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Anti-arrhythmic and electrophysiological effects of the endothelin receptor antagonists, BQ-123 and PD161721

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Abstract

The effects of the endothelin ET_A, (BQ-123) and endothelin $ET_{A/B}$ (PD161721) receptor antagonists were investigated on ischaemia-induced arrhythmias and on the maximum following frequency. The study was carried out in Langendorff perfused rat hearts subjected to coronary artery occlusion in which the severity of arrhythmias, coronary perfusion pressure and heart rate were measured. The % incidence of ischaemia-induced irreversible ventricular fibrillation (ventricular fibrillation) was reduced significantly from 58%, in control rat hearts, to 0% (at 10^{-7} and 10^{-6} M PD161721 and 10^{-6} M BQ-123 $P < 0.05$). Maximum following frequency was measured in guinea-pig isolated atria. In the presence of normal extracellular $[K^+]$, BQ-123 and PD161721, at 10^{-6} M, significantly decreased the maximum following frequency from 9.0 ± 0.7 to 7.2 ± 0.4 and from 8.3 ± 0.4 to 6.7 ± 0.3 Hz, respectively ($P < 0.05$). These effects were not potentiated by raising the extracellular $[K^+]$ with the exception of 10^{-9} M PD161721. In contrast, lignocaine's ability to reduce the maximum following frequency was greater in elevated (e.g. at 1.7×10^{-4} M from 8.4 ± 0.3 to 2.5 ± 0.6 Hz) than in normal $[K^+]$ (from 9.0 ± 0.3 to 4.9 ± 0.5 Hz). In conclusion, both BQ-123 and PD161721 had an anti-fibrillatory effect in isolated rat hearts that may be due, at least in part, to an ability to reduce the maximum following frequency. This latter effect is unlikely to be due to Na^+ channel blockade since it was not markedly potentiated by elevation of extracellular $[K^+]$. © 2001 Published by Elsevier Science B.V.

Keywords: Endothelin antagonist; Arrhythmia; Cardiac electrophysiology; BQ-123; PD161721

1. Introduction

Endothelin receptor antagonists have been shown to have beneficial effects in the setting of myocardial ischaemia. The endothelin ET_A receptor antagonists, BQ-123, LU135252 and PD155080, all reduced infarct size in animal models of ischaemia and reperfusion (Grover et al., 1993; Gonon et al., 1998; Brunner and Opie, 1998). Mixed endothelin ET_A/ET_B receptor antagonists have also been shown to reduce the extent of myocardial injury following coronary artery occlusion and reperfusion (Wang et al., 1995b; Vitola et al., 1996). The literature concerning the effects of endothelin receptor antagonists on ischaemia/reperfusion-induced arrhythmias is more controversial. The severity of reperfusion-induced arrhythmias has been shown in several studies not to be modified by endothelin ET_A (Grover et al., 1993) or mixed receptor antagonists (Richard et al., 1994; Wang et al., 1995b). However, the endothelin ET_A receptor antagonists BQ-123 and LU135252, as well as the mixed endothelin ET_A/ET_B receptor antagonist PD161721 (Bryant et al., 1996), have all been demonstrated to suppress the arrhythmias that occur during coronary artery occlusion in vivo (Garjani et al., 1995; Sharif et al., 1998; Raschack et al., 1998).

The mechanism(s) underlying this anti-arrhythmic of endothelin receptor antagonists has not been elucidated. Since exogenous endothelin has a pro-arrhythmic effect in normal (Yorikane and Koike, 1990) and ischaemic myocardium (Garjani et al., 1995) and it has been shown that myocardial release of endothelin occurs during ischaemia/reperfusion protocols (Tønnessen et al., 1993; Wang et al., 1995a), it is possible that endothelin-receptor antagonists exert their anti-arrhythmic action by blocking the pro-

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arrhythmic effect of endogenously released endothelin. Another possible mechanism of action is that these antagonists have direct cardiac electrophysiological effects that are independent of their ability to block endothelin receptors.

The aim of this study was to examine the effects of both the endothelin ET_A, antagonist BQ-123 and the mixed endothelin ET_A/ET_B receptor antagonist, PD161721 on ischaemia-induced arrhythmias in vitro, in the isolated Langendorff perfused rat heart. The effects of these antagonists on the maximum following frequency were also determined in guinea-pig atrial muscle, and compared with that of lignocaine, which is known to reduce the maximum following frequency via Na^+ channel blocking activity (Kane, 1980).

