Modification by hypoxia, hyperkalaemia and acidosis of the cardiac electrophysiological effects of a range of antiarrhythmic drugs

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1 The electrophysiological effects of a series of drugs with Class I antiarrhythmic activity were examined in sheep Purkinje fibres, superfused *in vitro* with either a normal or hypoxic, hyperkalaemic and acidotic physiological salt solution (PSS).

2 In normal sheep Purkinje fibres, lignocaine, disopyramide, nicainoprol and propranolol all significantly reduced action potential height and the maximum rate of depolarization of phase zero (MRD) and abbreviated the action potential, without modifying resting membrane potential (RMP).

3 Verapamil at the highest concentration studied, 8 μM , significantly reduced MRD with an associated slight membrane depolarization and abbreviated action potential duration measured at 50% repolarization (APD_{50}).

4 Superfusion of sheep Purkinje fibres with a hypoxic, hyperkalaemic and acidotic PSS resulted in marked reductions in resting membrane potential, upstroke and duration of the action potential.

5 In the presence of modified PSS, lignocaine, propranolol and verapamil all reduced MRD to a greater extent than in normal PSS. The effects of nicainoprol on MRD were not affected whereas those of disopyramide were significantly attenuated.

6 Under simulated ischaemic conditions, lignocaine, propranolol and nicainoprol did not produce a concentration-dependent reduction in action potential duration whereas disopyramide and verapamil, respectively, prolonged and abbreviated both APD_{50} and APD_{90} .

7 The Na^+ channel blocking actions of the different subtypes of Class I antiarrhythmic agents studied, as well as their effects on action potential duration, were modified differently by simulated ischaemia.

Keywords: Simulated ischaemia; cardiac electrophysiology; antiarrhythmic drugs

Introduction

Class I antiarrhythmic drugs, i.e. those that act principally by blocking Na^+ channels have been subdivided on the basis of their clinical electrophysiological effects (Harrison, 1985) and their effects *in vitro* on the kinetics of onset and offset interaction with the sodium channel (Campbell, 1983a) into 3 subgroups. Disopyramide (Campbell, 1983b), lignocaine (Bean *et al.*, 1983) and nicainoprol (Kimura *et al.*, 1989), a recently introduced antiarrhythmic drug, are examples of Class Ia, Ib and Ic agents, respectively. The sodium channel blocking activity of lignocaine is markedly potentiated by conditions that simulate the acute phase of myocardial ischaemia, i.e. hypoxia, hyperkalaemia and acidosis (Kimura *et al.*, 1982; Evans *et al.*, 1984). However, no studies have been carried out to compare how simulated ischaemia modifies the effects of each of the subtypes of Class I antiarrhythmic drugs. The aim of this study, therefore, was to compare the electrophysiological effects on sheep Purkinje fibres, of disopyramide, lignocaine, and nicainoprol, as examples of drugs of the Ia, Ib and Ic subgroups, under normal and simulated ischaemic conditions. In addition, the effects of other antiarrhythmic drugs that may in addition to their other pharmacological effects also block Na^+ channels, i.e. propranolol and verapamil, were also investigated. Propranolol was studied because its effects during simulated myocardial ischaemia have not been described and verapamil because conflicting results have been obtained with respect to its action under ischaemic conditions (Dersham & Han, 1981; Northover, 1987).

Methods

Action potential recording

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^{-1} with a normal PSS equilibrated with 95% O_2 /5% CO_2 . An equilibrium period of about 1 h in normal PSS was allowed. The composition of the control PSS was as follows (mM): 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. Acute ischaemia was simulated by a hypoxic, hyperkalaemic and acidotic PSS of the following composition (mM): NaCl 141.5, NaHCO_3 8.5, NaH_2PO_4 1.2, MgCl_2 1.0, KCl 8.0, CaCl_2 1.8 and glucose 5.5. This modified PSS was gassed with 95% N_2 /5% CO_2 which yielded a PO_2 and a pH in the organ bath of 33.9 ± 1 mmHg and 6.8 ± 0.01 u, respectively.

