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K_{ATP} channels and 'border zone' arrhythmias: role of the repolarization dispersion between normal and ischaemic ventricular regions

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1 In order to investigate the role of K_{ATP} channel activation and repolarization dispersion on the 'border zone' arrhythmias induced by ischaemia-reperfusion, the effects of glibenclamide and bimakalim, agents modifying action potential (AP) duration, were studied in an *in vitro* model of myocardial 'border zone'.

2 The electrophysiological effects of 10 μM glibenclamide and 1 μM bimakalim ($n=8$ each), respectively K_{ATP} channel blocker and activator, were investigated on guinea-pig ventricular strips submitted partly to normal conditions (normal zone, NZ) and partly to simulated ischaemic then reperfused conditions (altered zone, AZ).

3 By preventing the ischaemia-induced AP shortening ($P<0.0001$), glibenclamide reduced the dispersion of AP duration 90% (APD_{90}) between NZ and AZ ($P<0.0001$), and concomitantly inhibited the 'border zone' arrhythmias induced by an extrastimulus (ES), their absence being significantly related to the lessened APD_{90} dispersion ($\chi^2=8.28$, $P<0.01$).

4 Bimakalim, which also reduced the APD_{90} dispersion ($P<0.005$) due to differential AP shortening in normal and ischaemic tissues, decreased the incidence of myocardial conduction blocks (25% of preparations versus 83% in control, $n=12$, $P<0.05$) and favoured 'border zone' spontaneous arrhythmias (75% of preparations versus 25% in control, $P<0.05$).

5 During reperfusion, unlike bimakalim, glibenclamide inhibited the ES-induced arrhythmias and reduced the incidence of the spontaneous ones (12% of preparations versus 92% in control, $P<0.05$), this latter effect being significantly related ($\chi^2=6.13$, $P<0.02$) to the lessened ischaemia-induced AP shortening in the presence of glibenclamide ($P<0.0001$).

6 These results suggest that K_{ATP} blockade may protect the ischaemic-reperfused myocardium from 'border zone' arrhythmias concomitantly with a reduction of APD_{90} dispersion between normal and ischaemic regions. Conversely, K_{ATP} channel activation may modify the incidence of conduction blocks and exacerbate the ischaemia-induced 'border zone' arrhythmias.

Keywords: Cardiac muscle; ischaemia; reperfusion, action potential; arrhythmia, K_{ATP} channel

Abbreviations: ANOVA, analysis of variance; AP, action potential; APA, action potential amplitude; APD, action potential duration; $APD_{50,90}$, action potential duration measured at 50 and 90% of repolarization; AZ, altered zone; DAD, delayed afterdepolarizations; EAD, early afterdepolarizations; ES, extrastimulus; K_{ATP} channel, adenosine triphosphate dependent potassium channel; NZ, normal zone; RMP, resting membrane potential; V_{max}, maximal upstroke velocity of action potential; χ^2, Chi^2 test

Introduction

During myocardial ischaemia, the 'border zone' located between normal and hypoxic/ischaemic tissues (Hofman, 1985) has been described as a site which may favour the occurrence of arrhythmias such as automatic activity, focal reexcitation or reentry arrhythmia (El-Sherif *et al*., 1981; Duo *et al*., 1989). This cardiac region, presenting inhomogeneous distribution of electrophysiological properties and both anatomic and biochemical changes, has been associated with injury currents possibly responsible for the emergence of arrhythmias (Janse *et al*., 1980; Duo *et al*., 1989). Evidences have been provided for a link between the dispersion of action potential (AP) duration (APD) and the occurrence of triggered activities in sheep Purkinje fibres (Kupersmith *et al*., 1994) or the inducibility of monomorphic ventricular tachycardia in human heart (Yuan *et al*., 1995).

K_{ATP} channels are involved in several types of myocardial damage induced by ischaemia and reperfusion (Coetzee, 1992), such as myocardial conduction disturbances (Sawanabori *et al*., 1995), arrhythmias (Wilde, 1994), contractile failure (Bril *et al*., 1992), and biochemical impairment resulting in alteration of the cell membrane lipid composition (Freyss-Béguin *et al*., 1995). On the other hand, the ischaemia-induced K_{ATP} channel activation has been postulated as an endogenous protective mechanism (Cavero *et al*., 1995; Hearse, 1995), since the resulting mechanical arrest might protect myocardium from further ischaemia damage (Cole *et al*., 1991) by preservation of intracellular high energy phosphate (McPherson *et al*., 1993; Docherty *et al*., 1997). Nevertheless, if the activation of K_{ATP} channels may exhibit protective effects on both contractile dysfunction (Auchampach *et al*., 1992; Djellas *et al*., 1993) and

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infarct size (Yao & Gross, 1994; Ohno *et al*., 1997), it remains yet by far more controversial as regards arrhythmias induced by ischaemia and reperfusion (Siegl, 1994; Haverkamp *et al*., 1995; Sawanabori *et al*., 1995), and particularly those occurring around the myocardial 'border zone'.

Considering the ability of the K_{ATP} channel blocker glibenclamide to antagonize the ischaemia-induced AP shortening (Cole *et al*., 1991; Yao *et al*., 1993; Tanaka *et al*., 1996), we aimed at evaluating *in vitro* its effects on the APD dispersion between normal and ischaemic guinea-pig ventricular regions and at determining if it may so prevent the occurrence of 'border zone' arrhythmias. The effects of the K_{ATP} channel activator bimakalim, able to shorten cardiac AP in both normal and ischaemic tissues (Yao & Gross, 1994), were also investigated in an *in vitro* model of myocardial 'border zone' (Rouet *et al*., 1989; Picard *et al*., 1998b,c).

