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Effects of K^+ channel blockers on the action potential of hypoxic rabbit myocardium

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1 In order to assess the role of different ionic currents in hypoxia-induced action potential shortening, we investigated the effects of blockers of voltage-dependent and ATP-sensitive K^+-channels on the membrane potential of hypoxic rabbit hearts and papillary muscles. The response to blocking of the inward rectifier was studied at three external K^+ concentrations: 2.5, 5, and 7.5 mM.

2 Hypoxia produced a progressive decline in action potential duration (APD) that levelled off after 15 to 20 min. Steady state APD values at 25% and 95% repolarization (APD_{25} and APD_{95}) were 26.0 ± 1.9% and 42.2 ± 2.4% of controls respectively.

3 Tetraethylammonium (TEA, 10 mM) delayed but did not reduce APD shortening at the steady state.

4 Blocking of I_{K1} with a mixture of 0.2 mM Ba^{2+} and 4 mM Cs^+ lengthened APD in normoxia and prevented APD_{95} shortening in hypoxia. The APD_{25} shortening was significantly attenuated at all $[K]_o$.

5 Glibenclamide (Glib, 30 μM) did not prevent APD shortening, but produced a progressive action potential (AP) lengthening after 15 min of hypoxia. Steady levels of 48 ± 3.5% and 62 ± 5.0% of controls for APD_{25} and APD_{95} respectively were reached after 45 min.

6 The relation between APD_{25} and pacing rate was determined in normoxic and hypoxic papillary muscles and the effects of 2 mM 4-aminopyridine (4-AP) were examined. Hypoxia attenuated the APD_{25} shortening currently observed when the stimulation rate was lowered from 1 to 0.1 Hz without altering the plateau reduction occurring at frequencies above 2 Hz. These effects were potentiated by 4-AP.

7 Our data suggest that the accelerated AP repolarization in hypoxic rabbit myocardium represents a delicate balance of several outward currents: I_{K1}, $I_{K\text{-}ATP}$, and at least one yet unidentified current component rather insensitive to changes in $[K]_o$ and to K^+ channel blockers.

Keywords: Hypoxic cardiac muscle; K^+ channel blockers; action potential shortening; I_{K1}; $I_{K\text{-}ATP}$; I_{to}

Introduction

Hypoxia is not only an element of ischaemia but a common sequela of cardiovascular and respiratory disease as well as of anaemia. It has long been recognized that the earliest effect of hypoxia on the cardiac action potential is a marked shortening (Carmeliet, 1978) resulting from a great increase in outward current (Isenberg *et al.*, 1983). The discovery of adenosine 5'-triphosphate (ATP)-sensitive K^+ channels in myocardium by Noma (1983), and subsequent work led to the proposition that activation of these channels underlies the increased outward current observed during metabolic inhibition (Noma & Shibasaki, 1985) as well as the cellular K^+ loss produced by ischaemia (Gasser & Vaughan-Jones, 1990; Wilde *et al.*, 1990). However, discrepancies exist between threshold levels of ATP required for channel opening and the decline in cytosolic ATP measured in whole hearts (Elliot *et al.*, 1989). Cytosolic ATP compartmentation (Weiss & Lamp, 1987), inhomogeneities in ATP distribution (Nichols & Lederer, 1990), and variability of channel sensitivity to ATP (Findlay & Faivre, 1991) have been invoked to explain them. Since the direct and simultaneous measurement of the ATP level in close proximity to the channels and of the current resulting from their opening is not yet possible in intact cells or tissues, blocking of these channels with sulphonylureas has been used to assess the role of $I_{K\text{-}ATP}$ in the action potential (AP) shortening. Some inconsistencies were also found with this approach. Glibenclamide only partially reversed the AP shortening induced by DNP in cardiocytes (Fosset *et al.*, 1988), by hypoxia in papillary muscles (Wilde *et al.*, 1990), and by ischaemia in canine myocardium (Nakaya *et al.*, 1991) whereas AP shortening was completely blocked when induced by ATP depletion (Fosset *et al.*, 1988) or by pinacidil application (deLorenzi *et al.*, 1991). In addition, in hypoxic rabbit hearts blockade of the inward rectifying current I_{K1} antagonized AP shortening near full repolarization but much less so at plateau level (Ruiz-Petrich *et al.*, 1991). It is therefore possible that more than one K conductance is involved in the electrical response of myocardium to metabolic stress compatible with cell survival.

To test that hypothesis, we examined the effects of different K^+ channel blockers on the action potential of rabbit perfused hearts and papillary muscles subjected to mild hypoxia in the presence of glucose. Although tetraethylammonium (TEA) is a rather unspecific blocker of K channels (Stanfield, 1983), we used it as a first approach to assess the role of hypoxia-induced K^+ currents in plateau depression because: (a) 10 mM TEA reduces the K loss in hypoxic rabbit hearts (Leblanc *et al.*, 1987), (b) the plateau shortening is rather insensitive to blockade of I_{K1} with 40 μM Ba^{2+} (Ruiz Petrich *et al.*, 1991), and (c) at low concentrations, TEA should selectively block the delayed rectifier but not the early outward current or I_{K1} (Stanfield, 1983).

