis of pH 6.8 on sheep Purkinje fibres was to prolong the APD, especially the terminal repolarisation phase ($APD_{90}$). This finding is in agreement with previous work on dog Purkinje fibres (12) but the ionic mechanism underlying this prolongation has not been elucidated. In single ventricular cells, a more severe acidosis of approximately 4 pH units has been shown to increase rather than to reduce outward

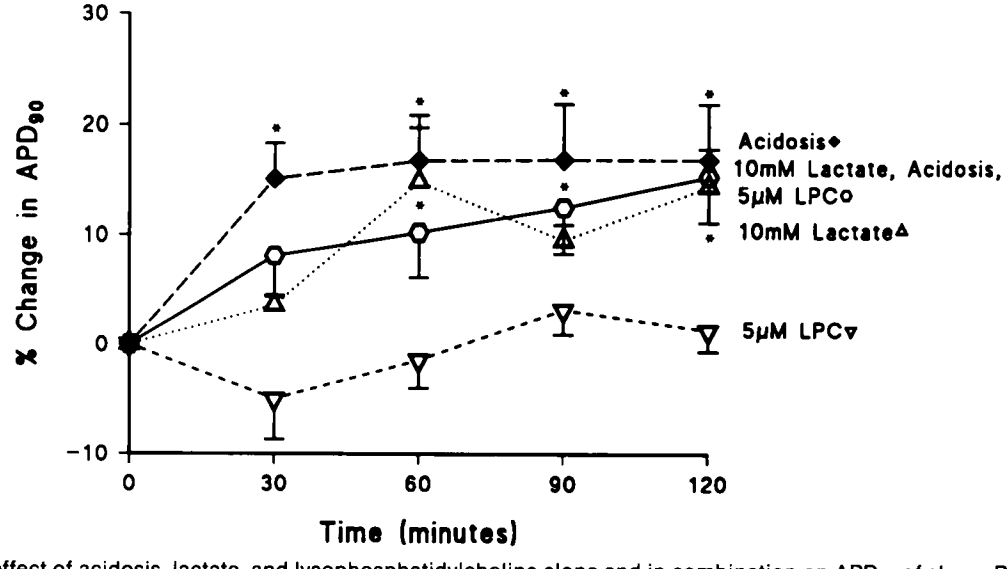


**FIG. 3.** The effect of acidosis, lactate, and lysophosphatidylcholine alone and in combination on $APD_{90}$ of sheep Purkinje fibres. n = 3–6; *significantly different from zero. Values of $APD_{90}$ for these experimental groups ranged from 261 ± 17 to 323 ± 55 ms (n = 3 for LPC group).

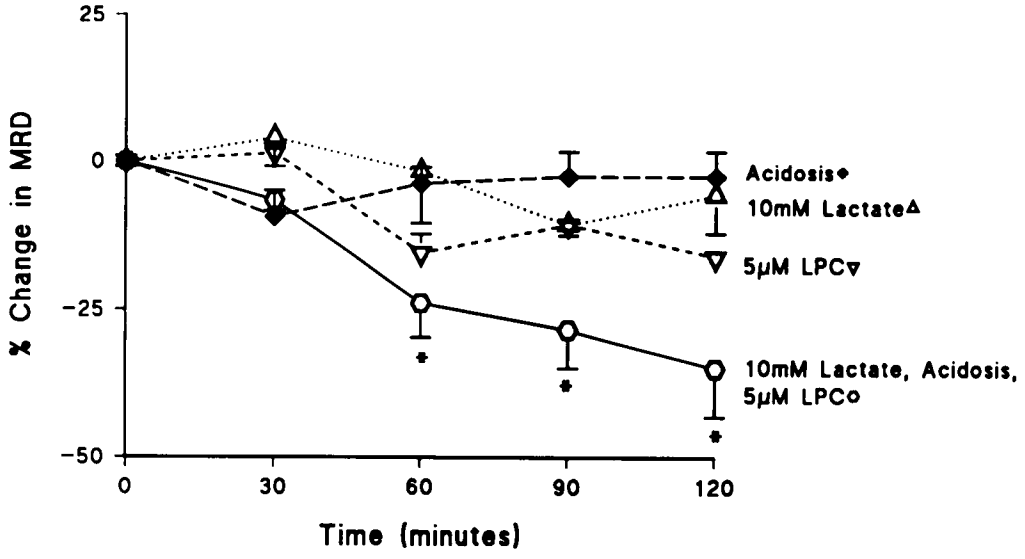


**FIG. 4.** The effect of acidosis, lactate, and lysophosphatidylcholine alone and in combination on MRD of sheep Purkinje fibres. n = 3–6; *significantly different from zero. Control values for MRD for these experimental groups ranged from 528 ± 32 to 604 ± 25 V/s.

current (13) and to reduce the inward calcium current, resulting in a shortening of the APD. Further work, therefore, is required on single Purkinje cells to investigate the ionic mechanisms that are responsible for the acidosis-induced prolongation of the action potential.

