Electrophysiological and antiarrhythmic effects of UK 52,046-27 during ischaemia and reperfusion in the guinea-pig heart

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1 We studied the antiarrhythmic and electrophysiological effects of UK 52,046-27 (10^{-8} M and 5×10^{-8} M), a highly selective α_1 -adrenoceptor antagonist, during global ischaemia (flow reduced to 10% of control for 30 min) and reperfusion, in isolated, buffer-perfused hearts of guinea-pigs.

2 The compound had few electrophysiological effects during normal perfusion, although action potential amplitude and $\dot{V}_{max}$ were reduced with 10^{-8} M (by 9% and 22%) and refractory period was increased with 5×10^{-8} M (by 13%) compared to control hearts.

3 Perfusion with 5×10^{-8} M UK 52,046-27 reduced the incidence of ventricular tachycardia during ischaemia from 67% to 25%, and during reperfusion reduced the incidence of ventricular tachycardia (from 83% to 17%) and ventricular fibrillation (from 67% to 8%).

4 The compound prolonged significantly action potential duration and refractory period during ischaemia and reperfusion. $\dot{V}_{max}$ was reduced to a greater extent during reperfusion in the treated hearts, while greater increases in QRS width and stimulation threshold occurred during ischaemia in the treated group.

5 These results confirm that blockade of the α_1 -adrenoceptor subpopulation during myocardial ischaemia and reperfusion decreases the incidence of arrhythmias and alters cellular electrophysiology during ischaemia and reperfusion.

Introduction

Several recent studies (Sheridan *et al.*, 1980; Stewart *et al.*, 1980; Penny *et al.*, 1985) have indicated that α -adrenoceptor blockade has significant antiarrhythmic effects during myocardial ischaemia *in vivo* and *in vitro*. These studies suggested that both selective and non-selective α -adrenoceptor antagonists were effective. Thandroyen *et al.* (1983) suggested that α_2 -adrenoceptor blockade may be more effective than α_1 , and proposed that the relatively high concentrations of α_1 -adrenoceptor blocking agents required to achieve an antiarrhythmic effect would indicate a direct myocardial action rather than one on adrenoceptors. Recent studies in the guinea-pig heart have demonstrated that selective α_1 -adrenoceptor stimulation is arrhythmogenic during myocardial ischaemia and reperfusion

(Culling *et al.*, 1987) and we considered that selective antagonism of this subpopulation of adrenoceptors during ischaemia and reperfusion should therefore be antiarrhythmic. To assess the efficacy of α_1 -adrenoceptor antagonism, we studied the antiarrhythmic and electrophysiological effects of UK 52,046-27, a highly selective α_1 -antagonist (M.J. Davey, personal communication) during global ischaemia and reperfusion in isolated, buffer-perfused hearts of guinea-pigs.

Methods

Hearts were rapidly removed from stunned male guinea-pigs (Dunkin-Hartley, 500-600 g) and mounted for perfusion by the Langendorff technique. Perfusion was performed with oxygenated aqueous Krebs-Henseleit buffer prewarmed to 32°C, containing (mM): NaCl 118, KCl 4.7, MgSO_4 1.2, KH_2PO_4

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Figure 1. Changes in action potential amplitude (a) and upstroke velocity ($\dot{V}_{max}$) (b) during ischaemia and reperfusion in control hearts (○) and in hearts treated with $10^{-8} M$ (□) and $5 \times 10^{-8} M$ (△) UK 52,046-27. The statistical significance levels indicated refer to differences between the control and drug-treated groups: * $P < 0.05$; ** $P < 0.02$ and *** $P < 0.01$, for $5 \times 10^{-8} M$; * $P < 0.05$; ** $P < 0.02$ and *** $P < 0.01$, for $10^{-8} M$ UK 52,046-27.

