Differential Class III and glibenclamide effects on action potential duration in guinea-pig papillary muscle during normoxia and hypoxia/ischaemia

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1 Microelectrode recording techniques were used to study the effects of several potassium channel blockers which are considered to be Class III antiarrhythmic compounds. The effects of (+)-sotalol, UK-66,914, UK-68,798 and E-4031 on action potential duration (APD) were determined in guinea-pig isolated papillary muscles. The compounds were evaluated under normoxic or hypoxic/ischaemic conditions at 36.5°C and compared to glibenclamide, which is considered to be a blocker of ATP-dependent potassium channels. Prolongation of action potential duration at 90% repolarization (APD₉₀) was taken as an indirect measure of potassium channel blockade.

2 Under normoxic conditions, the Class III compounds prolonged APD in a concentration-dependent manner. According to EC₁₅ values, the order of potency of the Class III compounds was found to be UK-68,798 > E-4031 > UK-66,914 > (+)-sotalol. Glibenclamide did not significantly prolong APD₉₀ under normoxic conditions.

3 Perfusion with an experimental hypoxic or ischaemic bathing solution produced qualitatively similar effects on action potentials. Over a period of 20–25 min in either of the experimental solutions, there was a small decrease in action potential amplitude (APA) and a prominent shortening of APD. The ischaemic solution also depolarized the resting membrane potential by about 15 mV.

4 (+)-Sotalol and UK-66,914 did not reverse the shortening of APD induced by perfusion with hypoxic Krebs solution. High concentrations of glibenclamide (10 µM) and UK-68,798 (30 and 60 µM) partially reversed the hypoxia-shortened APD. Glibenclamide was more potent and exhibited a greater time-dependent action than UK-68,798.

5 During experimental ischaemia, the Class III compound E-4031 (10 µM, *n* = 7) produced small, but significant, increases in the APD₉₀ (11 ± 3 ms after 20 min) which were not clearly time-dependent (14 ± 4 ms after 30 min). UK-68,798 (10 µM) also produced a small, but insignificant, increase in APD₉₀ (12 ± 6 ms at 20 min, *n* = 4). Higher concentrations of UK-68,798 (30 and 60 µM, *n* = 4) did not produce a consistently significant increase in APD₉₀ during ischaemia: significance was only attained after 20 min in the presence of 60 µM UK-68,798 (24 ± 12 ms). However, in marked contrast to the effects of the Class III compounds, glibenclamide (10 µM) produced large time-dependent increases in ischaemic APD₉₀ (34 ± 11 ms at 7 min, *n* = 9) which were significant 15 min or more after drug addition (52 ± 12 ms at 20 min, *n* = 7; 74 ± 5 ms at 30 min, *n* = 6).

6 The present microelectrode data suggest that blockers of ATP-dependent potassium channels, such as glibenclamide, might prove to be more effective than Class III compounds against ischaemia-induced shortening of cardiac action potentials.

Keywords: Antiarrhythmic drugs; ATP-dependent potassium channels; Class III drugs; glibenclamide; guinea-pig papillary muscle

Introduction

One of the principal causes of sudden death in the western world is the onset of lethal ventricular arrhythmias which follow myocardial ischaemia where partial membrane depolarization, a shortened action potential duration and impaired conduction velocity set up re-entrant arrhythmias (Janse & Wit, 1989). There has been much discussion as to the most appropriate form of therapy for these ischaemic arrhythmias since the sodium channel blockers flecainide and encainide increased mortality in a population of patients which had previous myocardial infarction (CAST Investigators, 1989). Therefore, attention has recently turned to the modulation of other ion channel subtypes, particularly potassium channels, for an antiarrhythmic compound which prolongs action potential duration in the ischaemic myocardium.

Vaughan Williams (1970) originally termed compounds which selectively prolonged action duration as Class III agents. Subsequently, these drugs were believed to prolong action potential repolarization by blockade of a delayed

rectifier (Carmeliet, 1985), a low-conductance potassium channel which is both voltage- and time-dependent (Giles & Shibata, 1985; Matsuura *et al.*, 1987; Shibasaki, 1987). Early Class III compounds in clinical use are typified by sotalol (Antonaccio & Gomoll, 1988; Nademanee & Singh, 1990). More recently, there have been other highly potent sotalol derivatives (such as UK-66,914, UK-68,798 and E-4031) which, under normoxic conditions, prolong ventricular action potential duration and refractoriness in electrophysiological studies (Tande *et al.*, 1990; Gwilt *et al.*, 1991a,b). These Class III drugs also prolong refractoriness in some *in vivo* models (Gwilt *et al.*, 1989; 1991b) and, additionally, exhibit antiarrhythmic effects in other *in vivo* models of prior myocardial infarction (Kato *et al.*, 1990; Lynch *et al.*, 1990; Zuanetti & Corr, 1991).