The preparations were stimulated at a frequency of 1.5 Hz by rectangular pulses, 1 ms in duration and twice threshold voltage, delivered through a bipolar silver electrode. Transmembrane action potentials were recorded by 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% repolarization levels (APD_{50} and APD_{90}).

Experimental protocol

To observe the drug effects on normal preparations, 8–10 consecutive action potentials were recorded before and

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30–40 min after cumulative addition of the drug, dissolved in reservoirs of gassed normal PSS to obtain the required final bath concentrations. In a different set of fibres, the effects of the modified PSS alone was measured; action potentials were recorded before and at 30, 60, 90 and 120 min following exposure to the modified PSS. Drug-induced effects in the presence of the modified PSS were examined by recording action potentials before, and 30 min after, superfusion with modified PSS alone and subsequently following the administration of three cumulative concentrations of the drug dissolved in modified PSS. Action potentials were again recorded 30 min after the addition of each concentration of the drug.

Analysis of data

The measurements from each set of 8 to 10 action potentials were meaned and the mean values used to represent the data from each preparation. Data are given either as mean values $\pm$ s.e. mean or mean percentage change from control values $\pm$ s.e. mean and are derived from four to eight experiments. Drug effects in the presence of the modified PSS were calculated as a % change from the values at 30 min superfusion with modified PSS alone. Multiple treatment and control mean values were analysed by a one-way analysis of variance for repeated measures and, where the *F* value permitted further analysis, individual treatment means were compared with respective control values by a modified Student's *t* test. For all other comparisons a two-tailed Student's *t* test was carried out. A value of $P < 0.05$ was considered to be statistically significant.

In order to assess the degree of potentiation of drug effects by simulated ischaemia on MRD, log concentration vs % reduction in the maximum response curves were drawn in the presence and absence of simulated ischaemia and the EC_{30} , i.e. the molar concentrations that produced a 30% reduction in the maximum response for MRD calculated. The EC_{30} rather than the EC_{50} was used because in some cases the log concentration-response curves were incomplete.

Results

Effect of hypoxia, hyperkalaemia, and acidosis

The effects of the combination of hypoxia, hyperkalaemia and acidosis on the action potential variables are given in Table 1. Hypoxia, hyperkalaemia and acidosis caused a fall in RMP, MRD and action potential amplitude, and markedly abbreviated the action potential. The changes are stable over the 30–120 min exposure period, i.e. the time period over which the action of the drug was measured.

Effects of lignocaine

Figure 1 illustrates the effects of lignocaine on MRD, APD_{50} and APD_{90} of paced sheep Purkinje fibres under normal and

Figure 1 The % reduction in maximum rate of depolarization of the upstroke (MRD), action potential duration measured at 50% (APD_{50}) and 90% of repolarization (APD_{90}) induced by lignocaine (3.5–28 μ M) in sheep Purkinje fibres superfused with normal (open columns) and modified (stippled columns) physiological salt solution. Control values for MRD, APD_{50} and APD_{90} were 523 ± 46 Vs $^{-1}$, 132.6 ± 13.4 and 206.9 ± 11 ms in normal fibres and 383 ± 41 Vs $^{-1}$, 114.5 ± 8.6 and 182.5 ± 8.5 ms in fibres after 30 min exposure to simulated ischaemia, respectively.

* $P < 0.05$ indicates a significant difference from zero. $n = 5-6$.

hypoxic, hyperkalaemic and acidotic conditions. On normal Purkinje fibres, only the two higher concentrations of lignocaine, i.e. 28 and 56 μ M (data not shown for 56 μ M), significantly reduced MRD and the action potential amplitude without modifying the resting membrane potential. In contrast, in the presence of hypoxia, hyperkalaemia and acidosis, lignocaine caused a concentration-dependent reduction in MRD over the full concentration-range studied (3.5 to 28 μ M) and this also occurred without modification of the resting membrane potential. This effect of lignocaine on MRD was greater under simulated ischaemia than in normal conditions. The log concentration vs % reduction in the maximum response curve was shifted to the left and the ratio of EC_{30} 's in the absence and the presence of simulated ischaemia was 4.2, i.e. lignocaine was about 4 times as potent in reducing MRD in fibres exposed to hypoxia, hyperkalaemia and acidosis as in normal fibres.