Methods

Care of the animals conformed to the recommendations of the Helsinki Declaration, and the study was performed in accordance with the regulations of the official edict of the French Ministry of Agriculture.

Material

Guinea-pigs of either sex weighing 300–400 g were sacrificed after a brief anaesthesia with ether. The hearts were quickly removed and a thin longitudinal strip of the right ventricle was pinned, the endocardial surface upward, in a special perfusion chamber (Rouet *et al*., 1989; Picard *et al*., 1998b). This chamber (5 ml) is bisected by a thin latex membrane containing a centrally located hole allowing the preparation to be passed carefully through and divided into two zones: one called Normal Zone (NZ) and the other Altered Zone (AZ), respectively. The two compartments were independently superfused at the rate of 2 ml min^{-1} with Tyrode's solution oxygenated with 95% O_2 and 5% CO_2 and maintained at 36.5 ± 0.5°C (Polystat 5HP, Bioblock, Illkirch, France). The composition of the Tyrode's solution is (in mM): Na^+ 135, K^+ 4, Ca^{2+} 1.8, Mg^{2+} 1, $H_2PO_4^-$ 1.8, HCO_3^- 25, Cl^- 117.8 and glucose 5.5 (pH 7.35 ± 0.05). At the end of each experiment, absence of leakage between the two compartments was tested by a dye injection (methylene blue) in one chamber.

Data acquisition and analysis

The preparations were stimulated at a frequency of 1 Hz either in the NZ or in the AZ, *via* a bipolar Teflon coated steel wire electrode. Rectangular pulses of 2 ms in duration and twice the diastolic threshold intensity were delivered by a programmable stimulator SMP 310 (Biologic, France). During the protocol, stimulation was stopped whenever sustained spontaneous arrhythmias occurred. An extrastimulus (ES) was applied every four stimulations in an attempt to elicit ES-induced repetitive responses by a progressive increase in 5 ms steps of the time interval between the stimulus and the ES. Transmembrane potentials were recorded simultaneously in both myocardial regions using glass microelectrodes filled with KCl 3 M (tip resistance: 10–30 megohms) and coupled to the input stages of an home-built high impedance capacitance-neutralizing amplifier. The recordings were displayed on a memory dual beam storage oscilloscope (Gould Ins. Sys. Inc. OH, U.S.A.). The following AP characteristics were automatically stored and measured by a system of cardiac AP automatic acquisition and processing device (DATAPAC, Biologic, France): resting membrane potential (RMP), AP amplitude (APA), AP duration at 50% of repolarization (APD_{50}), and at 90% of repolarization (APD_{90}), maximal upstroke velocity (V_{max}). Whenever it was possible the same impalement was maintained throughout the experiment; however, when it was lost readjustment was attempted. If the readjusted parameters were deviant not more than 10% from the previous ones experiments were continued otherwise they were terminated.

Experimental protocol

After a 120 min equilibration period, simulated ischaemia was induced for 30 min in one compartment (AZ) by superfusion with a modified Tyrode's solution while the other compartment remained in normal conditions (NZ). The modified Tyrode's solution differed from normal by elevated K^+ concentration (from 4 to 12 mM), decreased HCO_3^- concentration (from 25 to 9 mM) leading to a decrease in pH (from 7.35 ± 0.05 to 7.00 ± 0.05), decrease in pO_2 by replacement of 95% O_2 and 5% CO_2 with 95% N_2 and 5% CO_2 and withdrawal of glucose. As previously reported (Rouet *et al*., 1989; Bélichard *et al*., 1991; Schiariti *et al*., 1994) the present modifications combining hypoxia, hyperkalemia, acidosis and lack of substrates are similar to those reported by Morena *et al*. (1980), which reproduced *in vitro* electrophysiological abnormalities similar to those observed *in vivo* during ischaemia. The AZ then returned to superfusion with the normal Tyrode's solution for 30 min (reperfusion period).

During both simulated ischaemia and reperfusion, conduction disturbances (Monti *et al*., 1991; Picard *et al*., 1998b) and arrhythmias (Rouet *et al*., 1989; Picard *et al*., 1998c) were recorded: (i) conduction blocks between the AZ and the NZ, (ii) ES-induced repetitive responses defined as spontaneous extrasystoles induced by a single ES and (iii) spontaneous arrhythmias independent of the stimulation.

During the simulated ischaemia and reperfusion phases, Tyrode's solution with either 10 μM glibenclamide ($n=8$) previously diluted in ethanol ($<0.5\%$) or 1 μM bimakalim ($n=8$), or Tyrode's solution alone (control, $n=12$) was randomly superfused simultaneously in both compartments (NZ and AZ). These agents were used at relatively high concentrations, as pharmacological tools to investigate the role of K_{ATP} channels around the 'border zone' and to study the relation between repolarization dispersion and the occurrence of arrhythmias between normal and ischaemic-reperfused ventricular regions. The concentrations were thus chosen as efficient to modulate APD in normal and ischaemic tissues. Ethanol ($<0.5\%$) alone did not modify significantly AP parameters.

Statistical analysis

All results were expressed as mean $\pm$ standard error of the mean (s.e.m.). Analysis of variance (ANOVA for repeated measures) was performed to compare AP parameters to initial values (measured before initiation of the ischaemic period) and to compare variations of APD_{90} and APD_{90} NZ/APD_{90} AZ ratio among groups. Exact Fisher's test and χ^2 with the correction of Yates were used for comparison of nonparametric categorical data. Differences were considered significant when $P<0.05$.