Because of the known voltage- and time-dependence of I_{K1} blockade by Ba^{2+} (Hirano & Hiraoka, 1988), we used a combination of 200 μM $BaCl_2$ and 4 mM CsCl to achieve a time- and voltage-independent block of I_{K1}. The effect of varying the external K^+ concentration $[K]_o$ was also tested

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because of the marked influence of $[K]_o$ on electrogenesis through modifications of the resting membrane potential (RMP) and direct modulation of I_{K1}.

Because of its widely accepted specificity (Arena & Kass, 1989), we used glibenclamide as a blocker of $I_{K\text{-}ATP}$. Since in our hands, none of these blockers completely reversed the reduction of the plateau duration induced by hypoxia, we assessed the possible contribution of the transient outward current I_{to} to the plateau shortening by studying the frequency-dependence of the action potential duration in papillary muscles in normoxia and hypoxia with and without 4-AP. Because of its slow reactivation kinetics (Hiraoka & Kawano, 1987) the 4-AP-sensitive fraction of I_{to} plays a greater role in determining plateau duration at frequencies below 1 Hz to 2 Hz (Hiraoka & Kawano, 1989; Ruiz Petrich & Leblanc, 1989). Papillary muscles were chosen for these experiments because they allowed us to perform continuous recordings from the same cell during long-lasting impalements.

Preliminary accounts of part of this work have appeared in abstract form (Leblanc & Ruiz-Ceretti, 1986; deLorenzi *et al.*, 1990; 1991).

Methods

Experimental models

New Zealand rabbits of either sex weighing 1.5 to 2 kg were killed by cervical dislocation or anaesthetized with a mixture of ketamine (35 mg kg^{-1}) and xylazine (5 mg kg^{-1}) by intramuscular injection. The heart was rapidly removed and rinsed in cold (10°C), oxygenated (95% O_2 : 5% CO_2) Krebs solution.

Perfused hearts

After removal of the pericardium, great veins and connective tissue, the heart was quickly placed in the perfusion apparatus where retrograde aortic perfusion was started at a rate of 20 ml min^{-1} and constant temperature of 33 ± 1.0°C (monitored by an intraventricular thermistor).

The region of the sino-atrial node and adjacent right atrial tissue were removed and the ventricles were driven at a frequency of 2.5 Hz (2 ms long pulses of 1.5 times threshold strength) by means of bipolar platinum electrodes placed at the base of the right ventricle. The preparations were equilibrated for 20 min

Papillary muscle and trabeculae

Papillary muscles and ventricular trabeculae (2–3 mm long and less than 1 mm thick) were excised from the right ventricle. The dissection was carried out in a low calcium (0.2 mM) Krebs solution at room temperature. The preparation was then transferred into a 0.5 ml superfusion chamber lined with Sylgard (Dow Corning, Midland, U.S.A.). One end of the muscle was pinned to the bottom of the chamber and the other was attached to a platinum wire pinned down in the Sylgard. Superfusion was then started at the rate of 5–8 ml min^{-1}. The temperature (33 ± 1°C) was monitored by a thermistor placed near the preparation. The muscle was first superfused without stimulation with the low calcium solution for 10 min followed by 15 min with normal Krebs solution. Stimulation (square pulses of 1 ms duration and 1.5 times the threshold strength) was then applied across the preparation through two platinum electrodes at 1 Hz. The frequency was chosen because higher rates of stimulation resulted in rapid depression of action potential parameters (decreased amplitude, rate of rise, and duration) in these preparations. An equilibration period of at least 45 min preceded experimentation. Dissection and initial superfusion with low calcium medium increased the yield of viable preparations to more than 80%.

Solutions and drugs

The standard perfusate (Krebs solution) had the following composition (mM): NaCl 120, $NaHCO_3$ 25, KH_2PO_4 1.2, KCl 3.8, $MgSO_4$ 1.2, $CaCl_2$ 1.25 and dextrose 5.5. In the experiments in which the potassium concentration was modified, the potassium phosphate buffer was replaced by sodium biphosphate, and KCl was added to the desired final concentration (2.5, 5, or 7.5 mM) without compensation for osmolarity changes. When equilibrated with a gas mixture of 5% CO_2 in O_2 or N_2, the pH was 7.3–7.4. The P_{O_2} of the solution entering the heart (samples obtained anaerobically with a three way stopcock inserted immediately before the aortic cannula, Allied Instrumentation gas analyser model 1312) was between 570 and 640 mmHg (1 mmHg = 133.32 Pa) in normoxia corresponding to an O_2 content between 1.83 and 2.05 ml O_2 100 ml^{-1}. The P_{O_2} declined to 43 to 75 mmHg (0.14 to 0.24 ml O_2 100 ml^{-1}) when the solution was equilibrated with 5% CO_2 in N_2. The P_{CO_2} was between 45.5 and 46.8 mmHg throughout. The P_{CO_2} values indicate that the samples were not significantly contaminated with ambient air. In the experiments with papillary muscles, a constant flow of humidified gas mixture (5% CO_2 in O_2 or N_2) was maintained over the superfusing chamber.