Table 1 The effects of a modified (i.e. hypoxic, hyperkalaemic, and acidotic) salt solution on sheep Purkinje fibre action potential characteristics

Time (min)	RMP (mV)	MRD (Vs $^{-1}$)	APA (mV)	APD_{50} (ms)	APD_{90} (ms)
0	88.3 ± 1.2	486 ± 27	121.2 ± 2.3	162.1 ± 8.0	264.6 ± 13.7
15	$75.2 \pm 2.5^*$	$332 \pm 36^*$	$96.2 \pm 4.1^*$	$119 \pm 6.4^*$	$190.8 \pm 9.2^*$
30	$74.2 \pm 1.0^*$	$290 \pm 26^*$	$88 \pm 3.3^*$	$95.8 \pm 6.2^*$	$166.8 \pm 3.9^*$
60	$73.7 \pm 1.4^*$	$325 \pm 21^*$	$90.4 \pm 2.4^*$	$86.3 \pm 8.3^*$	$160.0 \pm 4.5^*$
90	$73.0 \pm 1.5^*$	$336 \pm 30^*$	$90.6 \pm 2.6^*$	$86.7 \pm 8.0^*$	$161.3 \pm 5.0^*$
120	$74.6 \pm 0.7^*$	$323 \pm 30^*$	$90.9 \pm 1.7^*$	$86.2 \pm 8.6^*$	$163.9 \pm 5.2^*$

$n = 6$; * $P < 0.05$ significantly different from values at 0 min. Between 30 and 120 min post simulated ischaemia there were no statistically significant differences in the variables measured.

On normal fibres, lignocaine reduced APD_{50} and APD_{90} at concentrations lower than those required to reduce MRD (Figure 1). In the presence of hypoxia, hyperkalaemia and acidosis, however, although all concentrations of lignocaine studied reduced APD_{50} and APD_{90} , no concentration-dependent effect on action potential duration was observed.

Effects of disopyramide

Figure 2 compares the effects of disopyramide (2.95–23.6 μM) under normal and simulated ischaemic conditions on MRD, APD_{50} and APD_{90} . On normal fibres, these concentrations of disopyramide caused a concentration-dependent reduction in MRD without altering the resting membrane potential. In contrast to lignocaine, this depressant effect on MRD was not potentiated by simulated ischaemia and indeed at the two lower concentrations studied (2.95 and 11.5 μM), the % reduction in MRD was significantly less ($P < 0.05$) in the presence of hypoxia, hyperkalaemia and acidosis than that seen in normal fibres.

Disopyramide also caused a concentration-dependent reduction in action potential duration in normal fibres, but under simulated ischaemic conditions the only statistically significant change was a prolongation of APD_{90} observed with the highest concentration studied i.e., 23.6 μM .

Effects of nicainoprol

The effects of nicainoprol on action potential characteristics of normal fibres and those exposed to hypoxia, hyperkalaemia and acidosis are given in Tables 2 and 3, respectively. The % changes in MRD, APD_{50} and APD_{90} induced by the drug under both sets of conditions are compared in Figure 3. Nicainoprol also reduced MRD and action potential amplitude without changing the resting membrane potential both under normal and simulated ischaemic conditions. Although simulated ischaemia tended to increase the depressant effect of nicainoprol on the upstroke velocity of the action potential, the difference between its effects on MRD under normal and simulated ischaemic conditions just failed to reach statistical significance. The dose-ratio for the EC_{30} 's in the presence and absence of simulated ischaemia was 2.2.