An ATP-dependent outward potassium conductance is activated in cardiac myocytes by a reduction in intracellular ATP concentration (Noma, 1983), which can be produced either by attenuation of the glycolytic pathway (e.g. by hypoxia), or by use of metabolic inhibitors (Trube &

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Hescheler, 1984; Weiss & Lamp, 1989). Promotion of this ATP-dependent potassium current by hypoxia or metabolic inhibition results in a shorter action potential duration (Kodama *et al.*, 1984; Fosset *et al.*, 1988). Thus, blockers of ATP-dependent potassium channels which could selectively prolong APD in metabolically compromised tissue represent a potential drug target with a pathophysiological relevance in myocardial ischaemia. Blockers of cardiac ATP-dependent potassium channels are typified by sulphonylureas such as glibenclamide (Fosset *et al.*, 1988). Glibenclamide also has a therapeutic effect on insulin secretion associated with its blocking action on pancreatic ATP-dependent potassium channels (Zünkler *et al.*, 1988). However, some of the differences in the biophysical and pharmacological properties of cardiac and pancreatic ATP-dependent potassium channels could be exploited to yield novel cardioselective compounds.

The present work has investigated the effects of the Class III compounds (+)-sotalol, UK-66,914 (N-[4-[1-Hydroxy-2-[4-(4-pyridyl)-1-piper-4-yl]ethyl]phenyl]methanesulphonamide), UK-68,798 (N-[4-[2-[N-methyl-2-[4-(methanesulphonamido)phenoxy]ethyl]amino]ethyl]phenyl]methanesulphonamide) and E-4031 (N-[4-[1-[2-(6-Methyl-2-pyridyl)ethyl]-4-piperidinyl]carbonyl]phenyl]methanesulphonamide dihydrochloride) on action potential duration in guinea-pig isolated papillary muscle under normoxic conditions. Maximally effective concentrations were determined and the compounds additionally tested under hypoxic or ischaemic conditions. These drug profiles were then compared with those of glibenclamide.

Some of this work has been communicated in preliminary form (MacKenzie *et al.*, 1990; Saville *et al.*, 1991).

Methods

Electrophysiological recording

Guinea-pigs (300–550 g) underwent cervical dislocation, or were stunned and exsanguinated. The heart was quickly removed and a papillary muscle (generally from the right ventricle) with attached piece of ventricular wall was pinned out in a tissue bath (2 ml, flow rate of 6 ml min⁻¹) containing oxygenated Krebs solution at 36.5°C. The preparations were stimulated at a frequency of 1 Hz (2 ms pulse width, threshold voltage plus 50%) by bipolar platinum electrodes placed against the preparation. After an initial stabilization period (1–1.5 h), action potentials were recorded from the papillary muscle using microelectrode recording techniques. Data were displayed on a digital oscilloscope (Gould 1604) where waveforms were overlaid and output as necessary. Figure 2 was taken from a chart recording (Gould 3400, pen frequency-response of 30–50 Hz) and other experiments used a DAT tape recorder (Biologic) in conjunction with a high-frequency recorder (Gould TA4000, with 10 kHz).

The temporal characteristics of the action potentials were recorded semi-automatically, at regular time intervals, from prolonged impalements of a single cell in the preparation. The effects of drugs on these steady-state determinations were then evaluated at fixed time points. The action potential duration at 90% repolarization (APD₉₀) from maximal amplitude (APA) was taken as an indirect measure of potassium channel activity. Drugs which prolonged the APD₉₀ without effects on the action potential upstroke velocity ($V_{\max}$) were considered to be potassium channel blocking agents. Action potential duration at 30 and 50% repolarization (APD₃₀ and APD₅₀, respectively) was also routinely measured. The effects of Class III compounds on the APD₉₀ were quantified by establishing concentration-response curves using a 7 min time cycle. This time cycle was chosen because preliminary experiments with the reference compound (+)-sotalol had shown that there was no further increase in the APD₉₀ with extended exposure. Due to difficulty in com-

pletely washing out the effects of the compounds, only one agent was studied in each preparation. From these experiments, EC₁₅ values were determined (i.e. the concentration of compound which prolonged the APD₉₀ by 15%) and used to establish the relative potencies of the Class III compounds. An EC₁₅ value was chosen for two reasons: firstly, because the prolongation induced by some compounds did not consistently exceed 20 or 25% and, secondly, because the errors associated with this value were small.