TEA-chloride was purchased from Aldrich Chemical Company (Milwaukee, U.S.A.). Glibenclamide and 4-AP were from Sigma Chemical Company (St Louis, U.S.A.). TEA and 4-AP were directly dissolved in the saline solution. Glibenclamide was dissolved in dimethylsulphoxide (DMSO) and appropriate amounts of the stock solutions were added to saline (0.05 to 0.5 ml l^{-1}) to reach the final concentrations. In 2 control experiments, addition of 0.5 ml l^{-1} DMSO did not modify cell electrical activity.

Recording techniques

In perfused hearts, the transmembrane potential of left ventricular subepicardial cells was recorded with floating glass microelectrodes and a silver-silver chloride reference electrode. Micropipettes were made of Pyrex glass (Corning 7740, 1 mm external diameter) with a vertical puller (David Kopf model 700 D; Tujunga, U.S.A.). When filled with 3 M KCl, the microelectrode resistance was 10 to 20 MΩ in Krebs solution. With papillary muscles and trabeculae, standard (non-floating) microelectrodes were used. The recording equipment consisted of a high impedance amplifier with capacity neutralization (WPI, model KS-700, Sarasota, U.S.A.) and a dual-beam storage oscilloscope (Tektronix 5113, Beaverton, U.S.A.). The traces were photographed with a C4 Grass camera and measured from enlargements under a microfilm reader. A Grass S88 stimulator with a stimulus isolation unit was used to drive the preparation.

Experimental protocols

Control experiments: after the equilbration period, recordings were obtained in normoxic conditions for 15 min followed by hypoxia for 1 h. Recovery in normoxia was tested for an additional 30 min. *Tetraethylammonium*: the perfusate contained 10 mM TEA during 20 min in normoxia followed by 30 min of hypoxia. *Experiments with Ba^{2+} – Cs^+*: three $[K]_o$ were tested: 2.5, 5 and 7.5 mM. Only one concentration was investigated per heart. An initial 20 min exposure to Ba^{2+} and Cs^+ in normoxia at a given $[K]_o$ was followed by 30 to 60 min of hypoxia at the same $[K]_o$. The rate of stimulation had to be lowered to 2 Hz because the blockers lengthened the late repolarization at all $[K]_o$ tested so that full repolarization could not be attained at higher rates. The same protocol was used in controls without blockers but with varied $[K]_o$. *Glibenclamide*: after a control period in normal

Krebs, glibenclamide (3, 10, or 30 μM) was added in normoxia and the action potential was monitored for 15 to 30 min. The effects of 60 min hypoxia in the presence of glibenclamide were then tested. *Papillary muscles*: control experiments were performed with the same protocol described for the perfused heart. In these preparations, continuous recordings of potential from the same cell could be maintained for up to 45 min. The frequency-dependence of the action potential duration was determined in single cells by step changes in frequency within the range 0.1 Hz to 4 Hz in normoxia and hypoxia with and without 2 mM 4-AP. Given the stability of the preparations and the reversibility of 4-AP effects, more than one cell could sometimes be studied in normoxic muscles. Only those relations where the APD value at 1 Hz was the same before and after changes in frequency were retained.

Statistical analysis

The data are presented as means ± s.e.mean from *n* experiments. In experiments with multiple impalements, individual values were measured and taken as data for that heart or muscle. For comparisons of two groups, the non-paired Student's *t* test was used. The s.e.mean of differences was calculated according to Meyer (1975). For comparisons involving more than two groups, an analysis of variance was used (Peritz F test) based on the advantages of Ryan's and Newman-Keuhl's tests for contentious comparisons. For both tests, a probability of error less than 0.05 was accepted as the level of significance. Some sets of data points were fitted with a least-square fitting method to a monoexponetial function.

Results

Perfused hearts

Action potential shortening in hypoxia: seven experiments were performed in normal Krebs (5 mM $[K^+]_o$) to determine the effects of reduced oxygen tension on action potential parameters. Stability of the preparations was assessed in pilot experiments where the membrane potential was monitored for 2 h in normoxia. No significant changes occurred during this observation period. The control record in Figure 1a shows the typical action potential of rabbit ventricle characterized by a fast upstroke and a well developed plateau. Hypoxia produced the well known AP shortening with early plateau depression (Figure 1a(ii)). By the end of 60 min of observation, the action potential had a triangular shape and the overshoot had markedly decreased (Figure 1a(iv)). No significant changes in RMP were observed. The maximum rate of rise of the upstroke ($\dot{V}_{max}$) was not significantly altered: 131 ± 4 Vs^{-1} in normoxia vs 122 ± 6 Vs^{-1} between 45 and 60 min of hypoxia. The change in APD with time in hypoxia was characterized by an early fast decline reaching new steady values after 10 to 20 min of hypoxia (Figure 1b, dashed curves). As a percentage, the decrease in APD was greater at the plateau level than at near full repolarization: after 60 min, APD_{25} was 26.6 ± 1.9% whereas APD_{95} was 42.2 ± 2.4% of the control. These effects were partly reversed (about 80%) by reperfusion with oxygenated solution (not shown).