Like lignocaine, nicainoprol abbreviated the APD_{50} of normal fibres at a concentration (1 μM) lower than that which depressed MRD. In the presence of hypoxia, hyperkalaemia and acidosis, the reduction in action potential duration was significantly less than that observed in normal fibres, e.g. at a concentration of 5 μM , APD_{50} was reduced from 171.2 ± 13.1 to 88.0 ± 15.3 ms in normal fibres and from 144.7 ± 4.7 to 110.9 ± 5.4 ms in the presence of simulated ischaemia.

Figure 2 The % reduction in maximum rate of depolarization of the upstroke (MRD), action potential duration measured at 50% (APD_{50}) and 90% of repolarization (APD_{90}) induced by disopyramide (2.95–23.6 μM) in sheep Purkinje fibres superfused with normal (open columns) and modified (stippled columns) physiological salt solution. Control values for MRD, APD_{50} and APD_{90} were $635 \pm 21 \text{ Vs}^{-1}$, 213 ± 19 and 320 ± 15 ms in normal fibres and $417 \pm 26 \text{ Vs}^{-1}$, 106.8 ± 6.1 and 167.5 ± 7.0 ms in fibres after 30 min exposure to simulated ischaemia, respectively.

* $P < 0.05$ indicates a significant difference from zero. $n = 4-5$.

Effects of propranolol

Figure 4 illustrates the effects of propranolol (0.35–2.8 μM) on the upstroke velocity and duration of the action potential under normal and hypoxic, hyperkalaemic and acidotic conditions. Like lignocaine, propranolol caused a reduction in MRD and the log concentration vs % maximum response curve was shifted to the left in the presence of simulated ischaemia. The ratio of the EC_{30} 's in the absence and presence of simulated ischaemia was 5.3. Also like lignocaine, propranolol caused an abbreviation of the action potential,

Table 2 Effects of nicainoprol (0.1–10 μM) on the action potential characteristics of normal sheep Purkinje fibres paced at 1.5 Hz

	Control	0.1	Nicainoprol (μM)		
			1	5	10
RMP (mV)	-84.0 ± 1.3	-84.3 ± 1.3	-85.5 ± 1.4	-85.0 ± 0.9	-84.9 ± 1.2
MRD (Vs^{-1})	476 ± 22	494 ± 36	444 ± 37	$359 \pm 36^*$	$271 \pm 32^*$
APA (mV)	114.7 ± 1.7	114.1 ± 1.5	112.7 ± 2.5	$103.5 \pm 2.4^*$	$91.6 \pm 2.2^*$
APD_{50} (ms)	171.2 ± 13.1	162.5 ± 14.4	$138.0 \pm 14.4^*$	$88.0 \pm 5.3^*$	$68.1 \pm 12.3^*$
APD (ms)	252.5 ± 17.4	249.4 ± 19.9	229.3 ± 17.3	$180.3 \pm 13.3^*$	$156.9 \pm 8.3^*$

$n = 5$; * $P < 0.05$ significantly different from control value.

Abbreviations are RMP: resting membrane potential; MRD: maximum rate of depolarization of phase 0; APA: action potential amplitude; APD_{50} and APD_{90} : action potential duration measured at 50 and 90% repolarization respectively.

Table 3 Effects of nicainoprol ($0.1 \pm 5 \mu\text{M}$) on sheep Purkinje action potential characteristics during simulated ischaemia (i.e. under conditions of hyperkalaemia, hypoxia and acidosis)

	Normal control	Ischaemia alone	Ischaemia + nicainoprol (μM)		
			0.1	1	5
RMP (mV)	-86.3 ± 0.8	$-78.9 \pm 0.4^*$	$-79.0 \pm 0.6^*$	$-79.9 \pm 0.4^*$	$-78.2 \pm 0.5^*$
MRD (Vs^{-1})	467 ± 19	$238 \pm 21^*$	$225 \pm 22^*$	$185 \pm 19^{*,\dagger}$	$157 \pm 21^{*,\dagger}$
APA (mV)	117.1 ± 1.5	$88.9 \pm 2.7^*$	$87.7 \pm 2.6^*$	$83.6 \pm 3.2^{*,\dagger}$	$80.0 \pm 3.1^{*,\dagger}$
APD ₅₀ (ms)	197.2 ± 8.1	$144.7 \pm 4.9^*$	$132.6 \pm 4.5^{*,\dagger}$	$123.4 \pm 5.4^{*,\dagger}$	$110.9 \pm 5.4^{*,\dagger}$
APD ₉₀ (ms)	279.5 ± 12.2	$205.9 \pm 5.8^*$	$197.4 \pm 6.6^*$	$192.3 \pm 8.4^{*,\dagger}$	$186.5 \pm 8.1^{*,\dagger}$