Illustrations show a continuous recording from a single cell in a preparation. Mean data ($\pm$ s.e.mean) were compared by Student's paired *t* test where appropriate with *P* < 0.05 considered statistically significant.

Bathing solutions and drugs

The Krebs solution was of the following ionic composition (mM): Na⁺ 149.1, K⁺ 5.4, Mg²⁺ 1.2, Ca²⁺ 2.0, Cl⁻ 144.2, HCO₃⁻ 15.5, H₂PO₄⁻ 1.2 and glucose 11. This 'normoxic' Krebs had a pH of 7.3 and was gassed by 95% O₂/5% CO₂. The 'hypoxic' Krebs solution was of the same composition as that above, except that it was glucose-free and gassed by 95% N₂/5% O₂ to reduce substantially the oxygen tension in the solution (*P*O₂ in bath 150 mmHg, by Corning 168 analyser). The 'ischaemic' Krebs solution was also glucose-free but the potassium concentration and pH were changed to simulate some of the changes measured in the *in vivo* myocardium during ischaemia (see Gettes, 1986). The ischaemic bathing solution was of the following composition (mM): Na⁺ 137.3, K⁺ 9.2, Mg²⁺ 1.2, Ca²⁺ 2.0, Cl⁻ 143.7, HCO₃⁻ 8.0, H₂PO₄⁻ 1.2 and glucose 0. The ischaemic bathing solution was gassed with 55% O₂/40% N₂/5% CO₂ to produce a less severe change in oxygen tension (*P*O₂ in bath 200 mmHg), and had a pH of 6.9.

Glibenclamide was obtained from Sigma. The Class III agents were synthesized in the Cardiovascular Chemistry Department at Roche Products Ltd. E-4031 was made up as a stock solution in distilled water and (+)-sotalol was dissolved initially in ethanol. Other drugs were dissolved in dimethylsulphoxide (DMSO), to a maximal solvent concentration of 0.5%, which had no effects on action potential configuration.

Results

Action potentials under normoxic conditions

Action potentials were usually recorded from preparations maintained for 2–4 h after equilibration. Within any experiment, a sample of cells tested in the absence of drugs generally exhibited action potentials of similar amplitude (mean APA was 113 $\pm$ 1 mV, *n* = 22) and duration. Between preparations, however, the duration of the action potential repolarization was quite variable (range 85–180 ms, mean APD₉₀ 130.5 $\pm$ 3.2 ms, *n* = 68). The average action potential upstroke velocity ($V_{\max}$) was 215 $\pm$ 14 mVs⁻¹ (*n* = 18) with an average APD₃₀ and APD₅₀ of 74.6 $\pm$ 6.3 ms and 99.3 $\pm$ 7.2 ms (*n* = 22), respectively. In any given preparation, once the time-course and amplitude of the action potentials had attained a steady-state level (usually 10–15 min after impalement), the effects of drugs on the action potential parameters were measured.

Prolongation of normoxic action potentials

The order of potency of the Class III compounds in this study, as determined using EC₁₅ values (see Methods) obtained from concentration-response relationships (Figure 1), was UK-68,798 > E-4031 > UK-66,914 > (+)-sotalol.

The mean EC₁₅ values together with their 95% confidence limits were calculated as 0.92 (0.18–4.68) μ M (*n* = 3) for UK-68,798, 2.25 (1.48–3.41) μ M (*n* = 7) for E-4031, 3.37

Figure 1 Concentration-dependent effects of UK-68,798 (●), E-4031 (▲), UK-66,914 (■) and (+)-sotalol (◆) on action potential duration recorded from guinea-pig papillary muscles under normoxic conditions and measured at 90% repolarization (APD₉₀). See text for details.

(2.08–5.45) μM ($n = 4$) for UK-66,914 and 29.8 (1.28–691) μM ($n = 3$) for (+)-sotalol. The mean maximal increase in APD₉₀ obtained with a 10 μM concentration of each compound was $20.6 \pm 3.1\%$ for UK-68,798 ($n = 3$, control APD₉₀ = 159.5 ± 2.0), $33.0 \pm 4.7\%$ for E-4031 ($n = 7$, control APD₉₀ = 95.0 ± 11.9), $18.5 \pm 4.7\%$ for UK-66,914 ($n = 5$, control APD₉₀ = 136.5 ± 8.7) and $5.9 \pm 3.0\%$ for (+)-sotalol ($n = 6$, control APD₉₀ = 132.8 ± 6.8). These agents prolonged the APD at 30, 50 and 90% repolarization. The percentage increase in APD₉₀ for a Class III compound was larger than corresponding increases in either APD₃₀ or APD₅₀ (for example 10 μM UK-68,798 prolonged the APD₉₀ by $20.6 \pm 3.1\%$, the APD₅₀ by $17.7 \pm 3.5\%$ and the APD₃₀ by $8.6 \pm 2.5\%$, $n = 3$). These drugs prolonged repolarization without any significant reduction in the $V_{\max}$. These observations confirm the reported Class III profiles of these compounds.