Delay caused by tetraethylammonium of AP shortening in hypoxia: the effects of 10 mM TEA on the hypoxia-induced AP shortening were tested in 4 hearts. TEA did not affect RMP or APD_{25} in normoxia but slightly lengthened APD_{95} from 219 ± 2.3 ms to 225 ± 1.6 ms ($P<0.05$). In hypoxia, TEA slowed down the rate of APD shortening at both levels of repolarization (Figure 1b, continuous lines). Moreover, the onset of the APD_{95} decline was delayed (no significant change during the first 5 min). However, after 30 min of hypoxia, the APD values approached those observed without TEA. The initial delay of APD_{95} shortening and its absence in APD_{25} reduction suggested that more than one outward current was involved in early AP shortening.

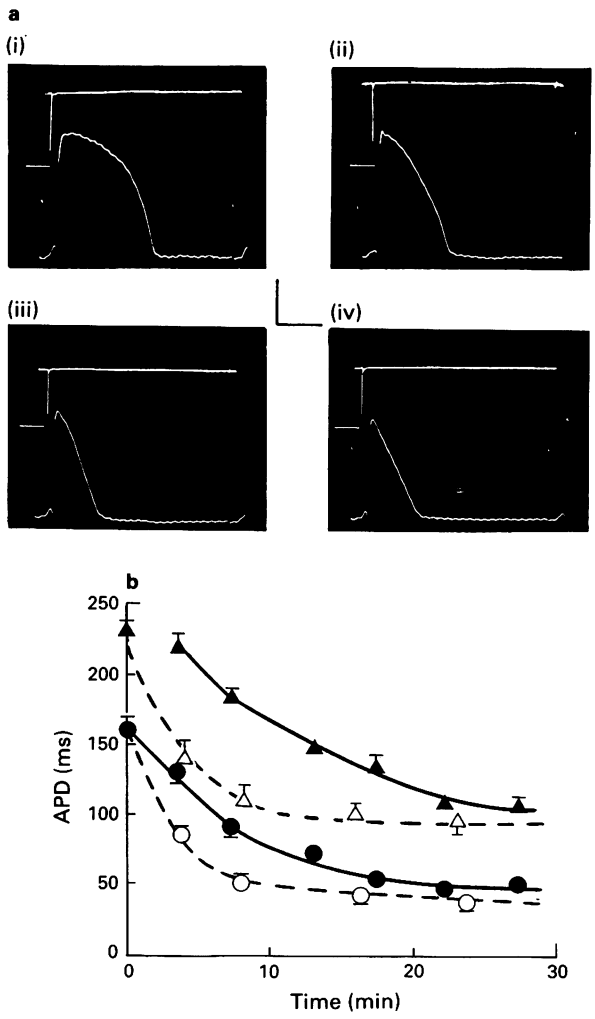

Figure 1 Time course of action potential changes during hypoxia in perfused hearts and the influence of tetraethylammonium (TEA, 10 mM). (a) Action potentials (lower traces) in normoxia (i) and after 4, 11 and 56 min of hypoxia ((ii), (iii) and (iv)). Upper traces are inverted first time derivates of the action potential. Horizontal bars: zero reference lines. Calibrations: vertical, 40 mV and 110 Vs^{-1}; horizontal, 100 ms. (b) Time course of action potential shortening at 25% and 95% repolarization (circles and triangles respectively) in control medium (dashed lines) and in the presence of TEA (solid lines). Control values indicated on the ordinate scale. Data points are means from 7 control hearts and 4 for TEA. Error bars indicated (unless within symbol size). The curves are least square fits of single exponentials with time constants of 4.0 and 4.4 min for APD_{25} and APD_{95} in control hypoxia and of 18.7 min for TEA.

K^+-dependence of APD and I_{K1} blockade: Figure 2 illustrates the steady state values of electrical parameters in normoxia and during the last 15 min of hypoxia at three different $[K]_o$ (7 hearts per concentration) as well as the effects of I_{K1} blockade with a mixture of 0.2 mM Ba^{2+} and 4 mM Cs^+ (4 hearts per $[K]_o$). In normoxia, the resting potential decreased significantly in the presence of Ba^{2+} and Cs^+ at 2.5 and 5 mM $[K]_o$ ($P<0.001$). The depolarization induced by raising $[K]_o$ from 2.5 to 7.5 mM was reduced from 23 ± 0.9 mV in controls to 17 ± 1.4 mV ($P<0.005$). Hypoxia did not affect RMP in the controls but in the presence of Ba^{2+} and Cs^+ a significant depolarization occurred. This effect was greater at low $[K]_o$ and the sensitivity of RMP to changes in $[K]_o$ was