2. Methods and materials

2.1. Surgical procedure for isolated rat heart study

All experimental procedures were carried out in accordance with the United Kingdom Home Office Animal Scientific Procedures Act, 1986. Male Sprague–Dawley rats (300–450 g) were anaesthetised with sodium pentobarbitone (60 mg kg^{-1} i.p.) and heparin (500 i.u.) was administered i.v. via the dorsal penile vein. A transverse laparotomy and a left and right anterolateral thoracotomy were performed. On removal of the pericardium, a 6/0 braided silk suture (attached to a 10-mm micropoint reverse cutting needle) was placed around the left main coronary artery. The heart was rapidly excised and immersed in heparinised (500 i.u.) ice-cold Krebs-Henseleit buffer (in mM: NaCl 118, KCl 3.2, $CaCl_2$ 2.5, $MgSO_4$ 1.7, $NaHCO_3$ 27, KH_2PO_4 1.2, D-glucose 5.6, Na-pyruvate 2). The aorta was attached to a metal cannula and the heart mounted on a Langendorff perfusion apparatus for retrograde perfusion. Temperature was maintained at 37 °C using a heated jacket and hearts were perfused with a Kreb's Henseleit solution (pH 7.4) at a constant flow rate (12 ml min^{-1}) using a roller pump (Watson-Marlow 505S). The coronary perfusion pressure (coronary perfusion pressure, mm Hg) was measured using a pressure transducer (Gould) via a side arm just proximal to the aortic cannula. A surface electrocardiogram was recorded via electrodes placed on the right atrium and left ventricle and the coronary perfusion pressure and electrocardiogram traces were continuously recorded on a two-channel chart recorder (Gould TA 240). Heart rate (in beats per minute) was calculated from the electrocardiogram trace. After a 30-min stabilisation period in normal Kreb's the perfusion medium was changed to a modified Kreb's Henseleit solution (in mM: NaCl 118, KCl 2, $CaCl_2$ 1.23, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, D-glucose 11) for the remainder of the experiment. This has been shown in our previous work to increase the incidence of ventricular fibrillation during coronary artery occlusion (Demiryürek et al., 1998). The hearts were then stabilised for a further 45-min period, to allow the equilibration of the coronary perfusion pressure and the extracellular K^+ concentration.

2.2. Induction of myocardial ischaemia and arrhythmia analysis

Rat hearts were allocated to one of five groups; vehicle infusion (control; $n = 12$), BQ-123 at 5×10^{-7} M ($n = 8$), or 10^{-6} M ($n = 8$), PD161721 at 10^{-7} M ($n = 8$) or 10^{-6} M ($n = 7$). These concentrations were chosen as the ones that would block endothelin ET_A (BQ-123, Ihara et al., 1992) and both endothelin ET_A and ET_B receptors (PD161721, Bryant et al., 1996). Following the stabilisation period described above, the perfusion solution was changed to one containing the drug or vehicle for 10 min before and throughout the occlusion protocol. The coronary artery was occluded by threading the ends of the ligature around the coronary artery, through a short length of polythene tubing to form a snare. The snare was then tightened, and held in place with a small artery clip. Successful coronary occlusion was determined by a rapid rise in coronary perfusion pressure within 1 min of occlusion. The consequent ventricular arrhythmias were monitored for 30 min. Ventricular arrhythmias were analyzed (from the electrocardiogram trace) according to the guidelines of the Lambeth Conventions for the determination of experimental arrhythmias (Walker et al., 1988). Arrhythmias were classified as single ventricular premature beats, salvos (couplets or triplets ventricular premature beats), and ventricular tachycardia (defined as a run of four or more ventricular ectopic beats). The total number of ventricular ectopic beats (calculated as the sum of individual arrhythmias) was only counted in hearts that survived the 30-min period of ischaemia. Ventricular fibrillation was defined as when individual QRS complexes could no longer be distinguished, and successive waves were inconsistent both in amplitude and rhythm. The percent incidence of ventricular tachycardia, total (reversible and irreversible) ventricular fibrillation and irreversible ventricular fibrillation (any period of ≥ 5 min ventricular fibrillation) was noted for each group. Any heart that developed persistent spontaneous ventricular arrhythmias before the administration of the endothelin ET-1 antagonists or vehicle was excluded from the study. Hearts were also excluded if the tightening of the ligature described in the methodology did not produce an immediate and sustained increase in coronary perfusion pressure. Under these criteria two hearts were excluded.

2.3. Determination of the maximum following frequency in isolated guinea pig atrium

The left atria from male Dunkin–Hartley guinea pigs (400–450 g) were suspended in 20 ml organ baths contain-