Due to loss of microelectrodes impalements, variations of AP parameters were analysed for eight preparations in Control, eight in Glibenclamide 1 μM group and seven in Bimakalim 1 μM group.

Results

Effects of glibenclamide and bimakalim on the AP parameters and the dispersion of APD_{90} in normoxic and simulated ischaemic/reperfused conditions

As summarized in Table 1, in normoxic conditions (Normal Zone), 10 μM glibenclamide and 1 μM bimakalim did not affect significantly RMP, V_{max} and APA. Whereas glibenclamide did not significantly modify AP duration, bimakalim reduced APD_{50} and APD_{90} by $80\pm5\%$ and $73\pm5\%$ respectively, after 60 min ($P<0.0001$ for *Time* and *Group*).

As shown in Table 2, simulated ischaemia (control) induced significant membrane depolarization and decreased V_{max}, APA, APD_{50} and APD_{90}, which was reversed by reperfusion ($P<0.0001$ for *Time*). The V_{max} and APA variations were

Table 1 Electrophysiological effects of glibenclamide and bimakalim on the action potential parameters in normoxic conditions (Normal Zone)

	Control (n=8)	*Glibenclamide* 10 μM (n=8)	*Bimakalim* 1 μM (n=7)	P *Time*	P *Group*
RMP (mV)				NS	NS
Initial	-87 ± 1	-87 ± 2	-86 ± 1		
30 min	-88 ± 1	-87 ± 2	-90 ± 2		
60 min	-87 ± 1	-86 ± 2	-90 ± 2		
V_{max} (V/s)				NS	NS
Initial	242 ± 30	239 ± 22	282 ± 28		
30 min	221 ± 38	235 ± 32	279 ± 50		
60 min	197 ± 34	258 ± 25	291 ± 41		
APA (mV)				NS	NS
Initial	115 ± 2	109 ± 3	114 ± 3		
30 min	111 ± 4	106 ± 2	109 ± 5		
60 min	112 ± 2	111 ± 3	110 ± 4		
APD_{50} (ms)				<0.0001	<0.0001
Initial	127 ± 6	128 ± 6	121 ± 4		
30 min	126 ± 8	134 ± 7	25 ± 4		
60 min	132 ± 9	136 ± 8	24 ± 5		
APD_{90} (ms)				<0.0001	<0.0001
Initial	151 ± 6	157 ± 7	148 ± 4		
30 min	152 ± 8	158 ± 7	43 ± 5		
60 min	157 ± 11	163 ± 8	40 ± 6		

Mean$\pm$s.e.mean. *P* refers to ANOVA for repeated measures on the three groups. Abbreviations: RMP, resting membrane potential; V_{max}, maximal upstroke velocity of action potential; APA, action potential amplitude; APD_{50} and APD_{90}, action potential duration at 50 and 90% of repolarization.

Table 2 Electrophysiological effects of glibenclamide and bimakalim on the action potential parameters in ischaemic/reperfused conditions (altered zone)

	Control (n=8)	*Glibenclamide* 10 μM (n=8)	*Bimakalim* 1 μM (n=7)	P *Time*	P *Group*
RMP (mV)				<0.0001	0.03
Initial	-88 ± 1	-89 ± 2	-88 ± 2		
Isch. 30 min	-60 ± 1	-60 ± 1	-64 ± 2		
Reperf. 60 min	-92 ± 1	-87 ± 1	-93 ± 2		
V_{max} (V/s)				<0.0001	NS
Initial	248 ± 17	266 ± 31	244 ± 33		
Isch. 30 min	52 ± 21	53 ± 12	39 ± 23		
Reperf. 60 min	248 ± 27	231 ± 18	244 ± 37		
APA (mV)				<0.0001	NS
Initial	120 ± 2	116 ± 4	118 ± 4		
Isch. 30 min	78 ± 6	82 ± 4	53 ± 17		
Reperf. 60 min	119 ± 3	112 ± 4	114 ± 8		
APD_{50} (ms)				<0.0001	<0.0001
Initial	125 ± 4	127 ± 7	117 ± 4		
Isch. 30 min	43 ± 8	87 ± 8	16 ± 5		
Reperf. 60 min	127 ± 5	129 ± 7	36 ± 6		
APD_{90} (ms)				<0.0001	<0.0001
Initial	151 ± 4	155 ± 7	143 ± 4		
Isch. 30 min	60 ± 7	102 ± 8	28 ± 8		
Reperf. 60 min	155 ± 5	160 ± 7	54 ± 7		

Mean$\pm$s.e.mean. *P* refers to ANOVA for repeated measures on the three groups. Abbreviations: RMP, resting membrane potential; V_{max}, maximal upstroke velocity of action potential; APA, action potential amplitude; APD_{50} and APD_{90} action potential duration at 50 and 90% of repolarization.

similar between treated and control groups (NS for *Group*), whereas the ischaemia-induced depolarization was lessened in the presence of bimakalim (RMP reduced by $25\pm3\%$ versus $31\pm2\%$ for Control after 30 min, $P<0.05$). The ischaemia-induced AP shortening was also significantly affected by both agents (APD_{50} and APD_{90}: $P<0.0001$ for *Group*). As illustrated on Figure 1, glibenclamide significantly lessened the AP shortening in AZ (Figure 1B, $P<0.0001$) with no effect in NZ (Figure 1A), whereas bimakalim significantly enhanced the ischaemia-induced APD_{90} reduction (Figure 1B, $P<0.0001$) and decreased APD_{90} in normoxic conditions (Figure 1A, $P<0.0001$ versus control). During reperfusion (Table 2), APD returned close to initial values in control and glibenclamide groups and remained reduced in the presence of bimakalim ($P<0.0001$ versus control) by $70\pm6\%$ and $62\pm5\%$ for APD_{50} and APD_{90} respectively, after 30 min of reperfusion.