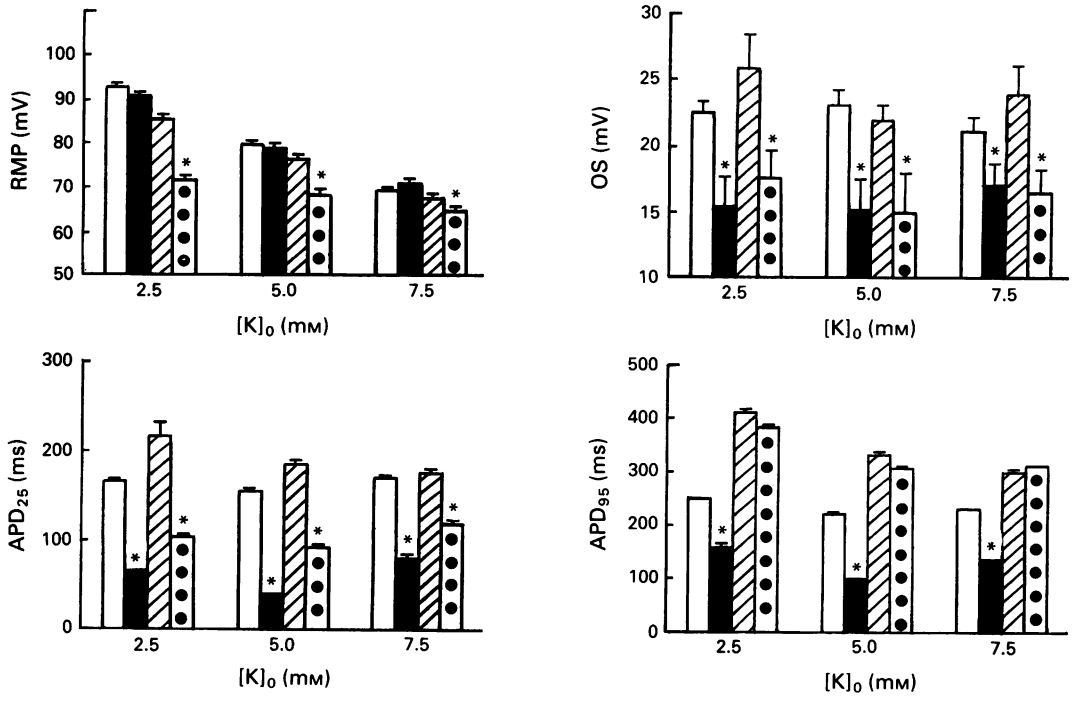

Figure 2 Effects of Ba^{2+} (0.2 mM) and Cs^+ (4 mM) on action potential parameters in normoxia and hypoxia at the steady state: RMP, absolute values of resting membrane potential, OS, overshoot; APD_{25} and APD_{95}, action potential duration at 25% and 95% repolarization at $[K]_o$ of 2.5, 5, and 7.5 mM, as indicated. Values are means from 7 hearts for the control experiments without blockers and 4 for each of the other $[K]_o$ in the presence of Ba^{2+}-Cs^+ . Error bars indicate s.e. unless within trace thickness. Open and solid columns, control normoxia and hypoxia respectively; cross-hatched and dotted columns, Ba^{2+}-Cs^+ in normoxia and hypoxia respectively. $*P<0.05$ or lower, paired t tests between hypoxia and normoxia for each experimental condition.

markedly attenuated (7.8 ± 1.6 mV between 2.5 and 7.5 mV) when compared with hypoxia without the blockers (19.2 ± 1.2 mV, $P<0.001$). Hypoxia significantly reduced the overshoot at all $[K]_o$ regardless of the presence of the blockers but the degree of the reduction was not related to $[K]_o$.

Blocking of I_{K1} with Ba^{2+}-Cs^+ in normoxia significantly increased APD_{25} at 2.5 and 5 mM $[K]_o$ and APD_{95} at all $[K]_o$ but the latter effect became smaller as $[K]_o$ was raised. Moreover, the blockers affected APD shortening and its $[K]_o$ dependence with a different pattern according to the repolarization level. The APD_{25} shortening was attenuated in the presence of the blockers: 55 ± 2.1% vs 61.6 ± 1.9%, 52 ± 2.9% vs 74 ± 1.8%, and 34 ± 4.9% vs 52.7 ± 3.9% at 2.5, 5 and 7.5 mM $[K]_o$ respectively. These differences were highly significant. Most interestingly, the Ba^{2+}-Cs^+ mixture prevented the APD_{95} shortening regardless of $[K]_o$.

Partial restoration of APD by glibenclamide: In 15 hearts, glibenclamide in normoxia did not alter AP configuration at any of the concentrations used (not shown) and at 3 μM the response to hypoxia was not affected. Pre-incubation with 10 or 30 μM glibenclamide for 30 min did not prevent AP shortening upon exposure to hypoxia (Figure 3a). On the contrary, APD values after 15 min were lower than in hypoxic controls. Thereafter, the action potential progressively lengthened and new steady state values were reached at about 45 min of hypoxia. Reoxygenation produced a fast but incomplete recovery of action potential parameters: 90 ± 4% for action potential amplitude, 73 ± 5% for APD_{25} and 85 ± 3.5% for APD_{95} after 30 min (traces not shown). Figure 3b shows that the partial recovery of APD_{25} induced by glibenclamide was dose-dependent and accompanied by a significant increase in APA at 30 μM. In contrast, there was no significant difference between the APD_{95} lengthening observed at 10 and 30 μM (138.6 ± 3.0 ms, vs 132.4 ± 2.9 ms respectively). Higher concentrations of glibenclamide (50 and 100 μM) did not produce further APD lengthening (data not shown). The resting potential slightly but significantly depolarized in hypoxia (3.4 ± 1.1 mV) in the presence of glibenclamide.