ing a Krebs Henseleit solution gassed with 95% oxygen and 5% carbon dioxide and maintained at a temperature of 32 °C. The composition (mM) of the bathing medium was as follows: NaCl, 117.4; $NaHCO_3$, 25; NaH_2 PO_4, 1.2; $MgSO_4$, 1.2; KCl, 5.4; $CaCl_2$, 2.5; and glucose, 11.1. The atrium was secured to a silver hook through which rectangular pulses of 5-ms duration at three times threshold voltage were delivered at a frequency of 4 Hz. A tension of 500 mg was applied and isometric contractions recorded on a pen recorder. After a 30-min equilibration period, two or three determinations of the maximum following frequency (Hz) were made to ensure the consistency of the measurement before the experimental protocol began. The maximum following frequency was determined by increasing the frequency of stimulation until the preparation no longer responded with individual contractions to each stimulation pulse (Winslow, 1984). BQ-123 (10^{-8}–10^{6} M, $n=6$), PD161721 (10^{-9}–10^{6} M, $n=6$) or lignocaine (0.43–$3.4.10^{-4}$ M, $n=10$) was added cumulatively to the organ bath and the maximum following frequency was determined 10 min after the addition of each concentration. Between the maximum following frequency measurements, the atria were stimulated at 4 Hz, at which frequency all determinations of developed tension were made. A similar procedure was carried out in three preparations to which vehicle (deionised water) alone was added. In these tissues, there was no significant change in either the maximum following frequency or developed tension. Since elevated extracellular potassium is known to enhance the effects of drugs with Na^+-channel blocking activity (Grant et al., 1999), a separate set of experiments was carried out in atria bathed in a physiological salt solution which was identical to that described above for the atrial experiments with the exception that the KCl concentration was 8 mM. The maximum following frequency and amplitude of contraction (mg) was measured before and following exposure to BQ-123 (10^{-8}–10^{6} M, $n=5$), PD161721 (10^{-9}–10^{6} M, $n=7$) or lignocaine (0.43–3.4×10^{-4} M, $n=6$) using an identical protocol to that described for experiments in 5.4 mM KCl.

2.4. Statistical analysis

The total numbers of ventricular ectopic beats are expressed as median (Q1–Q3), and were compared using Mann–Whitney non-parametric test. The incidences of ventricular tachycardia, total ventricular fibrillation and irreversible ventricular fibrillation are expressed as a percentage incidence for the group, and statistical significance was assessed using Fisher's exact (X^2 with Yate's correction) test. Coronary perfusion pressure, heart rate, maximum following frequency and amplitude of contraction (mean ± S.E.M.) were assessed within the group by one-way analysis of variance (ANOVA) and Dunnett's multiple comparison test determined significant differences. Variations in coronary perfusion pressure, heart rate and the maximum following frequency between groups were compared by a two-tailed unpaired Student's *t*-test. All differences were taken as significant if $P \leq 0.05$.

2.5. Drugs and materials

Sodium chloride and sodium pyruvate were purchased from Sigma. Potassium dihydrogen orthophosphate, sodium hydrogen carbonate, potassium chloride, calcium chloride, magnesium sulphate, and glucose were all purchased from BDH Laboratory Supplies (Poole, BH15 1TD, England). Sodium pentobarbitone and heparin were purchased from Rhone Merieux (Ireland) and Leo Laboratory (Ireland), respectively. PD161721, [Sodium3-cyclohexylmethyl-4-(2,3-dihydro-benzo-[1,4]dioxin-6-yl)-2-(7-methoxy-benzo [1,3]dioxo-5-yl)-4-oxo-but-2-enoate] was obtained as a gift from Parke-Davis (USA). BQ-123 [cyclo(D-Asp-L-Pro-D-Val-L-Leu-D-Trp) was purchased from Alexis (England). The antagonists were dissolved in distilled water to yield a stock solution and subsequently diluted in the perfusing solution to yield the required concentrations. All stock solutions were stored in the freezer at −25 °C.

3. Results

3.1. Effects of BQ-123 and PD161721 on ischaemia-induced arrhythmias

The effects of BQ-123 (5×10^{-7} and 10^{-6} M) on the % incidences of ventricular tachycardia, irreversible and total ventricular fibrillation produced by coronary artery occlusion are compared with the vehicle group in Fig. 1. In the vehicle-treated group, during the 30-min period of coronary artery occlusion, 58% of the hearts exhibited ventricular tachycardia and irreversible ventricular fibrilla-

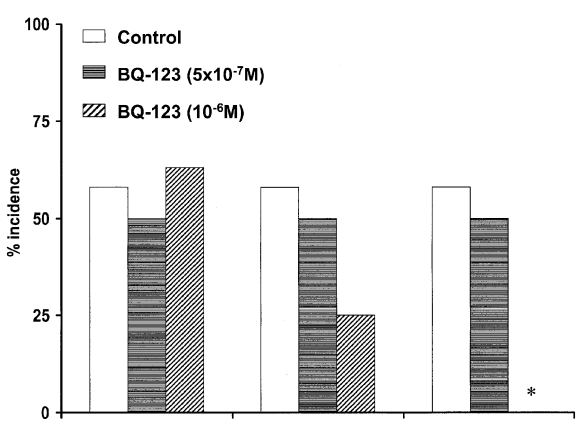

Fig. 1. The incidences (%) of ventricular tachycardia, total ventricular fibrillation (VF) and irreversible VF in the 30-min post occlusion period in vehicle ($n=12$) and BQ-123 (5×10^{-7}, $n=8$, and 10^{-6} M, $n=8$) treated rat hearts. * $P \leq 0.05$ compared to the control group.