As illustrated in Figure 2A, the ischaemia-induced AP shortening led to a dispersion of APD_{90} between both adjacent myocardial regions ($P<0.0001$ for *Time*), measured as ratio APD_{90} NZ/APD_{90} AZ. This APD_{90} dispersion was significantly reduced in presence of 10 μM glibenclamide ($P<0.0001$ versus control) or 1 μM bimakalim ($P<0.005$ versus control), resulting respectively from the preventive effects of glibenclamide on the ischaemic-induced APD shortening (Figure 1B) and the marked APD_{90} reduction induced by bimakalim in NZ (Figure 1A).

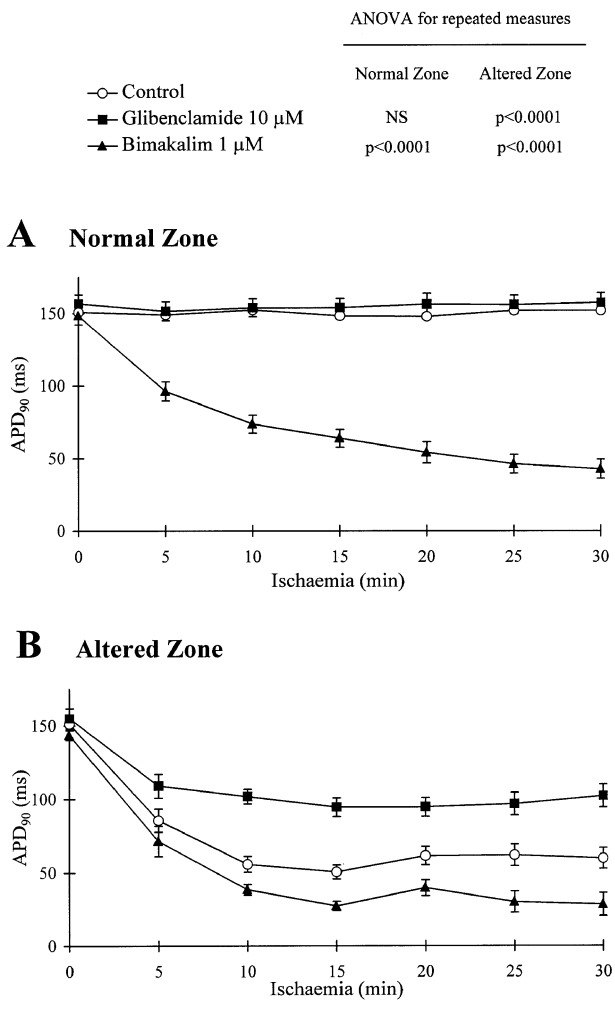

Figure 1 Effects of glibenclamide and bimakalim on action potential duration 90% (APD_{90}) in normal and ischaemic conditions. Data are expressed as mean$\pm$s.e.mean. APD_{90} values were measured simultaneously on both normal zone (A) and altered zone (B) during 30 min of the ischaemic phase. Results of ANOVA (for repeated measures) are given for both treated groups: Glibenclamide 10 μM ($n=8$) and Bimakalim 1 μM ($n=7$), versus control ($n=8$). Note that glibenclamide lessened the ischaemic-induced action potential (AP) shortening (B) whereas bimakalim reduced APD_{90} in normoxia (A) and worsened the AP shortening induced by ischaemia (B).

Effects of glibenclamide and bimakalim on the incidence of electrical disturbances during simulated ischaemia/reperfusion

As summarized in Table 3, simulated ischaemia-induced conduction blocks in 83% of preparations: 66% with unidirectional blocks from NZ towards AZ or conversely and 17% with bidirectional blocks between both regions. ES-induced arrhythmias and spontaneous repetitive responses occurred in 25% of preparations. During the ischaemic period, the occurrence of arrhythmias decreased as conduction blocks occurred (Figure 2B, control). In presence of glibenclamide, few spontaneous repetitive responses and no ES-induced ones occurred during the ischaemic phase. The absence of ES-induced arrhythmias was significantly related to the lessened APD_{90} dispersion between AZ and NZ in presence of 10 μM glibenclamide (Figure 2A) as compared to control ($\chi^2=8.28$, $P<0.01$). Unlike glibenclamide group, the incidence of conduction blocks was significantly reduced ($P<0.05$, Table 3, Figure 2B) in presence of 1 μM bimakalim (in 25% of preparations: 12.5% with unidirectional blocks and 12.5% with bidirectional ones). Bimakalim enhanced significantly ($P<0.05$) the incidence of ES-induced spontaneous responses, and worsened the severity of arrhythmias occurring around the 'border zone' along the ischaemic period as illustrated in Figure 3. This figure shows representative recordings obtained from the same preparation and illustrating that arrhythmia severity was progressively worsened by 1 μM bimakalim: the ES-induced extrasystole occurring during the early ischaemic phase (Figure 3A, at 6 min) became salvos of APs as the simulated ischaemia prolonged (Figure 3B, at 17 min) and led to sustained spontaneous arrhythmias independent of the stimulation during the late ischaemic phase (Figure 3C, at 28 min).