Papillary muscles

Figure 4 illustrates the effects of hypoxia on the membrane potential of a single cell in a typical experiment. As in whole hearts, RMP was not significantly altered and the slow decline of APA and the overshoot (Figure 4a) contrasts with the faster decrease in APD (Figure 4b). Similar results were obtained in 4 other preparations. Whereas the decrease in OS was comparable to that seen in rapidly paced hearts, the APD decline was smaller and the new steady state values were reached only after 20 to 30 min of hypoxia.

Figure 5 illustrates the effect of hypoxia and 4-AP on the frequency-dependence of APD_{25}. The control curve (composed from steady state values measured in the same cell at frequencies between 0.1 and 4 Hz) showed a maximum at 1 Hz (Figure 5a). A downward, parallel shift between 1 and 4 Hz was observed after 5 min of hypoxia. Because of the rapid decline of APD, steady state values could not be obtained for low frequency range (8 to 10 stimuli were required to attain the final APD at any given frequency, Ruiz Petrich & Leblanc, 1989). The parallel shift of the APD-frequency curve is compatible with the development of a time-independent outward current. An early depression of $I_{Ca\text{-}L}$ would have selectively depressed the response to higher frequencies. As APD further shortened with time in hypoxia, the influence of the pacing rate diminished, as shown by the curves obtained after 35 and 45 min, once more stable values of APD had been reached. The APD shortening produced by lowering the frequency from 1 to 0.1 Hz was then reduced from 41 ± 2.4% in controls to 25 ± 2.5% in hypoxia (Figure 5b). In addition, the maximum APD shifted from 1 to 2 Hz but the reduction with higher frequencies was maintained. Exposure to 4-AP in normoxia reduced AP shortening at low rates of stimulation and enhanced it as high frequencies. These effects are compatible with the blocking of the 4-AP

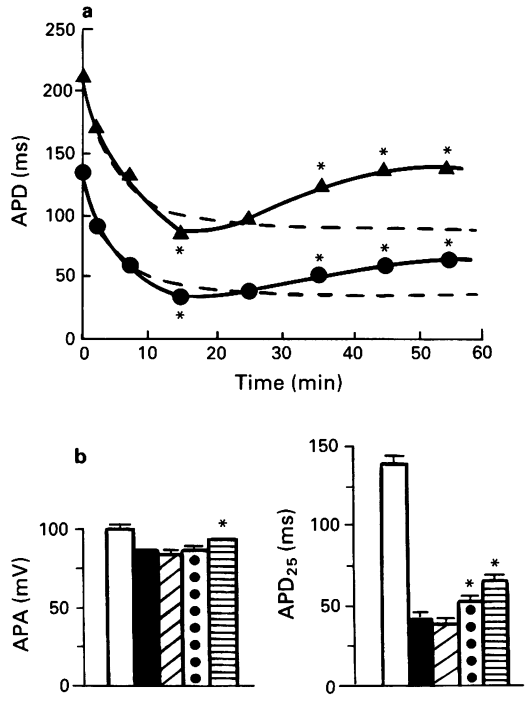

Figure 3 (a) Effects of 30 μM glibenclamide on the time course of action potential shortening during hypoxia (solid lines). Dashed lines: time course of APD decline in the absence of the blocker, same data as in Figure 1b. Control APD values in normoxia in the presence of the drug indicated on the ordinate scale. Data points represent means from 5 hearts with s.e. included in the size of the symbol: (▲) APD_{95}; (●) APD_{25}. Asterisks indicate statistical significance, with respect to hypoxia without glibenclamide, paired *t*-test. (b) Steady state values of action potential amplitude (APA) and APD_{25} in normoxia and hypoxia without drug (open and solid columns respectively) and hypoxia in the presence of 3 (cross hatched), 10 (dotted), and 30 μM (horizontal stripes) glibenclamide. Values are means from 3 hearts for the control and 5 for each glibenclamide concentration with s.e. shown by error bars, except when included within column. Asterisks indicate statistical significance with respect to hypoxia without drug (tested by analysis of variance).

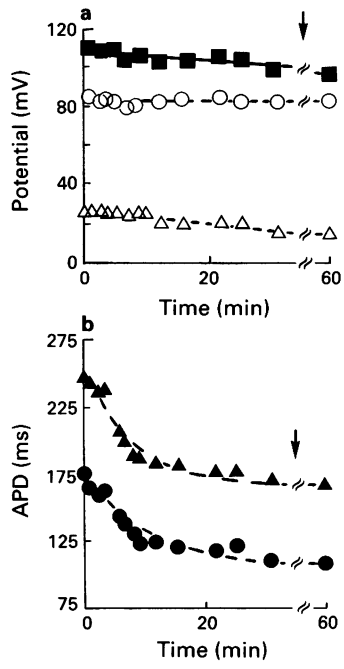

Figure 4 Changes in action potential parameters as a function of time of hypoxic superfusion in a papillary muscle paced at 1 Hz. Control values indicated on the oridinate scale. Data points are individual measurements from the same cell up to 31 min. The arrow indicates spontaneous dislodgement of the electrode. Data at 60 min are from another cell. (a) Action potential amplitude (■); absolute value of membrane resting potential (○), and overshoot (△). (b) Action potential duration at 25% (●) and 95% repolarization (▲). The dashed lines are fitted to single exponential functions with time constants of 9.3 and 7.9 min respectively.

sensitive component of I_{to} (Tseng *et al.*, 1987; Hiraoka & Kawano, 1989). In hypoxia with 4-AP, the AP shortening at 0.1 Hz was only 8 ± 2.7% but the response to higher frequencies was similar in normoxic controls and in hypoxia without 4-AP. These data suggest that the contribution of the 4-AP-sensitive fraction of I_{to} to plateau duration is diminished during metabolic inhibition.