tion. BQ-123, at 10^{-6} but not 5×10^{-7} M, significantly reduced the incidence of irreversible ventricular fibrillation but not of total ventricular fibrillation or ventricular tachycardia. Ventricular ectopic beats which occurred as singles, salvos and ventricular tachycardia were counted in those hearts which did not exhibit irreversible ventricular fibrillation. The total number of ventricular ectopic beats was significantly reduced from 49 (21–72) to 4 (2–5) by the lower concentration of BQ-123 and this was a consequence of a reduction in the number of such beats which occurred as ventricular tachycardia (12 (0–30) vs. 0 (0–0) $P < 0.05$) and as single ectopic beats (14 (9–29) vs. 3 (2–3) $P < 0.05$). However, the higher concentration of BQ-123, 10^{-6} M, did not significantly reduce the total or individual ectopic counts.

Both concentrations of PD161721 (10^{-7} and 10^{-6} M) abolished the incidence of irreversible ventricular fibrillation and only one out of eight hearts, exposed to the lower concentration of PD161721, had a period of reversible ventricular fibrillation (Fig. 2). Neither dose of PD161721 significantly reduced the incidence of ventricular tachycardia nor the number of ectopic beats that occurred as ventricular tachycardia, singles or salvos.

3.2. Effects of BQ-123 and PD161721 on coronary perfusion pressure and heart rate

Fig. 3 illustrates coronary perfusion pressure, measured throughout the experimental protocol, in vehicle- and BQ-123-treated hearts. Coronary artery occlusion caused an immediate significant (from 55 ± 8 to 79 ± 10 mm Hg) increase in coronary perfusion pressure in vehicle-treated hearts. This increase in coronary perfusion pressure was sustained in the hearts that survived the 30-min period of coronary artery occlusion. Neither concentration of BQ-123 had an effect on coronary perfusion pressure before coronary artery occlusion. At 10- and 15-min post occlusion only, in hearts treated with 10^{-6} M BQ-123, coronary perfusion pressure was significantly higher than in the vehicle-treated hearts. Heart rate did not vary from the value of 305 ± 21 beats min^{-1} (pre occlusion in control hearts) throughout the experimental protocol in vehicle- or BQ-123-treated hearts.

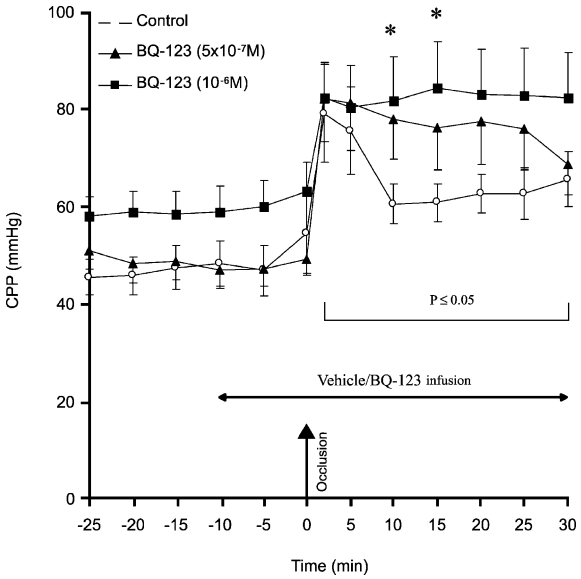

Fig. 3. The effects of infusion of BQ-123 (5×10^{-7}, $n = 8$ and 10^{-6} M, $n = 8$), or vehicle ($n = 12$) prior to and during coronary artery occlusion, on coronary perfusion pressure (CPP) in rat isolated hearts. $^{*}P \leq 0.05$ compared to the same time point in the vehicle group. In all three groups, coronary artery occlusion caused a significant and sustained increase in coronary perfusion pressure ($P \leq 0.05$). By 10-min post occlusion, n had reduced to 5 and 4 in vehicle and BQ-123 (5×10^{-7} M) treated hearts due to the occurrence of irreversible VF.