1.1, $NaHCO_3$ 24, $CaCl_2$ 2.5, glucose 9 and pyruvate 2 (pH 7.4) at a constant flow rate of $5 ml min^{-1} g^{-1}$. Hearts were supported horizontally in the organ bath and superfused with aqueous buffer (as above) at $32^\circ C$ to avoid alterations in temperature associated with myocardial ischaemia. Heart rate was maintained constant to 3.3 Hz (just above the spontaneous sinus rate) by right ventricular pacing at twice the diastolic pacing threshold. Action potentials, refractory periods, electrocardiograms and perfusion pressure were recorded as previously described (Penny & Sheridan, 1983). Action potentials were analysed with an on-line microcomputer, designed to provide real-time analogue reconstruction of digitised data prior to analysis (Sheridan *et*

al., 1983). Transmembrane action potentials were recorded by the floating microelectrode technique from epicardial cells in the apical region of the right ventricle. With this method, the heart is impaled at each time point and, once a stable impalement is achieved, ten action potentials are analysed. This is repeated in each heart, at each time point and means obtained during normal perfusion, global myocardial ischaemia and reperfusion ($n = 11$ impalements per heart).

Myocardial ischaemia was induced by reducing global coronary flow to $0.5 ml min^{-1} g^{-1}$ (10% of control) for 30 min, and reperfusion was initiated by restoring flow to control. The time of onset and duration of spontaneously occurring ventricular tachycardia (VT) and ventricular fibrillation (VF) were noted during ischaemia and reperfusion. The incidence of VT and VF, and electrophysiological changes were studied in 12 control hearts, in 15 hearts perfused with buffer containing $10^{-8} M$ UK 52,046-27, and in 12 hearts which were perfused with buffer containing $5 \times 10^{-8} M$ UK 52,046-27 during ischaemia and reperfusion.

Electrophysiological measurements are expressed as the mean and standard error of the mean for each group. Comparisons are made between the groups using the non-paired *t* test or chi-squared analysis, and within groups using the paired *t* test, with $P < 0.05$ defining the probability value indicating statistical significance.

UK 52,046-27 (4-amino-6,7-dimethoxy-2-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl)quinoline methanesulphonate) was obtained from (Pfizer Central Research, Sandwich, Kent).

Results

Perfusion with $5 \times 10^{-8} M$ UK 52,046-27 reduced the incidence of ventricular tachycardia during ischaemia from 67% to 25% ($P < 0.05$), and during reperfusion reduced the incidence of ventricular tachycardia from 83% to 17% and reduced the incidence of ventricular fibrillation from 67% to 8%. During ischaemia, ventricular fibrillation occurred in 25% of control hearts and 17% of treated hearts. Spontaneous onset of arrhythmias occurred during ischaemia at 15.9 ± 1.0 min in the control group and at 20.3 ± 2.3 min in the treated group. Perfusion with $5 \times 10^{-8} M$ reduced the duration of ventricular tachycardia during ischaemia from 9.9 ± 2.1 min to 2.8 ± 1.1 min. During normal perfusion of the hearts, refractory period was greater in hearts treated with the $5 \times 10^{-8} M$ UK 52,046-27 (145 ± 3 ms vs. 128 ± 4 ms).

During perfusion with $10^{-8} M$ UK 52,046-27, ventricular tachycardia occurred during ischaemia in 40% and during reperfusion in 60%. Ventricular

fibrillation occurred in no hearts during ischaemia and in 47% during reperfusion with this concentration. Spontaneous onset of arrhythmias during ischaemia was delayed from 15.9 ± 1.0 min to 21.7 ± 2.0 min with 10^{-8} M UK 52,046-27. During ischaemia, ventricular tachycardia lasted for 9.9 ± 2.1 min in control hearts and 8.3 ± 2.0 min in hearts perfused with 10^{-8} M UK 52,046-27. During normal perfusion of the hearts with this concentration of the compound, action potential amplitude was reduced from 98 ± 2 mV in the control group to 89 ± 2 mV and $\dot{V}_{max}$ was similarly reduced from 91 ± 5 V s $^{-1}$ to 71 ± 4 V s $^{-1}$.