In contrast to the Class III compounds described above, the ATP-dependent potassium channel blocker, glibenclamide (10 μM for up to 30 min) did not significantly prolong APD₉₀ under normoxic conditions. The values for APD₉₀, in the presence of 10 μM glibenclamide, over a 30 min incubation period were as follows: control = 146.6 ± 6.3 ms, 7 min glibenclamide 145.2 ± 6.7 ms, 15 min glibenclamide 151.4 ± 5.6 ms, 20 min glibenclamide 152.7 ± 5.3 ms, 25 min glibenclamide 152.6 ± 5.8 ms and 30 min glibenclamide 151.8 ± 7.7 ms ($n = 5$).

Effect of drugs under hypoxic conditions

When the Krebs bathing solution was exchanged for the 'hypoxic' bathing solution (see Methods), there was a small but consistent reduction in the APA of a few millivolts and a marked reduction in the APD₉₀. These changes attained a near steady-state after 20–25 min. Thereafter, drugs were added during continued hypoxia to assess whether they had any effect on action potential configuration.

A high concentration of the Class III compound (+)-sotalol (30 μM) had no effect on the APD₉₀ under hypoxic conditions (Figure 2a), which contrasted with its Class III activity under normoxic conditions (Figure 1). The more potent Class III compounds, UK-66,914 and UK-68,798, were used in another series of experiments at concentrations which were maximal for their Class III effects. UK-66,914 (30 μM) had no effect under hypoxic conditions (Figure 2b). However, UK-68,798 (30 μM) had some effect on the hypoxia-shortened APD (Figure 3). There was a small increase in the mean APD₉₀ produced by 30 μM UK-68,798 which was significant only after 15 min (the hypoxic APD₉₀ was increased by 22 ± 5.5 ms, $n = 3$) and this increase did not become larger with time (23 ± 11 ms, $n = 3$, after 20 min). At a higher concentration (60 μM UK-68,798, $n = 3$) there was no significant increase in APD₉₀.

Figure 2 Lack of effect of (a) (+)-sotalol (30 μM) and (b) UK-66,914 (30 μM) during hypoxic conditions. Action potentials were recorded initially under normoxic conditions (controls) and then progressively shortened under hypoxic conditions until they attained a steady-state. The subsequent addition of (a) (+)-sotalol or (b) UK-66,914 did not prolong the hypoxic APD₉₀ in these experiments. (In this and subsequent figures of this type, the hypoxic action potential illustrated was measured at the annotated time after the onset of hypoxia. The APD₉₀ had then reached a steady-state and drugs were subsequently added to the hypoxic solution for the times shown below these drug-treated responses). There was no apparent effect of hypoxia on resting membrane potential in these experiments.

The ATP-dependent potassium channel blocking agent glibenclamide also prolonged the hypoxia-shortened APD₉₀. The threshold concentration for this effect was about 10 μM. One striking feature of the action of glibenclamide was its clear latency (around 10–15 min) before an initial effect was apparent (compare the insignificant increase of 4 ± 2.1 ms at 7 min, $n = 7$, with that of 28 ± 7.3 ms at 15 min, $n = 6$, significant at $P < 0.01$). Thereafter, the effect of glibenclamide at a fixed concentration (generally 10 μM in these studies) progressively increased as the period of drug incubation was extended (Figure 4). A significant ($P < 0.01$) net increase in the APD₉₀ of 50 ± 9.6 ms ($n = 4$) was observed after 20 min exposure to 10 μM glibenclamide.

Effect of drugs under ischaemic conditions

Although the experimental hypoxia methodology is often used in cardiac electrophysiological studies, we also used an alternative experimental stimulus which would better simulate some of the ionic and chemical changes which arise within the myocardium during ischaemia. A simulated ischaemic bathing solution (see Methods) was used to shorten the APD, probably by activating ATP-dependent potassium channels. The ability of compounds to reverse the shortening effect of ischaemia on the APD was then assessed.