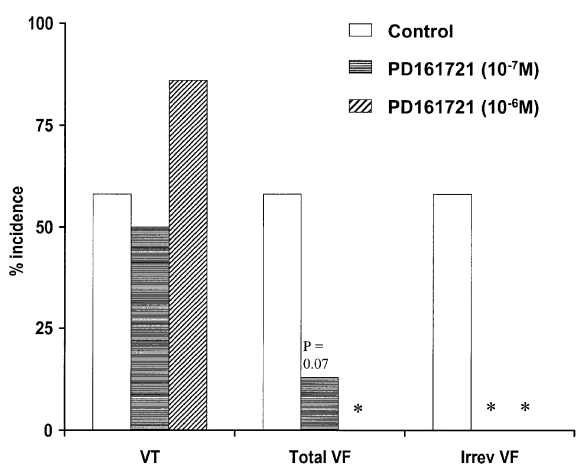

Fig. 2. The incidences (%) of ventricular tachycardia, total ventricular fibrillation (VF) and irreversible VF in the 30-min post occlusion period in vehicle ($n = 12$) and PD161721 (5×10^{-7}, $n = 8$, or 10^{-6} M, $n = 7$) treated rat hearts. $^{*}P \leq 0.05$ compared to the control group.

The infusion of PD161721 (at either concentration) had no effect on coronary perfusion pressure before coronary artery occlusion. Coronary perfusion pressure post occlusion, in hearts treated with 10^{-6} M PD161721, tended to be higher than in vehicle-treated hearts although the difference (61 ± 4 vs. 91 ± 12 mm Hg) only reached significance at 10-min post occlusion. PD16171, like BQ-123, had no effect on heart rate either before or during coronary artery occlusion.

3.3. Effects of BQ-123, PD161721 and lignocaine on the maximum following frequency in atrial muscle

Fig. 4 shows the effect of BQ-123 (10^{-8}–10^{-6} M) on the maximum following frequency (expressed as a % increase from control) in the presence of 5.4 and 8.0 mM KCl. Maximum following frequency in normal extracellular $[K^{+}]$ was significantly decreased from 9.0 ± 0.7 to a maximum of 7.2 ± 0.4 Hz ($P < 0.05$) at the highest concentration studied, 10^{-6} M. Elevating the extracellular

$[K^+]$ did not modify the effect of BQ-123 on the maximum following frequency.

Similarly, as shown in Fig. 5, PD161721 at concentrations of 10^{-8} M and above significantly decreased the maximum following frequency. In the presence of normal extracellular $[K^+]$, the maximum following frequency decreased from 8.3 ± 0.4 to 6.7 ± 0.3 Hz at a concentration of 10^{-6} M PD161721. In the presence of elevated extracellular $[K^+]$, the effects of PD161721 on the maximum following frequency were similar to those in normal $[K^+]$ with the exception that at 10^{-9} M there was a significant difference between the maximum following frequency measurements in 5.4 and 8.0 mM KCl.

Lignocaine (0.43 to 3.4×10^{-4} M) caused a marked concentration-dependent decrease in the maximum following frequency under both normal and elevated $[K^+]$. In normal extracellular $[K^+]$, the maximum following frequency decreased from 9.0 ± 0.3 to 4.9 ± 0.5 and 3.2 ± 0.4 Hz at concentrations of 1.7 and 3.4×10^{-4} M, respectively. This effect of lignocaine was potentiated in the presence of elevated extracellular $[K^+]$ so that the maximum following frequency decreased from 8.4 ± 0.3 to 2.5 ± 0.6 ($1.7\ 10^{-4}$ M) and 1.5 ± 0.3 Hz (3.4×10^{-4} M).

3.4. Effect of BQ-123, PD161721 and lignocaine on force of contraction in atrial muscle

Neither of the endothelin receptor antagonists had any significant effect on heart force in the presence of normal or elevated extracellular potassium. In contrast, lignocaine caused a concentration-dependent reduction in heart force.

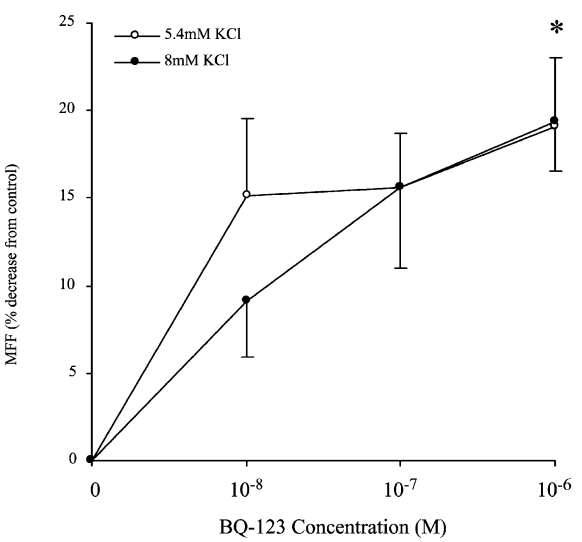

Fig. 4. The effects of BQ-123 (10^{-8}–10^{-6} M) on the maximum following frequency (MFF) expressed as a % decrease from the control value, in paced guinea-pig atria in the presence of 5.4 mM ($n = 6$) or 8.0 mM ($n = 5$) KCl. $^{*}P \leq 0.05$ indicates a statistically significant difference from the respective control MFF value which was 9.0 ± 0.7 and 8.2 ± 0.2 Hz in 5.4 and 8.0 mM KCl, respectively.