Figure 1 shows that action potential amplitude was similarly and significantly reduced by ischaemia in both control and treated hearts. UK 52,046-27 5×10^{-8} M prevented the spontaneous recovery in action potential amplitude seen in the control hearts at 20 min of ischaemia (73 ± 7 mV vs. 55 ± 3 mV). Ischaemia significantly reduced $\dot{V}_{max}$ to about 50% of control values in both groups (Figure 1). Recovery of $\dot{V}_{max}$ during reperfusion was prevented in the hearts treated with 5×10^{-8} M UK 52,046-27 (76 ± 11 V s $^{-1}$ in control hearts vs. 47 ± 4 V s $^{-1}$ in the treated hearts at 5 min of reperfusion (Figure 1)).

Figure 2 shows that action potential duration (APD) was significantly reduced in all groups during ischaemia. UK 52,046-27 5×10^{-8} M modified this, prolonging APD at 10 and 15 min of ischaemia and after 30 s, 5 and 10 min of reperfusion. Thus, although the compound had no effect on APD during normal perfusion, it prolonged APD during ischaemia (145 ± 3 ms vs 131 ± 4 ms, $P < 0.01$, at 10 min and 133 ± 4 ms vs 118 ± 5 ms, $P < 0.05$ at 15 min), and increased APD during early and later reperfusion (111 ± 8 ms vs 134 ± 4 ms, $P < 0.02$ at 30 s, and 162 ± 3 ms vs 177 ± 3 ms, $P < 0.01$ at 5 min, Figure 2). A rapid, significant, early increase in refractory period occurred during ischaemia in all groups (24% in control hearts, 41% in 10^{-8} M and 17% in 5×10^{-8} M treated hearts all at 5 min, Figure 2). Refractory periods increased to a similar extent during ischaemia in all groups, and at 10 min were longer in the drug-treated groups before returning towards control values. UK 52,046-27, at the higher concentration increased refractory periods throughout reperfusion (162 ± 4 ms vs 120 ± 9 ms, at 2 min, Figure 2).

Conduction time increased similarly and significantly in all groups during ischaemia (compared to normal perfusion), Figure 3. QRS width followed a similar pattern (Figure 3), increasing significantly during ischaemia by 36%, 41% and 57% in control, 10^{-8} M treated and 5×10^{-8} M treated hearts respectively at 10 min but with greater values in the group perfused with the higher concentration compared to control hearts during early ischaemia (42 ± 4 ms vs

Figure 2 Changes in action potential duration (APD) (a) and refractory period (RP) (b) during ischaemia and reperfusion in control hearts (○) and hearts treated with 10^{-8} M (□) and 5×10^{-8} M (△) UK 52,046-27. Note that the higher concentration of the drug prolonged APD and RP during ischaemia and reperfusion. The statistical significance levels indicated refer to differences between the control and drug-treated groups: * $P < 0.05$, ** $P < 0.02$ and *** $P < 0.01$ for 5×10^{-8} M; * $P < 0.05$; ** $P < 0.02$ and *** $P < 0.01$, for 10^{-8} M UK 52,046-27.

32 ± 2 ms after 5 min). Stimulation threshold was also significantly increased by ischaemia (Figure 3). This effect was also more pronounced in the hearts treated with 5×10^{-8} M UK 52,046-27 at 15 and 20 min of ischaemia (5.94 ± 0.61 V vs 3.90 ± 0.63 V at 15 min), and during early and later reperfusion when stimulation threshold was elevated in these hearts (5.40 ± 0.42 V vs 2.90 ± 0.95 V at 2 min and 5.07 ± 0.52 V vs 3.00 ± 0.75 V at 10 min).