Discussion

Our control experiments showed once more that high flow hypoxia produces a rapid and marked abbreviation of the cardiac action potential (Figure 1). Although the action potential duration is a complex parameter that reflects a delicate balance between outward and inward currents, it is accepted that its shortening during metabolic inhibition results from an early and pronounced increase in outward current (Isenberg *et al.*, 1983; Trube & Hescheler, 1984; Carmeliet *et al.*, 1990; Nichols & Lederer, 1990; Nichols *et al.*, 1991). Our study suggests that more than one ionic conductance needs to be invoked to account fully for the different responses to APD_{25} and APD_{95} to changes in $[K]_o$ and to K^+ channel blockers.

Contribution of I_{K1} to AP shortening

Effective blockade of I_{K1} by the Ba^{2+}-Cs^+ mixture was indicated by the decreased sensitivity of RMP to changes in $[K]_o$

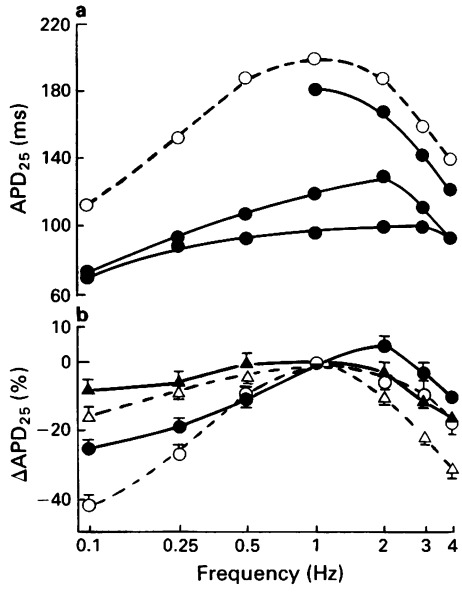

Figure 5 Effects of hypoxia and 4-aminopyridine (4-AP, 2 mM) on the relation between APD_{25} and frequency of stimulation. (a) Typical example of the rate dependence of APD_{25} in normoxia (○) and at different times in hypoxia (●): 5, 35 and 45 min, from top to bottom. Data points represent single measurements from the same cell for each curve. (b) Percentage changes of APD_{25} with frequency (with respect to 1 Hz) at the steady state for different experimental conditions: normoxia (○); hypoxia (●); 4-AP in normoxia (△), and 4-AP in hypoxia (▲). Data points are means from 7 preparations in normoxia and 8 in hypoxia. Error bars indicate s.e. unless included in the size of the symbol.

and the APD lengthening that was greater at 95% than at 25% repolarization, as expected from selective blockade of I_{K1} (Giles & Imaizumi, 1988). The suppression of APD_{95} shortening and the depolarization observed in hypoxia with Ba^{2+}-Cs^+ supports our previous findings that an increase in g_{K1} determines the maintenance of RMP and the AP shortening near full repolarization (Ruiz Petrich *et al.*, 1991). An increased g_{K1} can also explain the inhibitory effects of Ba^{2+} on the hypoxia-induced increase in K^+ efflux (Jiang *et al.*, 1991) and myocardial K^+ loss (Ruiz Petrich *et al.*, 1991). The attenuation of APD_{25} shortening by Ba^{2+}-Cs^+ indicates that either the I_{K1} current was also enhanced at plateau levels or that, at the concentration used, Ba^{2+} was no longer specific for I_{K1} but was also blocking K-ATP channels (Nelson *et al.*, 1990). In either case, these data strengthen the view that more than one outward current is involved in abbreviating APD.

Role of $I_{K\text{-}ATP}$

The involvement of K-ATP channels in AP shortening, increased K^+ efflux, and interstitial K^+ accumulation of hypoxic and ischaemic myocardium has been extensively documented in recent years (Venkatesh *et al.*, 1989; Carmeliet *et al.*, 1990; Wilde *et al.*, 1990; Gasser & Vaughan Jones, 1990; Hicks & Cobbe, 1991; Nakaya *et al.*, 1991; Jiang *et al.*, 1991) and most of these authors agree that blocking of these channels with sulphonylureas does not fully reverse the effects of metabolic inhibition. In our experiments, the enhanced AP shortening observed at about 15 min of hypoxia with glibenclamide (Figure 3) can be explained by a concomitant reduction of coronary flow (Wilde *et al.*, 1990) or to lack of the vasodilatation produced by low O_2 tensions (Daut *et al.*, 1990). The subsequent partial recovery of APD supports the view, also held by others (Hicks & Cobbe, 1991; Nakaya *et al.*, 1991) that K-ATP channels are not the only pathway involved in the response to metabolic stress.