Reperfusion removed all conduction blocks during the first 2 min of reperfusion similarly among control and treated groups (Figure 4). During reperfusion (Table 3 and Figure 4), the incidence of both ES-induced and spontaneous arrhythmias was not significantly modified by 1 μM bimakalim whereas 10 μM glibenclamide abolished the ES-induced repetitive responses and prevented significantly ($P<0.05$) the occurrence of the spontaneous ones. The prevention of reperfusion-induced spontaneous arrhythmias was significantly related to a reduced AP shortening in AZ in presence of 10 μM glibenclamide at the end of the ischaemic period (Figure 1B), as compared to control ($\chi^2=6.13$, $P<0.02$).

Discussion

The main results of this *in vitro* study are: (1) the prevention of the APD_{90} dispersion between both normal and ischaemic myocardial zones by K_{ATP} channel blockade with 10 μM glibenclamide, concomitantly with an antiarrhythmic efficacy around the simulated 'border zone' against both ES-induced and spontaneous repetitive responses; (2) despite a reduction of the APD_{90} dispersion, K_{ATP} channel activation with 1 μM bimakalim led to proarrhythmic effects on 'border zone' spontaneous arrhythmias during ischaemia, concomitantly

with a decreased incidence of myocardial conduction blocks; (3) unlike bimakalim, glibenclamide was antiarrhythmic during reperfusion in this *in vitro* model of 'border zone' arrhythmias.

The expected preventive effect of glibenclamide on the ischaemia-induced AP shortening confirmed data already obtained *in vitro* (Cole *et al*., 1991; McPherson *et al*., 1993; Tanaka *et al*., 1996) and *in vivo* during coronary artery occlusion in dogs (Yao *et al*., 1993). No benefit were found however on the ischaemia-induced AP shortening after 5 min of coronary occlusion in rabbits (Barrett & Walker, 1998). Although in the present study ischaemia was performed by using a modified Tyrode's solution, these ischaemic conditions have been shown to reproduce the electrical alterations on cardiac AP observed in more complex *in vivo* animal models during acute myocardial ischaemia (Janse *et al*., 1979). The different results might be merely due to the model used. Otherwise, the absence of glibenclamide effects in the normal ventricular region was expected considering the K_{ATP} channels in their closed state under normoxia, contributing to reduce the APD_{90} dispersion between normal and ischaemic tissues.

Concomitantly, the K_{ATP} channel blockade by glibenclamide prevented the occurrence of ischaemia-induced spontaneous arrhythmias and inhibited the ES-induced repetitive responses occurring around the simulated 'border zone'. This antiarrhythmic efficacy on ES-induced arrhythmias might be explained by the benefit of glibenclamide on the repolarization dispersion which might contribute to impair reentry movements, likely responsible for the emergence of these arrhythmic events, as previously discussed (Rouet *et al*., 1989). Indeed,

Table 3 Effects of glibenclamide and bimakalim on the incidence of conduction blocks and arrhythmias during simulated ischaemia and reperfusion (% of preparations with disturbances)

	Control (n = 12)	*Glibenclamide* 10 μM (n = 8)	*Bimakalim* 1 μM (n = 8)
Ischaemia			
Conduction blocks	83	50	25*
ES-induced repetitive responses	25	0	37
Spontaneous repetitive responses	25	25	75*
Reperfusion			
ES-induced repetitive responses	17	0	25
Spontaneous repetitive responses	92	12*	62

*$P<0.05$, Fisher's exact test versus control. Abbreviation: ES, extrastimulus.