Discussion

In recent years, evidence has accumulated which suggests that α -adrenoceptors play an important role in

Figure 3 Changes in conduction time (a), QRS width (b) and stimulation threshold (c) during ischaemia and reperfusion in control hearts (○) and hearts treated with 10^{-8} M (□) and 5×10^{-8} M (△) UK 52,046-27. The higher concentration of the drug prolonged QRS width and increased stimulation threshold during ischaemia. The statistical significance levels indicated refer to differences between the control and drug-treated groups: * $P < 0.05$; ** $P < 0.02$ and *** $P < 0.01$ for 5×10^{-8} M; * $P < 0.05$; ** $P < 0.02$ and *** $P < 0.01$ for 10^{-8} M UK 52,046-27.

mediating the arrhythmogenic effects associated with myocardial ischaemia and reperfusion (Sheridan *et al.*, 1980; Stewart *et al.*, 1980; Thandroyen *et al.*, 1983; Penny *et al.*, 1985). α -Adrenoceptor antagonists produce haemodynamic effects, although these can be reversed without attenuating the antiarrhythmic action (Sheridan *et al.*, 1980), suggesting that their antiarrhythmic effect is independent of such peripheral actions. Cellular electrophysiological studies support this (Penny *et al.*, 1985; Culling *et al.*, 1984), and although direct myocardial electrophysiological effects can be demonstrated for some

α -adrenoceptor antagonists in high concentrations (Rosen *et al.*, 1971) which might explain their antiarrhythmic activity, these effects are not apparent at lower concentrations which are potentially antiarrhythmic (Penny & Sheridan, 1982; Penny *et al.*, 1985). Further evidence supporting a role for α -adrenoceptors in mediating the arrhythmogenic effects of myocardial ischaemia comes from studies in which α_1 -adrenoceptor stimulation has been shown to be arrhythmogenic, but without effect during normal perfusion (Culling *et al.*, 1987).

Thandroyen *et al.* (1983) suggested that antiarrhythmic effects of α_2 -adrenoceptor blockade may be more effective than α_1 . The present results indicate that selective blockade of the α_1 subpopulation of adrenoceptors during myocardial ischaemia and reperfusion is effective in decreasing the incidence of arrhythmias and further demonstrates that a relatively low concentration (5×10^{-8} M) is required for antiarrhythmic activity. Possible changes in the numbers of α -adrenoceptors during myocardial ischaemia which may complicate the interpretation of data from such studies have been suggested (Corr *et al.*, 1981), but not always demonstrated by other workers (Broadley *et al.*, 1985; Crome *et al.*, 1985). The high affinity of UK 52,046-27 for myocardial α_1 -adrenoceptors and its selectivity as an α_1 -adrenoceptor antagonist ($IC_{50} = 6$ pM for α_1 -adrenoceptors vs 25 nM for α_2 -adrenoceptors vs 8.1μ M for β -adrenoceptors vs $> 10 \mu$ M for 5-HT₂-receptors from radioligand binding studies; $pA_2 = 8.91$ in rabbit papillary muscles; M.J. Davey, personal communication) may help to explain its efficacy in low concentrations compared to other non-specific antagonists. The results obtained also demonstrate that this antiarrhythmic action is mediated by altering cellular electrophysiology during ischaemia and reperfusion with relatively little effect on normally perfused myocardium. The prolongation in APD, and prevention of spontaneous recovery of action potential amplitude and upstroke velocity during ischaemia and reperfusion are likely to be important in the prevention of arrhythmias, but would not have been predictable from a study of the compound's action during normal perfusion at a concentration which has antiarrhythmic effects during myocardial ischaemia and reperfusion (5×10^{-8} M). The electrophysiological effects of UK 52,046-27 during ischaemia and reperfusion are broadly similar to those seen with other α -adrenoceptor antagonists (Penny *et al.*, 1985). In previous studies we have shown that these effects are similar to those produced by depletion of myocardial catecholamines and that additional electrophysiological effects are not seen in the absence of endogenous myocardial catecholamines (Culling *et al.*, 1984). Such findings indicate that adrenergic mecha-

nisms, rather than a direct action on sarcolemmal properties, mediate their effects.

Although some early anecdotal reports suggest that α -adrenoceptor blockade may be antiarrhythmic in man (Gould *et al.*, 1969) there are as yet no convincing studies available. The electrophys-

iological effects of UK 52,046-27 demonstrated here, and its claimed cardioselectivity (M.J. Davey, personal communication) if substantiated, suggest that it may be a suitable agent with which to test this important hypothesis in man.

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