The 'ischaemic' bathing solution prolonged qualitatively similar effects on action potential configuration to those seen with the 'hypoxic' solution. There was a small decrease in the APA and a pronounced shortening of the APD₉₀, both of which reached a steady-state after about 20 min (Figures 5

Figure 3 The effect of UK-68,798 on the APD₉₀ recorded from a guinea-pig papillary muscle following exposure to the hypoxic bathing solution. There was a significant reduction in the APD₉₀ after 20 min experimental hypoxia. UK-68,798 (30 μ M) elicited a small increase in APD₉₀ (see text). In this recording, there was some instability in the resting membrane potential as the hypoxic solution entered the bath. This quickly restabilized, at the lower level shown, and remained constant for the rest of the experiment.

Figure 4 The effect of glibenclamide (10 μ M) on hypoxia-induced shortening of APD₉₀ in guinea-pig papillary muscle. There was a latency of 10–15 min following the infusion of glibenclamide before an increase in the APD₉₀ was observed. Thereafter, glibenclamide progressively increased the duration of the hypoxic APD₉₀ as its period of incubation was extended.

and 6). The following data demonstrate the stability of the response under ischaemic conditions and shows that DMSO (the solvent used to dissolve some of the drugs) had no effect on APD₉₀. The control APD₉₀ was 137.8 ± 8.5 ms, after 20 min ischaemia it was reduced to 63.8 ± 18.8 ms and in the presence of 0.1% DMSO the APD₉₀ values obtained over time were 66.0 ± 17.7 ms (7 min), 65.8 ± 18.2 ms (15 min), 66.7 ± 15.3 ms (20 min), 66.7 ± 15.1 ms (25 min) and 67.6 ± 15.8 ms (30 min) ($n = 4$ in all cases). In most experiments, test drugs were added after 20 min of ischaemia, although a few preparations required a further 5 min for APD₉₀ readings to attain steady-state. One marked difference between the use of hypoxic and ischaemic bathing solutions was the depolarization of the cell membrane potential (by about 15 mV) which occurred with the ischaemic bathing solution. The depolarization preceded the shortening of the APD₉₀ by several minutes, and was presumably a consequence of the elevated extracellular K⁺ (9.2 mM) in the ischaemic solution.

The aim of this series of experiments was to obtain more quantitative data about the effects of high (near-maximally effective) concentrations of E-4031 and UK-68,798, which

were the two most potent Class III compounds previously studied under normoxic conditions. As far as we are aware, this is the first time that these Class III drugs have been compared with glibenclamide under ischaemic conditions for their effects on APD. Neither E-4031 (10 μ M, Figure 5a) nor UK-68,798 (10, 30 and 60 μ M, Figure 5b) produced substantial reversal of the ischaemia-induced shortening of the action potential. E-4031 (10 μ M, $n = 7$) produced a net mean increase of 7 ± 3 ms (at 7 min), 11 ± 3 ms (at 20 min) and 14 ± 4 ms (at 30 min); these small increases in the APD₉₀ after 20 and 30 min were significant (Figures 5a and 6a). UK-68,798 (10 μ M, $n = 4$) produced net increases in the APD₉₀ (Figure 5b) similar to those observed with the same concentration of E-4031 (see above data) with increases of 7 ± 4 ms (at 7 min) and 12 ± 6 ms (at 20 min). Even with higher concentrations of UK-68,798 (30 and 60 μ M, $n = 4$), a significant net increase in APD₉₀ was only attained after 20 min in the presence of 60 μ M UK-68,798, when the increase was 24 ± 12 ms (Figures 5b and 6b).

Glibenclamide prolonged ischaemic action potentials in a similar manner to that described above for the hypoxic solu-

Figure 5 Comparison of the effects of (a) E-4031 (10 μ M), (b) UK-68,798 (10, 30 and 60 μ M) and (c) glibenclamide (10 μ M) on APD₉₀ recorded from guinea-pig papillary muscles under conditions of experimental ischaemia (see Methods). The APD₉₀ recorded under ischaemic conditions (open columns show measurements after 7, 15, and 20 min of ischaemia, respectively) was smaller than a corresponding control value (solid column). Once the action potentials under ischaemic conditions had attained a steady-state a test drug was added (hatched columns for times indicated). (a) E-4031 produced a small, but significant, increase in the ischaemic APD₉₀ after 15 min or more (shaded columns show data 7, 15, 20, 25 and 30 min after drug treatment, respectively). (b) UK-68,798 was studied at three concentrations (10 μ M [left hand group of shaded columns], 30 μ M [central group of shaded columns] and 60 μ M [right hand group of shaded columns]) and changes in APD₉₀ were measured after 7, 15 and 20 min. There were small increases in APD₉₀ which were only significant at the highest concentration used (60 μ M for 20 min). (c) Glibenclamide induced larger increases in APD₉₀ during ischaemia which was clearly a time-dependent effect (columns represent measurements made 7, 15, 20, 25 and 30 min after drug addition, respectively). The time-dependent action of glibenclamide contrasts with the effects of E-4031 and UK-68,798. Asterisks represent significant increases in the ischaemic APD₉₀ (measured after 20 min ischaemia) by drug treatment: * P < 0.05; ** P < 0.01; *** P < 0.001.