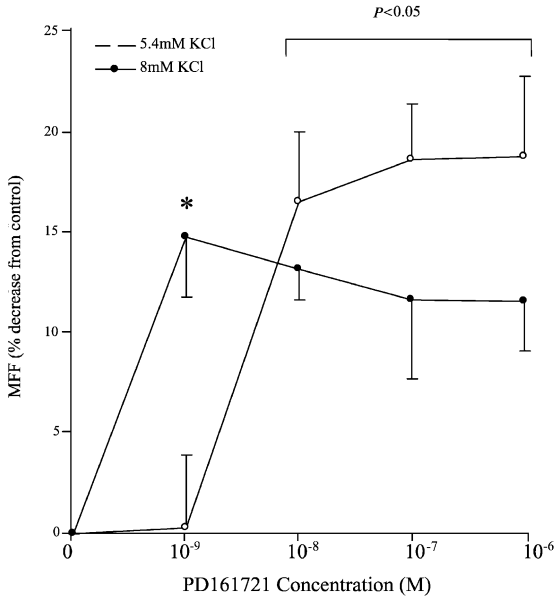

Fig. 5. The effects of PD161721 (10^{-9}–10^{-6} M) on the maximum following frequency (MFF), expressed as a % decrease from the control value, in paced guinea-pig atria in the presence of 5.4 mM ($n = 6$) or 8.0 mM ($n = 7$) KCl. $P \leq 0.05$ all values, with the exception of 10^{-9} M in 5.4 mM KCl, were significantly different from the respective control MFF value which was 8.3 ± 0.4 and 7.9 ± 0.4 Hz in 5.4 and 8.0 mM KCl, respectively. $^{*}P \leq 0.05$, indicates a significant difference between MFF values in 5.4 and 8.0 mM KCl.

The highest concentration of lignocaine studied, 3.4×10^{-4} M, reduced heart force from 104 ± 18 to 40 ± 5 ($P < 0.05$) and from 186 ± 27 to 21 ± 3 mg ($P < 0.05$) in the presence of normal and elevated extracellular $[K^+]$, respectively.

4. Discussion

In this study, we have demonstrated that both endothelin receptor antagonists studied, BQ-123 and PD161721, had anti-fibrillatory effects in isolated rat hearts subjected to coronary artery occlusion. BQ-123, at the higher concentration of 10^{-6} M, but not at 5×10^{-7} M, reduced the incidence of irreversible ventricular fibrillation, i.e. that which did not spontaneously revert to sinus rhythm. Similarly, PD161721 abolished the occurrence of irreversible ventricular fibrillation and this effect was seen with both concentrations used, i.e. 10^{-7} and 10^{-6} M. BQ-123 had a further anti-arrhythmic action, at 5×10^{-7} M but not 10^{-6} M of reducing the number of ectopic beats during coronary artery occlusion. These results, therefore, show that BQ-123 and PD161721, in addition to reducing ischaemia-induced arrhythmias in anaesthetised rats (Sharif et al., 1998), exert a similar action in vitro.

One possible mechanism for the observed anti-fibrillatory effect of the endothelin receptor antagonists is a direct

cardiac electrophysiological effect. On guinea-pig left atrial muscle, we observed that both antagonists, at concentrations which were anti-fibrillatory, caused a small but statistically significant decrease of the maximum following frequency. Such an effect would be expected to prevent the occurrence of re-entry circuits which are thought to underlie the occurrence of ventricular fibrillation during myocardial ischaemia (Wolk et al., 1999). The ability of both drugs to decrease the maximum following frequency may, therefore, explain their anti-fibrillatory activity. It should be noted, however, that this effect for both drugs was small, with a maximum decrease of about 18% being observed. This is in contrast to the effect of the Na^+ channel blocking drug, lignocaine, which albeit over a higher concentration range, caused a marked decrease of the maximum following frequency. The effects of both endothelin receptor antagonists to decrease the maximum following frequency also differed from that of lignocaine in that raising the extracellular concentration of potassium did not markedly potentiate their effects. Lignocaine is known to decrease the maximum following frequency by blocking Na^+ channels in their inactivated form. Raising the extracellular potassium concentration increases the proportion of Na^+ channels in the inactivated form and, consequently, potentiates the effect of a Na^+ channel blocking drug-like lignocaine (Grant et al., 1999). Na^+ channel blocking drugs also have a negative inotropic effect, as evidenced by the reduction in the force of contraction caused by lignocaine. Neither BQ-123 nor PD161721 modified the force of contraction over the concentration range studied. Taken together, this data supports the view that the endothelin receptor antagonists studied did not reduce the maximum following frequency via Na^+ channel blockade. There is no literature on the cardiac electrophysiological effects of PD161721 but there is one report on BQ-123 (Kelso et al., 1998). That study showed that BQ-123, at 10^{-6} M, prolongs the action potential duration of isolated rabbit ventricular myocytes via an increase of the L-type Ca^{2+} current. An increase in the action potential duration would, by prolonging the refractory period, produce a decrease of the maximum following frequency. It is of interest to note that Kelso et al. (1998) concluded that the effect of BQ-123 on Ca^{2+} current was not mediated via endothelin receptor antagonism since three other endothelin receptor antagonists studied did not share this effect. Electrophysiological studies would need to be carried out to determine the ionic basis of the ability of BQ-123 and PD161721 to decrease the maximum following frequency.