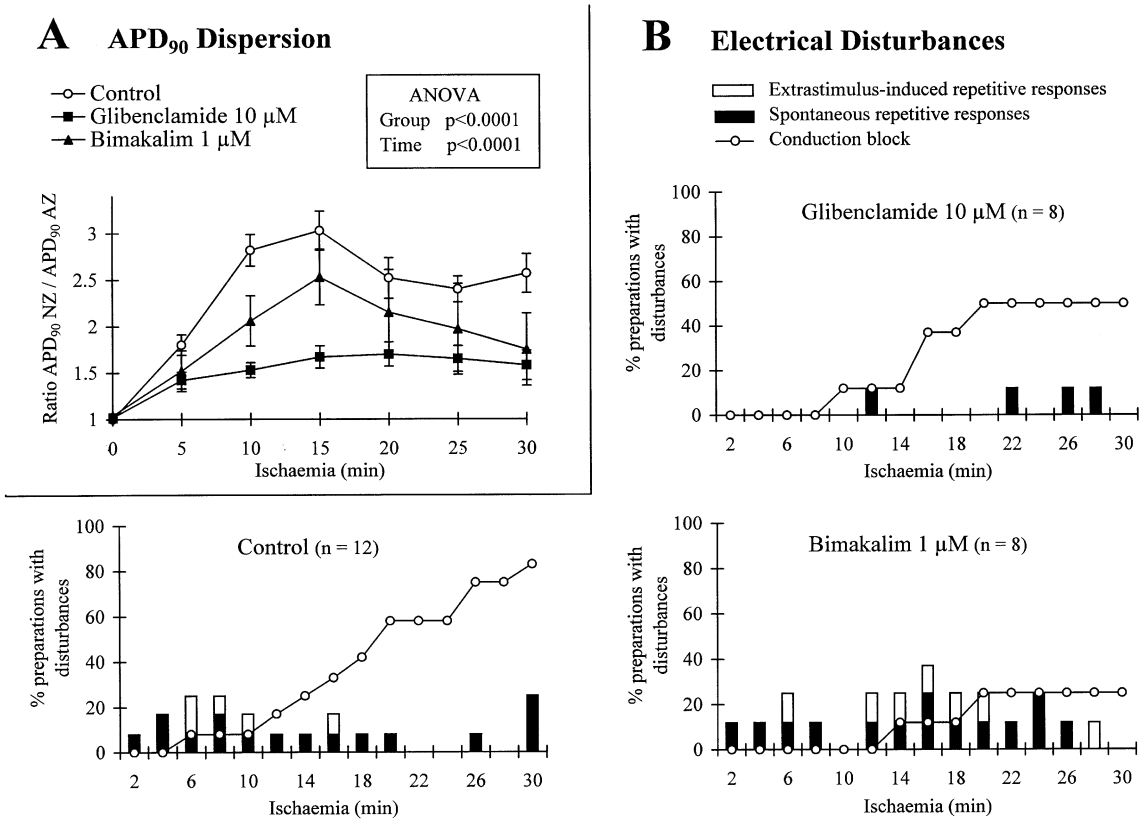

Figure 2 Effects of glibenclamide and bimakalim on the dispersion of action potential duration 90% (APD_{90}) between normal and ischaemic myocardial zones (A) and on the incidence of electrical disturbances during simulated ischaemia (B). In (A) data are expressed as mean ± s.e.mean and the dispersion of APD_{90} is represented by ratio APD_{90} normal zone/APD_{90} altered zone. Results of ANOVA (for repeated measures) are given for group and time factors. In (B), for each time interval (2 min) of the ischaemic period (30 min), values are expressed as a percentage of preparations presenting (i) conduction block between the two myocardial regions, (ii) Extrastimulus-induced repetitive responses and (iii) spontaneous arrhythmias. Patterns are shown in absence of drug (control) and in presence of 10 μM glibenclamide or 1 μM bimakalim. Note that the APD_{90} dispersion between AZ and NZ was effectively lessened by glibenclamide, concomitantly with a reduced occurrence of arrhythmias. Note also that the APD_{90} dispersion was slightly reduced in presence of 1 μM bimakalim and, concomitantly with a decreased incidence of conduction blocks, the occurrence of arrhythmias was not prevented.

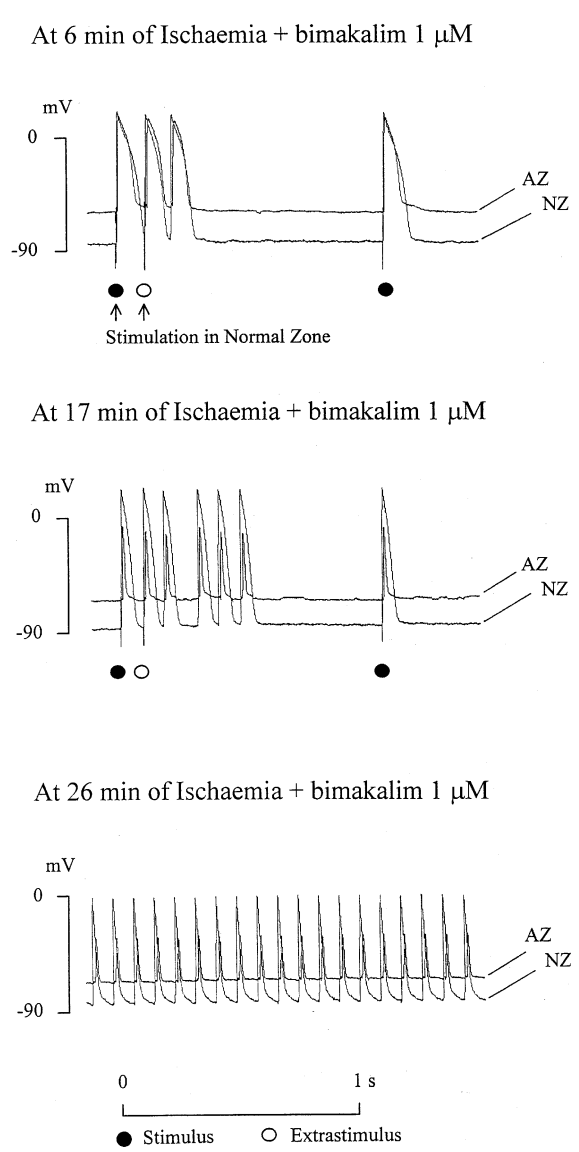

Figure 3 Representative action potentials (APs) recordings illustrating the arrhythmia severity progressively worsened by 1 μM bimakalim during simulated ischaemia. APs were recorded simultaneously in normal zone (NZ) and altered zone (AZ). During the early phase of ischaemia (A, at 6 min), the Extrastimulus (ES) induced a unique repetitive response, namely an extrasystole following the ES-induced AP. As the simulated ischaemia prolonged (B, at 17 min), the ES-induced repetitive response became a salvos of four extrasystoles. Finally, during the late ischaemic period (C, at 28 min), the arrhythmia was a sustained spontaneous activity (frequency $\approx$10 Hz) which persisted in absence of stimulation.

reentry movements require a site of unidirectional conduction block and a slow retrograde conduction throughout the cardiac tissue, similarly to the conduction disturbances observed in this 'border zone' model. On the other hand, the ischaemia-induced spontaneous repetitive responses observed in the present model are unlikely related to early (EAD) or delayed (DAD) afterdepolarization, not seen in our experiments, but may also be attributed to reentry, explaining the protective effect of glibenclamide. Supporting this, other investigators have reported differential effects of K_{ATP} channel blockers as regards the type of arrhythmia, namely antiarrhythmicity against reentries (Pasnani & Ferrier, 1992; D'Alonzo *et al*., 1994a), and enhancement of arrhythmic events related to EAD or DAD (Spinelli *et al*., 1991; D'Alonzo *et al*., 1994b). The diversity of mechanisms underlying the ischaemia-induced arrhythmias and the diversity of experimental models might explain the contradictory findings of the literature as concerns pro- and antiarrhythmic effects of glibenclamide in isolated cardiac preparations (Pasnani & Ferrier, 1992), isolated hearts (Bril *et al*., 1992; Bernauer, 1997), and anaesthetized rabbits (Barrett & Walker, 1998).