tion. The threshold concentration for glibenclamide was about 6 μ M and it exhibited a pronounced and progressive time-dependent action (Figure 5c and see Figure 4, in hypoxia). Glibenclamide (10 μ M) produced net increases in the ischaemic APD₉₀ of 34 ± 11 ms (at 7 min, $n = 9$), 52 ± 12 ms (at 20 min, $n = 7$) and 74 ± 5 ms (at 30 min, $n = 6$). These increases in the APD₉₀ were significant 15 min or more after drug addition (Figures 5c and 6a) and were larger than those observed with either E-4031 or UK-68,798 (see above data). Despite prolonged exposure to glibenclamide, ischaemic action potentials were incompletely restored to their control (pre-ischaemic) values. For example, after 30 min of glibenclamide treatment (10 μ M), there was $85 \pm 8\%$ ($n = 6$) restoration of the APD₉₀.

Discussion

In the present experiments, the electrophysiological effects of several Class III compounds have been studied under either normoxic conditions or conditions designed to simulate experimental hypoxia or myocardial ischaemia. The Class III compounds evaluated here exhibited different pharmacological profiles in comparison to the ATP-dependent potassium channel blocking agent, glibenclamide. Under normoxic conditions, the Class III compounds prolonged the intracellularly recorded APD, whereas glibenclamide had no significant effect. In contrast, during hypoxia, the ability of both (+)-sotalol and another more potent methanesulphonamide, UK-66,914, to prolong APD was lost whereas glibenclamide significantly prolonged the shortened action potentials. Similarly, during experimental ischaemia, a near-threshold (10 μ M) concentration of glibenclamide produced larger increases in the APD₉₀ than did high concentrations of E-4031 or UK-68,798. This is particularly pertinent since UK-68,798 is the most potent Class III compound studied to date. In addition, this is the first time that the effects of glibenclamide on APD have been compared with those of E-4031 and UK-68,798 during simulated ischaemia.

Effect of Class III compounds during normoxia and ischaemia

The present data agree with the earlier characterization of the compounds studied ((+)-sotalol, UK-66,914, UK-68,798 and E-4031) as Class III agents (Carmeliet, 1985; Sawada *et al.*, 1988; Tande *et al.*, 1990; Gwilt *et al.*, 1991a,b) since they prolonged the APD₉₀ without any changes in the V_{max} , although there was generally a small increase in the APA of a few millivolts. The threshold concentrations for Class III effects in this study were similar to those obtained in other tests used within our laboratory (for example, changes in the refractory period, Cooper, Lad & MacKenzie, unpublished observations), although they are at variance with other studies which have shown that UK-66,914 and UK-68,798 are about 30 times more potent than the present data. For example, in experiments which used similar experimental conditions (36–37°C and a stimulation frequency of 1 Hz) UK-68,798 and UK-66,914 elicited threshold increases in the canine ventricular muscle APD at ≥ 5 nM and ≥ 0.1 μ M, respectively (Gwilt *et al.*, 1991a,b). In addition, Tande *et al.* (1991) used continuous microelectrode recordings from guinea-pig papillary muscles (32°C and 1 Hz) to show that ≥ 10 nM UK-68,798 prolonged the APD.

Although some differences in methodology are apparent between the present microelectrode work and that of other groups (for example, slight differences in bathing solution composition, cell sampling technique or experimental temperature), the differences in threshold potency between the present data and that from other studies cannot be fully explained. Like Tande *et al.* (1991), we studied action potential parameters from prolonged recordings of a single, representative, cell within the preparation in the absence and

Figure 6 The effect of (a) E-4031 (10 μ M) followed by glibenclamide (10 μ M) and (b) UK-68,798 (10, 30 and 60 μ M) on ischaemia-induced shortening of APD₉₀ in guinea-pig papillary muscle. (a) In this cell, there was only a small effect of E-4031, which showed no time-dependency, over a 30 min period (see Figure 5a for quantified data). After 30 min treatment with E-4031, this drug was removed from the ischaemic bathing solution and replaced with glibenclamide (10 μ M for 30 min). During this period, glibenclamide induced a prominent and characteristic time-dependent prolongation of APD₉₀. There was a latency of 10–15 min following the infusion of glibenclamide before an increase in the APD₉₀ was observed. (b) In this cell, there was a small effect of UK-68,798 which was more prominent at the lowest concentration used (10 μ M); further increases in concentration (30 and 60 μ M) produced comparatively small increases in APD₉₀ (see Figure 5b for quantified data).