It is also possible that BQ-123 and PD161721 exert their anti-arrhythmic action via antagonism of a pro-arrhythmic effect of endogenously released endothelin. In isolated rat hearts, it has been demonstrated that there is a measurable release of endothelin under basal conditions but that this release is reduced during low flow hypoxic ischaemia for 180 min (Brunner et al., 1992). Under our experimental conditions, neither antagonist, at concentrations which are known to block endothelin receptors (Peter and Davenport, 1996; Bryant et al., 1996), modified coronary perfusion pressure or heart rate before coronary artery occlusion. This suggests that any basally released endothelin, in isolated rat hearts, has little or no functional effect, at least on the coronary circulation or the sinus node. However, it does remain a possibility that endogenously released endothelin could have a direct electrophysiological effect on the myocardium which is blocked by the antagonists. Further experiments are currently underway to test this possibility. During coronary artery occlusion, it appeared that both antagonists prevented the fall in coronary pressure seen in vehicle-treated hearts after 10 min of occlusion. However, this can be explained by the fact that a high percentage of the vehicle-treated hearts did not last throughout the 30-min period of coronary artery occlusion whereas the drug-treated hearts did.

There is no evidence, therefore, from this study to support the view that BQ-123 or PD161721 is anti-fibrillatory due to an action of antagonising the effect of endogenously released endothelin on vascular endothelin ET_A or ET_B receptors. That said, we cannot exclude a direct electrophysiological effect on the ventricular myocardium of endothelin, similar to that described by Merkely et al. (1998), which may be antagonised by either BQ-123 or PD161721. We cannot also exclude the possibility that the anti-fibrillatory effect of both antagonists is secondary to an effect on the extent of ischaemic damage. However, studies have reported that BQ-123 reduces infarct size in anaesthetised rats and dogs (Grover et al., 1992; Grover et al., 1993) but not in isolated rat hearts (Sleph et al., 1992) suggesting that blood borne mediators may be a necessary component of any effect of endogenously released endothelin on infarct size. Further studies are clearly needed to establish the mechanism by which both BQ-123 and PD161721 are antiarrhythmic. A limitation of our work is that the studies on the maximum following frequency were carried out in guinea-pig atrial tissue whereas the antiarrhythmic effect was observed in rat ventricle. Thus, in future work it would be necessary to study the electrophysiological and antiarrhythmic actions of these antagonists in the same tissue type.

In conclusion, both endothelin receptor antagonists, BQ-123 and PD161721, had an anti-fibrillatory effect in isolated rat hearts that may be due, at least in part, to an ability to decrease the maximum following frequency. The electrophysiological basis of the effect of the antagonists on the maximum following frequency remains to be determined.

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References

Brunner, F., Opie, L.H., 1998. Role of endothelin ET_A receptors in ischemic contracture and reperfusion injury. Circulation 97, 391–398.

Brunner, F., Du Toit, E.F., Opie, L.H., 1992. Endothelin release during ischemia and reperfusion of isolated perfused rat hearts. J. Mol. Cell. Cardiol. 24, 1291–1305.

Bryant, J.W., Welch, K.M., Flynn, M.A., Walker, D.M., Repine, J., Doherty, A.M., Haleen, S.J., 1996. Characterization of PD161721, a non-selective competitive endothelin ET_A /ET_B antagonist. FASEB J. 10, 3808.

Demiryürek, A.T., Çakici, I., Wainwright, C.L., Wadsworth, R.M., Kane, K.A., 1998. Effects of free radical production and scavengers on occlusion–reperfusion induced arrhythmias. Pharmacol. Res. 38, 433–439.

Garjani, A., Wainwright, C.L., Zeitlin, I.J., Wilson, C., Slee, S.J., 1995. Effects of endothelin-1 and the endothelin ET_A receptor antagonist, BQ123, on ischaemic arrhythmias in anaesthetized rats. J. Cardiovasc. Pharmacol. 25, 634–642.

Gonon, A.T., Wang, Q.-D., Shimizu, M., Pernow, J., 1998. The novel non-peptide selective endothelin A receptor antagonist LU 135252 protects against myocardial ischaemia and reperfusion injury in the pig. Acta Physiol. Scand. 163, 131–137.