In combination, acidosis, hypoxia, and elevated extracellular $K^+$ produced stable and marked reductions in all of the measured action potential variables. The quantitative changes were not significantly different from those observed with hyperkalaemia alone. The only additional effect of the combination was that a reduction in excitability, not seen with elevation of extracellular $K^+$ alone, was also obtained. This particular combination of factors has been used by other workers (14,15) to mimic the characteristic changes induced by early ischaemia. As the changes are stable from 30 to 120 min of superfusion with the modified PSS, drug-induced effects under simulated ischaemic conditions can be easily studied over this time period.

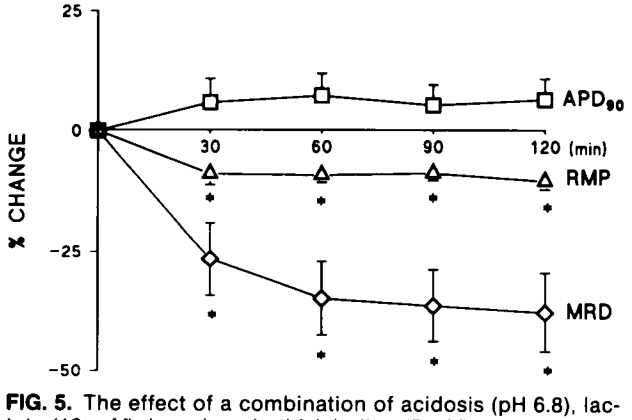


**FIG. 5.** The effect of a combination of acidosis (pH 6.8), lactate (10 m*M*), lysophosphatidylcholine (5 μ*M*), zero glucose, and hypoxia on RMP, MRD, and $APD_{90}$ of sheep Purkinje fibres. n = 5; *significantly different from zero. Control values for RMP, MRD, and $APD_{90}$ were −85.3 ± 1.1 mV, 548 ± 17 V/s, and 225 ± 9 ms, respectively.

Lactate (10 m*M*) increased the $APD_{90}$ but did not affect any of the other action potential characteristics. These concentrations of lactate are similar to those detected in vivo during myocardial ischaemia (16). A higher concentration of lactate (40 m*M*) has been reported to cause a shortening of sheep Purkinje fibre action potentials concomitant with a reduction in the RMP (17), suggesting that there are different ionic effects of lactate dependent upon the concentration used.

LPC in concentrations (20–50 μ*M*) similar to those measured during myocardial ischaemia (18) produced marked depolarisation and resultant "slow-response" action potentials or in excitability. A lower concentration of 5 μ*M* caused less marked effects on the upstroke and abbreviated the plateau phase of the action potential. LPC has been shown to reduce the conductance of the background potassium channel (19) and to reduce the inward calcium current (20) actions, which would explain the fall in RMP and abbreviation of the plateau, respectively.

The combination of the above factors that most closely mimicked the changes seen in vivo in Purkinje fibres following 24 h of ischaemia, namely a fall in RMP and in upstroke and a prolongation of the terminal phase of action potential, was lactate (10 m*M*), acidosis (pH 6.8), and LPC (5 μ*M*). Individually, none of these factors produced this profile

of effects. Acidosis has been shown to potentiate the effects of LPC (21), which probably explains why depressant effects on the RMP and upstroke were observed in the combination, but not when given alone at this concentration. The increase in $APD_{90}$ produced by this combination was no greater than the individual effects of lactate and acidosis, which suggests that both factors lengthen the APD by the same mechanism(s). Glucose removal and hypoxia, when combined with the above factors, produced a slight prolongation of the $APD_{90}$ but the changes were too small to be statistically significant. Elevating the extracellular $K^+$ in combination with the above factors led to a marked shortening of the APD, suggesting that Purkinje fibres are unlikely to be exposed to hyperkalaemia during "late" ischaemia.

It is of interest to note that this combination of factors, i.e., acidosis (pH 6.8), lactate (10 m*M*), and LPC (5 $\mu M$), produced a similar profile of effects on single ventricular myocytes (22) to that seen on Purkinje fibres in the present study. These findings suggest that combined acidosis, lactate, and LPC may serve as a useful in vitro model of the delayed phase of myocardial ischaemia in both Purkinje and ventricular tissue. In the case of the Purkinje tissue, the changes induced by the mixture simulating "late" ischaemia were shown to be relatively stable between 60 and 120 min of superfusion, during which time the effects of drugs could be monitored. It would be of interest to investigate whether the effects of antiarrhythmic drugs are modified by this "late" ischaemic mixture as indeed many of them are by conditions simulating early ischaemia (15). However, it should also be noted that attempts to simulate myocardial ischaemia in vitro do not fully reproduce the situation in vivo and as a result extrapolation from in vitro to in vivo findings is of limited value.

In conclusion, in sheep Purkinje fibres, modification of the PSS to combine acidosis, lactate, and LPC was shown to mimic the electrophysiological changes observed in vivo following a prolonged period of myocardial ischaemia.

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