The bimakalim-induced worsening of the AP shortening during ischaemia also confirmed data obtained with other K_{ATP} channel openers *in vitro* (McPherson *et al*., 1993; Tanaka *et al*., 1996) and *in vivo* (Yao *et al*., 1993; Yao & Gross, 1994). Nevertheless the extent of the AP shortening induced by these agents in ischaemic myocardial muscle as compared with normal region and their resulting action on APD dispersion in inhomogeneous cardiac tissues were not clarified. The less potent action of bimakalim in ischaemic region as compared to normal tissue is likely due to K_{ATP} channels already activated in ischaemic conditions. This differential efficacy to shorten AP led to a lessened APD_{90} dispersion around the simulated 'border zone', as did glibenclamide, whereas ischaemia-induced arrhythmias were not prevented.

Otherwise, the absence of protective effect of the K_{ATP} channel activator on the 'border zone' ES-induced arrhythmias might be considered in regard of its concomitant action on the incidence of conduction blocks. Indeed, as illustrated on Figure 3B, the ES-induced repetitive responses occurred mainly when the myocardial conduction was slowed but before the total block of the signal conduction occurred (until 16–18 min of ischaemia), since no ES-induced arrhythmias were observed after the myocardial conduction around the 'border zone' was completely blocked. By preventing the occurrence of conduction block, bimakalim may contribute to maintain a slowed myocardial conduction and favour reentrant circuits, overriding so any potential antiarrhythmic benefit of the reduced APD_{90} dispersion between normal and simulated ischaemic zones. Supporting this, we recently observed in this 'border zone' model that the slowing of the myocardial conduction induced by 1 μM bupivacaine, a local anaesthetic, was accompanied by an enhanced incidence of arrhythmias whereas neither ES-induced nor spontaneous repetitive responses occurred when the myocardial conduction was completely blocked by higher concentrations of this agent (Picard *et al*., 1998b). As concerns the 'border zone' spontaneous arrhythmias, the proarrhythmicity induced by bimakalim is likely due to the dramatic AP shortening observed in the ischaemic myocardial region, leading to spike-like APs which might thus facilitate the emergence of abnormal automaticity (Figure 4C). Moreover, this proarrhythmic effect confirms that the spontaneous repetitive responses observed in our model are unlikely based on EAD or DAD, since several K_{ATP} channel openers have been shown as efficient *in vitro* and *in vivo* against arrhythmias related to these repolarization abnormalities (Fish *et al*., 1990; Spinelli *et al*., 1991; Takahashi *et al*., 1991).

During reperfusion also, the K_{ATP} channel blockade by glibenclamide was accompanied by antiarrhythmic efficacy around the simulated 'border zone' in relation to the lessened AP shortening during the previous ischaemic phase. Glibenclamide might therefore indirectly prevent reperfusion-induced arrhythmias by its protective effects during ischaemia. However, it cannot be ruled out that the K_{ATP} channel blockade acts also during reperfusion, since it has been demonstrated in guinea-pig isolated ventricular walls that these channels may remain activated during the early reperfusion (Shigematsu *et al*., 1995). Otherwise, we recently provided evidence that glibenclamide was able to prevent the