$n = 6$; $^*, \dagger P < 0.05$ significantly different from normal control value and from ischaemic control value respectively.

Abbreviations are RMP: resting membrane potential; MRD: maximum rate of depolarization of phase 0; APA: action potential amplitude; APD₅₀ and APD₉₀ - action potential duration measured at 50 and 90% repolarization respectively.

Figure 3 The % reduction in maximum rate of depolarization of the upstroke (MRD), action potential duration measured at 50% (APD₅₀) and 90% of repolarization (APD₉₀) induced by nicainoprol ($0.1\text{--}5 \mu\text{M}$) in sheep Purkinje fibres superfused with normal (open columns) and modified (stippled columns) physiological salt solution. Control values for MRD, APD₅₀ and APD₉₀ were $476 \pm 22 \text{ Vs}^{-1}$, 171.2 ± 13.1 and $252.5 \pm 17.4 \text{ ms}$ in normal fibres and $238 \pm 21 \text{ Vs}^{-1}$, 144.7 ± 4.9 and $205.9 \pm 5.8 \text{ ms}$ in fibres after 30 min exposure to simulated ischaemia, respectively.

$^*P < 0.05$ indicates a significant difference from zero. $n = 5\text{--}6$.

under normal conditions, at a concentration that did not significantly reduce MRD. Under simulated ischaemic conditions, propranolol did not abbreviate the action potential in a concentration-dependent manner. Propranolol did not significantly change the resting membrane potential in these experiments.

Effects of verapamil

The effects of verapamil ($0.2\text{--}8 \mu\text{M}$) on MRD and action potential duration in normal and simulated ischaemic fibres

Figure 4 The % reduction in maximum rate of depolarization of the upstroke (MRD), action potential duration measured at 50% (APD₅₀) and 90% of repolarization (APD₉₀) induced by propranolol ($0.35\text{--}2.8 \mu\text{M}$) in sheep Purkinje fibres superfused with normal (open columns) and modified (stippled columns) physiological salt solution. Control values for MRD, APD₅₀ and APD₉₀ were $657 \pm 55 \text{ Vs}^{-1}$, 180 ± 18 and $281.5 \pm 30 \text{ ms}$ in normal fibres and $435 \pm 57 \text{ Vs}^{-1}$, 106.6 ± 9.2 and $170.4 \pm 10.1 \text{ ms}$ in fibres after 30 min exposure to simulated ischaemia, respectively.

$^*P < 0.05$ indicates a significant difference from zero. $n = 4\text{--}6$.

are shown in Figure 5. Only the highest concentration studied, i.e. $8 \mu\text{M}$, significantly reduced MRD in normal fibres and this was accompanied by a slight fall in resting membrane potential from -86.5 ± 0.4 to $-81.3 \pm 0.3 \text{ mV}$ and a reduction in action potential amplitude from 125 ± 3 to $100 \pm 4 \text{ mV}$ ($P < 0.05$). A concentration-dependent shortening of the action potential duration measured at 50% of repolarization was observed whereas APD₉₀ was unaffected (Figure 5).

The effect of verapamil on MRD was potentiated in the presence of hypoxia, hyperkalaemia and acidosis (Figure 5).