presence of drugs, rather than use a serial sampling technique. This is because we wanted to use each cell as its own control to study the effect of several drug concentrations, measured at predetermined time-points, once the cell had completely resealed after impalement. The action potential parameters usually attained a steady-state 10–15 min after impalement. Although Tande *et al.* used a 40 min exposure period for each concentration of Class III agent, they also saw significant effects of lower concentrations within 10 min (which was similar to the 7 min time cycle adopted here).

Our rank order of potency, with UK-68,798 as the most potent of these compounds, is in good agreement with the literature. An EC₁₅ value was used only as an indicator of potency – it did not, however, take into account the different maximal responses to the Class III compounds. The newer methanesulphonamide derivatives produced a larger maximal prolongation of APD₉₀ than (+)-sotalol, suggesting their greater efficacy or a slightly different mechanism of action. (+)-Sotalol was used in these experiments because the (+)-stereoisomer has less activity as a β -adrenoceptor blocking agent compared to the (–)-stereoisomer (Woosley *et al.*, 1990).

Several questions could not be addressed using data from the present microelectrode experiments. Firstly, which potassium channel(s) is (are) associated with the drug-induced Class III effects under normoxic conditions? We can

speculate about some possibilities based upon voltage-clamp data from guinea-pig isolated myocytes. Under normoxic conditions, time- and voltage-dependent potassium currents and their associated tail currents are mediated by different mechanisms (Sanguinetti & Jurkiewicz, 1990; Wettwer, 1990). It has also been suggested that there are two components of this 'delayed rectifier' current which exhibit differential drug sensitivity to the Class III compounds E-4031 and (+)-sotalol (Sanguinetti & Jurkiewicz, 1990). This interpretation differs from the more established view that Class III compounds act only on the time- and voltage-dependent currents or their tails (often collectively described as the 'delayed rectifier' or I_K). Secondly, how was the slight prolongation of the APD₉₀ produced by Class III drugs during experimental hypoxia or ischaemia? This could have arisen from incomplete block of a single component of outward current. Alternatively, the high concentrations of Class III agents used could have had a small effect on other potassium channel subtypes, or they could have promoted some inward current which might explain some of the small increases in APA which we see during ischaemia or hypoxia.

The implications of the present experiments are that Class III compounds may have limited effects against ischaemic arrhythmias in some experimental or clinical settings. Cobbe (1988) reviewed available evidence which suggested that Class III compounds largely lost their ability to prolong action

potential duration in abnormal (hypoxic or ischaemic) myocardium. Much of the experimental support for this suggestion (see Cobbe, 1988) relied on observations made with sotalol, but the limited effects of the newer and more potent Class III compounds studied in the present microelectrode experiments endorse these views. Additionally, recent *in vivo* data suggest that these newer Class III compounds do not provide significant protection against the lethal ischaemic arrhythmias which follow coronary artery occlusion in the anaesthetized cat, even at relatively high doses (Lad & MacKenzie, 1991). However, E-4031 has been reported to have antagonistic effects towards chronic ischaemic arrhythmias *in vivo* in canine post-infarction models (Kato *et al.*, 1990; Lynch *et al.*, 1990). This could be due to either its primary Class III effect in the normoxic myocardium, rather than an effect on ATP-dependent potassium channels, or even some other effect *in vivo*.

Effect of glibenclamide under hypoxic/ischaemic conditions

Under hypoxic or ischaemic conditions, ATP-dependent potassium channels are activated by falls in the intracellular ATP concentration and the cardiac action potential is shortened as a result of the promotion of a time-independent outward K^+ current (Noma, 1983; Fosset *et al.*, 1988). When such a low intracellular ATP concentration was produced by internal perfusion of guinea-pig cardiac myocytes, glibenclamide (20–50 nM) prolonged the shortened APD and also reduced the opening probability of the ATP-dependent single channels (Fosset *et al.*, 1988). In the present work, the electrophysiological consequences of hypoxia (shortening of APD and small reduction in APA) were largely the same as those following ischaemia, with the exception that the elevated potassium concentration in the ischaemic bathing solution elicited an additional partial membrane depolarization, and these effects were largely antagonized by the subsequent addition of glibenclamide. The significant effect of glibenclamide on APD under hypoxic or ischaemic conditions contrasted with its lack of effect under normoxic conditions. Glibenclamide antagonized the effects of ischaemia at a slightly lower threshold concentration (about 6 μ M) than under hypoxic conditions (about 10 μ M) but the effects of the drug were qualitatively similar. The incomplete restoration of ischaemic APD to control values by glibenclamide (approximately 85% at 10 μ M) could either be interpreted as a substantial but partial block of ATP-dependent potassium channels by glibenclamide, or could indicate the presence of other minor components of potassium current activated during ischaemia.