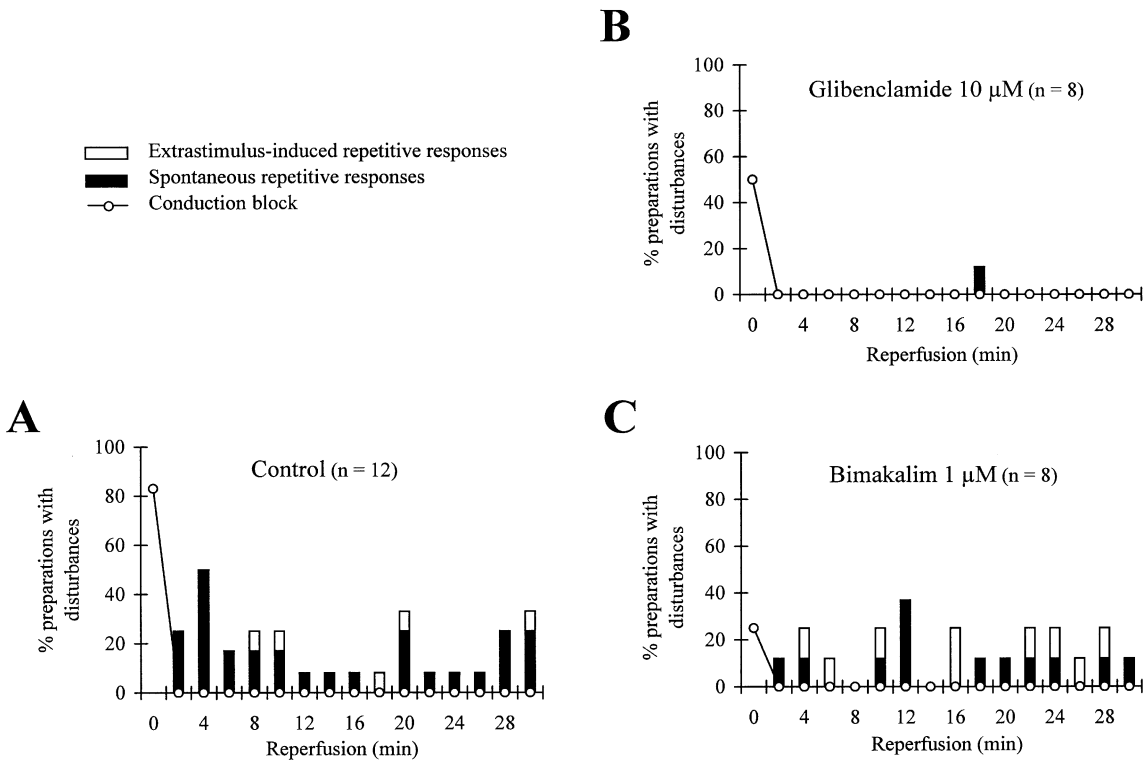

Figure 4 Effects of glibenclamide and bimakalim on the incidence of conduction disturbances and arrhythmias during simulated reperfusion. For each time interval (2 min) of the reperfusion period (30 min), values are expressed as a percentage of preparations presenting (i) conduction blocks between the two myocardial regions, (ii) Extrastimulus (ES)-induced repetitive responses and (iii) spontaneous arrhythmias. Patterns are shown in absence of drug (A, control) and in presence of 10 μM glibenclamide (B) or 1 μM bimakalim (C). Note that, unlike bimakalim, glibenclamide prevented the occurrence of both spontaneous and ES-induced arrhythmic events.

reperfusion-induced accumulation of free arachidonic acid released from the cell membrane phospholipids in guinea-pig ventricular preparations (Picard *et al*., 1998a). Considering the arrhythmogenic properties of free fatty acids accumulated in the cardiac muscle (Oliver & Opie, 1994), an indirect antiarrhythmic action of the K_{ATP} channel blockade, involving a protective action against the degradation of cell membrane lipids, cannot be excluded also.

Unlike the ischaemia-induced arrhythmias, the incidence of reperfusion repetitive responses around the simulated 'border zone' was not modified by K_{ATP} channel activation. These results differ from those previously obtained in our laboratory on guinea-pig ventricular preparations with the K_{ATP} channel opener cromakalim, which favoured the occurrence of reperfusion spontaneous arrhythmias without affecting those occurred during ischaemia (Picard *et al*., 1998a). Interestingly, these latter findings were obtained in a model of global simulated ischaemia-reperfusion, suggesting that the mechanisms underlying the emergence of arrhythmias around the myocardial 'border zone' are likely different from those responsible for arrhythmias observed in cardiac tissue rendered globally ischaemic. This might help to explain the controversial data of the literature supporting either profibrillatory (Tosaki *et al*., 1993) or antiarrhythmic effects (Tanaka *et al*., 1996) of K_{ATP} channel openers, since in addition to different species and drug concentrations, different experimental models of myocardial ischaemia were used.

In conclusion, this study provided evidence for an antiarrhythmic efficacy during simulated ischaemia and reperfusion of the reduction of repolarization dispersion induced by K_{ATP} channel blockade in an *in vitro* model of 'border zone'. Conversely, K_{ATP} channel activation favoured the emergence of ischaemia-induced spontaneous arrhythmic events around the simulated 'border zone' and did not confer any protection during reperfusion. Some limitations for the interpretation of our findings and relative to the *in vitro* model have now to be taken into account, since the demarcation between normal and ischaemic-reperfused myocardial zones is likely more narrow and regular than it might occur *in vivo* in diseased hearts. As previously discussed in detail (Rouet *et al*., 1989), this 'border zone' model has been shown to reproduce arrhythmias similar to those seen *in vivo* during coronary artery occlusion and after abrupt reperfusion (Corr & Witowski, 1983) or in man during coronary transluminal angioplasty and after thrombolytic therapy (Tzivoni *et al*., 1983). However, it suits to keep in mind that an *in vitro* model using superfused cardiac tissues under simulated ischaemic-reperfused conditions is largely distant from *in vivo* experiments and the clinic. As pertinently discussed by Rosen (1988), an *in vitro* model as complex as possible cannot reproduce exactly *in vivo* situations and may provide interesting information on the limits of a cell ability to perform in a controlled environment simulating pathological conditions rather than its usual behaviour in healthy and diseased heart. Caution is therefore needed when confronting *in vitro* and *in vivo* data and overall for all attempts to extrapolate *in vitro* findings to *in vivo* and clinical settings. Now, the present study might help to understand the contributory role of the ischaemia-induced K_{ATP} channel activation as regards the electrical disturbances occurring during simulated ischaemia in this *in vitro* model of 'border zone' and the potential benefits of K_{ATP} channel blockers in this peculiar myocardial region.

These findings might also explain why controversial results have been obtained with K_{ATP} channel activators and blockers in amount of experimental studies using *in vitro* simulated pathophysiological conditions or *in vivo* coronary artery occlusion. It also supports the interest that must be placed, in the context of the anti and proarrhythmic effects of cardioactive agents, in the study of their electrophysiological effects in myocardial regions presenting inhomogeneous distribution of electrical properties and anatomic and biochemical changes, as it may occur around the 'border zone'.

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