Figure 5 The % reduction in maximum rate of depolarization of the upstroke (MRD), action potential duration measured at 50% (APD₅₀) and 90% of repolarization (APD₉₀) induced by verapamil (0.2–8 μM) in sheep Purkinje fibres superfused with normal (open columns) and modified (stippled columns) physiological salt solution. Control values for MRD, APD₅₀ and APD₉₀ were 653 ± 28 V s⁻¹, 171 ± 247 and 320 ± 15 ms in normal fibres and 412 ± 32 V s⁻¹, 105.8 ± 9.7 and 171.2 ± 12.3 ms in fibres after 30 min exposure to simulated ischaemia respectively.

* $P < 0.05$ indicates a significant difference from zero. $n = 4-8$.

Half (4 out of 8) of those fibres exposed to simulated ischaemia and the highest concentration of verapamil became inexcitable. The log concentration vs % maximum response curve was shifted to the left in fibres exposed to simulated ischaemia and the ratio of the EC₅₀'s was 2.9 in the absence and presence of simulated ischaemia. In contrast to its effect on normal action potential duration, verapamil also abbreviated the APD₉₀ of fibres exposed to hypoxia, hyperkalaemia and acidosis. The effect of the highest dose of verapamil was variable, due in part, to the small number of fibres surviving this concentration of the drug.

Discussion

On normal sheep Purkinje fibres, all of the drugs studied reduced the upstroke of the action potential, albeit that with verapamil, for example, such an effect was only observed with the highest concentration studied. This reduction in MRD and action potential amplitude was observed without concomitant changes in resting membrane potential and is indicative of Na⁺ channel blocking activity. The action potential duration at both 50 and 90% of repolarization was also reduced by all of the drugs with the exception of verapamil which abbreviated APD₅₀ only. Such effects on Purkinje action potential duration have previously been reported for lignocaine (Coraboeuf *et al.*, 1988), nicanoprol (Hamon *et al.*, 1984), propranolol (Davis & Tempte, 1968) and verapamil (Dersham & Han, 1981). Disopyramide has previously been shown to produce complex effects on Purkinje action potential duration which depend upon whether or not a gate region is studied and the external potassium concentration (Kus & Sasyniuk, 1975). The effects of these

drugs on action potential duration may be caused by an action on several ionic currents. Lignocaine and disopyramide have been shown to be blockers of the tetrodotoxin-sensitive sodium 'window' current that maintains the plateau in these Purkinje cells (Coraboeuf *et al.*, 1988). Our results with lignocaine, propranolol and nicanoprol suggest that these drugs may block this 'window' current at concentrations lower than those required to block the fast Na⁺ current as the plateau phase of the action potential was shortened at lower concentrations than those required to reduce MRD. However, voltage clamp experiments to measure the drug effects on these two currents would be necessary to provide evidence for this suggestion. Verapamil is thought to attenuate the plateau by reducing the inward calcium current (Tytgat *et al.*, 1988) but as it also reduces the delayed outward current the terminal phase of repolarization is not shortened (Kass & Tsien, 1975).

Superfusion of sheep Purkinje fibres with a hyperkalaemic, hypoxic and acidotic PSS caused marked reductions in the resting membrane potential, the upstroke and duration of the action potential. Similar effects of this combination of factors in *in vitro* preparations have been reported previously (Evans *et al.*, 1984) and such changes have also been shown *in vivo* during the early phase of myocardial ischaemia (Downar *et al.*, 1977). The reduction in resting membrane potential and upstroke of the action potential are principally due to the raised extracellular concentration of K⁺ whereas both hypoxia and the elevated K⁺ concentration contribute to the shortening of the duration in this preparation (Pacini & Kane, 1991). Raising the extracellular concentration of K⁺ causes depolarization (due to the reduction in the ratio of intracellular to extracellular K⁺ concentrations) which, in turn, would cause partial inactivation of the sodium current and reduce the upstroke velocity of the action potential. Elevation of the extracellular K⁺ concentration also induces an increase in the background K⁺ current (I_{K1} ; Sakmann & Trube, 1984) which would also lead to shortening of the action potential duration, although a depolarization-induced inactivation of the Na⁺ window current may also play a role in this observed effect. Although hypoxia has been shown to shorten Purkinje action potential duration under our experimental conditions the ionic basis of this has not been elucidated.