The variability of the glibenclamide effects has been attributed to the drug binding only to open channels (Carmeliet *et al.*, 1990) and to decreased sensitivity of the channels to glibenclamide because of ADP accumulation (Venkatesh & Weiss, 1990). However, glibenclamide blocks $I_{K\text{-}ATP}$ to the same degree whether the channels are open or closed at the time of application (Venkatesh & Weiss, 1990). Moreover, ADP accumulation would result in effects vanishing with time in hypoxia instead of building up, as we and others observed (Carmeliet *et al.*, 1990; Hicks & Cobbe, 1991). The data from our experiments therefore suggest that although $I_{K\text{-}ATP}$ contributes to AP shortening in high flow hypoxia, it may not be a major component of the increased outward current responsible for the early changes, as proposed by Trube & Hescheler (1984).

We have so far identified two current systems (I_{K1} and $I_{K\text{-}ATP}$) the relative contributions of which to APD_{25} and APD_{95} shortening can be estimated by adding up the difference in APDs resulting from their blockade in hypoxia (Figure 6). If these two currents were the only ones involved in APD shortening, their addition should add up to the APD shortening without blockers, which is not the case for APD_{25} where a fraction remains to be accounted for. In contrast, the addition of the Ba^{2+}-Cs^+ and glibenclamide-sensitive fractions exceeds APD_{95} shortening which suggests a certain overlapping of their blocking effects.

Outward currents other than I_{K1} *and* $I_{K\text{-}ATP}$ *involved in plateau shortening*

A delayed rectifier like I_K is very seldom found in rabbit ventricle (Giles & Imaizumi, 1988) and activates too slowly to contribute significantly to AP repolarization (Hiraoka & Kawano, 1989). It seems that this current would also be absent during hypoxia because of the transitory effects of TEA that at 10 mM are believed to be quite specific for I_K (Stanfield, 1983). The remaining K^+ current activated at

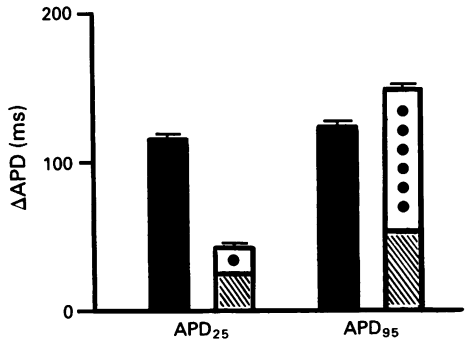

Figure 6 Relative contributions of the Ba^{2+}-Cs^+ and glibenclamide-sensitive currents to AP shortening at 25% and 95% repolarization. Solid columns, APD shortening without blockers; cross hatched columns, glibenclamide-sensitive fraction (difference between filled and dashed curves in Figure 3); spotted columns, Ba^{2+}-Cs^+ sensitive fraction (difference between hypoxia with and without blcokers, corrected for the increase in APD produced by the blockers in normoxia), see Figure 2. The greater size of the 2nd column for APD_{95} could imply a certain degree of overlapping between the blocks induced by glibenclamide and by Ba^{2+}-Cs^+. Means with s.e. (vertical bars); differences between columns were significant.

plateau potentials, I_{to}, determines the fast, early repolarization and the rate-dependent changes of APD. The downward, parallel shift of the relation between APD_{25} and pacing rate at the onset of hypoxia (Figure 5) suggests the rapid development of a time-independent current but the progressive flattening of this curve, particularly in the low frequency range speaks against an increase in I_{to} and rather in favour of another time-independent component of slower development. Inhibition of the 4-AP-sensitive component of I_{to} further enhanced the flattening of the APD_{25} vs frequency curve indicating the persistence of this component in hypoxia. On the other hand, if this current were a major contributor to plateau shortening, the response of APD_{25} to low stimulation rates would be expected to be enhanced, instead of depressed. Our data do not allow us to speculate on the role of the Ca^{2+}-dependent component of I_{to} in APD_{25} shortening at high rates but its contribution could be diminished because AP shortening produces a substantial decrease of the $[Ca^{2+}]_i$ transient (Stern *et al.*, 1988). The Na^+ activated K^+ current is not likely to play a major role in early plateau changes because cytosolic Na^+ accumulation is unlikely to occur within a few minutes of hypoxia.

Other current systems that could be involved in plateau shortening are the catecholamine-induced instantaneous I_{Cl} (Harvey *et al.*, 1990) and (but less probable for the reasons invoked above) a Ca^{2+}-activated transient I_{Cl} (Zygmunt & Gibbons, 1991) that is also enhanced by β-adrenoceptor stimulation. Further experimentation is needed to elucidate the role that these currents could play in AP shortening in hypoxia. Their involvement appears plausible because endogenous catecholamine release occurs early during metabolic inhibition. Moreover, a catecholamine-mediated mechanism could partly explain why APD shortening was less and developed more slowly in papillary muscles (where catecholamine pools should be much smaller) than in whole hearts. Given the multiple regulatory effects of sympathetic innervation on current systems acting at plateau potentials, and on $[Ca^{2+}]_i$, early adrenergic stimulation can markedly affect the delicate balance of inward and outward currents determining plateau duration. Such a mechanism could also explain the controversial evidence reported for perfused hearts vs myocytes where $I_{K\text{-}ATP}$ plays a predominant role in AP shortening.

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