Numerous investigators have shown that glibenclamide pretreatment has antagonistic effects in isolated cardiac muscle (usually at micromolar concentrations) against subsequent events elicited by metabolic inhibition or experimental hypoxia/ischaemia. Nakaya *et al.* (1991) showed that 60 min pretreatment of guinea-pig isolated papillary muscle with 10 μ M glibenclamide partially prevented the subsequent shortening effects of experimental hypoxia or metabolic inhibition on APD; this effect of glibenclamide was maximal at 20 μ M. In the present work, continuous microelectrode recordings of action potentials have extended these observations. For the first time, our recordings from cardiac muscle under normoxic and, subsequently hypoxic or ischaemic conditions, have highlighted a time-dependent component of the action of glibenclamide. This effect was apparent 7–10 min after the addition of glibenclamide during ischaemia (or hypoxia) and was significant and progressive after 15 min or more. This might have implications for experiments where long periods of pretreatment with glibenclamide are studied.

The time-dependent action of glibenclamide raises some

questions as to the precise site and mechanism of action of this drug. Although glibenclamide and other sulphonylureas have high-affinity binding sites typified by low K_d values (with potent compounds having activity in the nanomolar range, see Fosset *et al.*, 1988), the precise relationship between the concentrations required for binding at these sites and those required for their pharmacological effects in cardiac tissue (usually in the micromolar range or higher) have yet to be determined for a wide range of compounds. In guinea-pig myocytes, in the presence of low intracellular ATP concentrations, glibenclamide has been shown to have a potent action (20–50 nM) on both APD and single ATP-dependent potassium channels (Fosset *et al.*, 1988). However, other groups have found that much higher concentrations of glibenclamide are required to modify either hypoxic/ischaemic APD (Nakaya *et al.*, 1991 and present work), ATP-dependent potassium current from whole-cell recordings of guinea-pig myocytes during metabolic inhibition (Venkatesh *et al.*, 1991), or single-channel recordings from inside-out membrane patches (Venkatesh *et al.*, 1991) or open-cell patches (Nakaya *et al.*, 1991). Sulphonylureas have a comparatively short latency of action at single channels in heart (Nakaya *et al.*, 1991; Venkatesh *et al.*, 1991) and pancreas (Belles *et al.*, 1987) and a slower time-course in whole-cell experiments (Belles *et al.*, 1987) so this might have implications for the present microelectrode study. Perhaps, in addition to an immediate action on channels in isolated membrane patches, glibenclamide has some delayed effect on membrane conductance associated with slower second-messenger systems or by some metabolic pathway compartmentalised within the cardiac cells. These second messenger systems might be better preserved by use of microelectrode techniques.

While physicochemical properties of sulphonylureas may contribute to components of their biological activity in pancreatic β -cells (Zünkler *et al.*, 1989), it is at present unclear to what extent these factors come into play in myocardial tissue. Since sulphonylureas are generally more potent in electrophysiological terms in pancreatic tissue compared to cardiac muscle (Trube *et al.*, 1986; Belles *et al.*, 1987), ATP-dependent potassium channel blockers with greater cardioselectivity would obviously be desirable for the management of some ischaemic cardiac rhythm disturbances.

Conclusion

Class III agents clearly have an important role to play in the management of certain atrial and ventricular rhythm disturbances in normoxic situations, whereas glibenclamide would be ineffective. In a pro-arrhythmic ischaemic environment, a compound with a glibenclamide-like profile (showing decreased dispersion of refractoriness) would probably be more effective than a Class III agent. Indeed, the Class III agent might still prolong APD in normoxic tissue and exacerbate the dispersion in refractoriness between normoxic and ischaemic tissue. However, a more complete understanding of the functional effects of glibenclamide against ischaemic arrhythmias is desirable in a wider range of species *in vivo* before ATP-dependent potassium channel blockers can be used clinically in the treatment of lethal ventricular arrhythmias which arise after acute myocardial ischaemia.

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