In the presence of simulated ischaemia, the depressant effects on the upstroke velocity of the action potential of lignocaine, propranolol and verapamil were all potentiated with the potentiation being greatest for propranolol and least for verapamil. In the case of lignocaine it has been shown that this potentiation of its Class I effect is mainly due to the marked depolarization produced by hyperkalaemia (Furuta *et al.*, 1982). This depolarization leads to an increase in the proportion of the sodium channel population in the inactivated state and since lignocaine is thought to have a greater affinity for this state of the channel than the activated or rested state (Hondeghe & Katzung, 1977; Wasserstrom & Solata, 1988) this results in potentiation of its blocking activity on the channel. As the depressant effect of propranolol and verapamil on MRD was potentiated in the presence of hypoxia, hyperkalaemia and acidosis this may suggest that these drugs, like lignocaine, may also bind preferentially to the Na channel in its inactivated rather than open or rested state. However, we have no other direct evidence to support this suggestion. In this study we did not observe, as has been previously reported (Kimura *et al.*, 1982; Northover, 1987), that verapamil could attenuate the reduction in MRD produced by simulated ischaemia. The differences in the choice of tissue (cardiac muscle as compared with Purkinje tissue) and in the experimental protocol (the drug being given before rather than during simulated ischaemia) may explain the differences noted in the previous studies compared to our own. Although the effects of nicanoprol on MRD tended to be potentiated in the presence of simulated ischaemia, the difference was not statis-

tically significant. It has, nevertheless, been reported that nicainoprol does preferentially bind to inactivated Na channels (Weirich & Antoni, 1988). The reason why a marked potentiation of its Class I effect was not observed in the present study is not known but it is perhaps of importance that the preferential binding of the drug to inactivated Na channels was noted with concentrations higher than those used in this study. Of the five drugs studied, disopyramide was the only one which exhibited an attenuation rather than a potentiation of its Class I effect during simulated ischaemia. This finding is in line with the observations of Gruber & Carmeliet (1989) who have shown that disopyramide preferentially blocks the Na channel in its open state.

The effects of all the drugs studied on action potential duration were modified in the presence of simulated ischaemia. The abbreviation of the Purkinje action potential (APD₅₀ and APD₉₀) induced by lignocaine, propranolol and nicainoprol was attenuated in the presence of hyperkalaemia, hypoxia and acidosis. The ionic mechanism(s) underlying this difference in action is not known. Assuming that these drugs share a common mechanism of action in reducing the Na⁺ window current which maintains the plateau in Purkinje fibres, it is possible that if this current is inactivated during simulated ischaemia (as discussed above) the ability of the drugs to reduce action potential duration under simulated ischaemic conditions, would be expected to be attenuated. In

the case of disopyramide, a slight prolongation with the highest concentration studied rather than abbreviation of the action potential was noted to occur in the presence of simulated ischaemia. Disopyramide has been shown to reduce both the slowly inactivating Na⁺ current and the delayed rectifying outward K⁺ current, *I_K* (Coraboeuf *et al.*, 1988). These two effects would have opposing effects on action potential duration. It is likely that in the presence of simulated ischaemia, disopyramide's effect on the plateau Na⁺ current is reduced and consequently the effect on *I_K* to prolong APD predominates. Verapamil caused a slight but concentration-dependent shortening of the terminal phase of repolarization under simulated ischaemic but not under normal conditions. The ionic basis of this effect of verapamil is not known since the appropriate voltage clamp experiments have not been carried out.

In conclusion, the Class I effects on sheep Purkinje fibres of lignocaine (Subclass Ib) but not disopyramide (1a) nor nicainoprol (1c) were potentiated by a combination of factors mimicking myocardial ischaemia, i.e. hyperkalaemia, hypoxia and acidosis. It would be of interest to examine the effects of other drugs in these subgroups of Class I activity to determine if these observations are general to the subgroups or specific to the drugs selected in this study.

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