Grant, A.O., Whalley, D.W., Wendt, D.J., 1999. Pharmacology of the cardiac sodium channel. In: Zipes, D.P., Jalife, J. (Eds.), Cardiac Electrophysiology: From Cell to Bedside. W.B. Saunders, Philadelphia, pp. 133–141.

Grover, G.J., Sleph, P.G., Fox, M., Trippodo, N.C., 1992. Role of endothelin-1 and big endothelin-1 in modulating coronary vascular tone, contractile function and severity of ischaemia in rat hearts. J. Pharmacol. Exp. Ther. 263, 1074–1082.

Grover, G.J., Dzwonczyk, S., Parham, C.S., 1993. The endothelin-1 receptor antagonist BQ-123 reduces infarct size in a canine model of coronary occlusion and reperfusion. Cardiovasc. Res. 27, 1613–1618.

Ihara, M., Noguchi, K., Saeki, T. et al., 1992. Biological profiles of highly potent novel endothelin antagonists for the endothelin ET_A receptor. Life Sci. 50, 247–255.

Kane, K.A., 1980. Comparative electrophysiological effects of Org 6001, a new orally active antidysrhythmic agent, and lignocaine on human ventricular muscle. Br. J. Pharmacol. 68, 25–31.

Kelso, E.J., Spiers, J.P., McDermott, B.J., Scholfield, C.N., Silke, B., 1998. Stimulation of L-type Ca^{2+} current by the endothelin $receptor_A$-selective antagonist, BQ-123, in ventricular cardiomyocytes isolated from rabbit myocardium. Biochem. Pharmacol. 55, 897–902.

Merkely, B., Geller, L., Toth, M., Kiss, O., Kekesi, V., Solti, F., Vecsey, T., Horkay, F., Tenczer, J., Jughsz-Nagy, A., 1998. Mechanism of endothelin-induced malignant ventricular arrhythmias in dogs. J. Cardiovasc. Pharmacol. 31, S437–S439.

Peter, M.G., Davenport, A.P., 1996. Characterization of the endothelin receptor selective agonist, BQ3020 and antagonists BQ123, FR139317, BQ788, 50235, ro462005 and bosentan in the heart. Br. J. Pharmacol. 117, 455–462.

Raschack, M., Juchelka, F., Rozek-Schaefer, G., 1998. The endothelin $ET\text{-}_A$ antagonist LU 135 252 suppresses ischemic ventricular extrasystolic and fibrillation in pigs and prevents hypoxic cellular decoupling. J. Cardiovasc. Pharmacol. 31, S145–S148.

Richard, V., Kaeffer, N., Hogie, M., Tron, C., Blanc, T., Thuillez, C., 1994. Role of endogenous endothelin in myocardial and coronary endothelial injury after ischaemia and reperfusion in rats: studies with bosentan, a mixed endothelin ET_A–ET_B antagonist. Br. J. Pharmacol. 113, 869–876.

Sharif, I., Kane, K.A., Wainwright, C.L., 1998. Endothelin and ischaemic arrhythmias—antiarrhythmic or arrhythmogenic? Cardiovasc. Res. 39, 625–632.

Sleph, P.G., Hunt, J.T., Grover, G.J., 1992. The role of endothelin-1 and big endothelin-1 in modulating coronary vascular tone, contractile function and time to contracture in ischemic and non-ischemic isolated rat hearts. FASEB J. 6, A1245.

Tønnessen, T., Naess, P.A., Kirkebøen, K.A., Offstad, J., Ilebekk, A., Cheart rateistensen, G., 1993. Release of endothelin from the porcine heart after short-term coronary artery occlusion. Cardiovasc. Res. 27, 1482–1485.

Vitola, J.V., Forman, M.B., Holsinger, J.P., Kawana, M., Atkinson, J.B., Quertermous, T., Jackson, E.K., Murray, J.J., 1996. Role of endothelin in a rabbit model of acute myocardial infarction: effects of receptor antagonists. J. Cardiovasc. Pharmacol. 28, 774–783.

Walker, M.J., Curtis, M.J., Hearse, D.J., Campbell, R.W., Janse, M.J., Yellon, D.M., Cobbe, S.M., Coker, S.J., Harness, J.B., Harron, D.W. et al., 1988. The Lambeth Conventions: guidelines for the study of arrhythmias in ischaemia, infarction and reperfusion. Cardiovasc. Res. 22, 447–455.

Wang, Q.D., Hemsén, A., Li, X.S., Lundberg, J.M., Uriuda, Y., Pernow, J., 1995a. Local overflow and enhanced tissue content of endothelin following myocardial ischaemia and reperfusion in the pig: modulation by L-arginine. Cardiovasc. Res. 29, 44–49.

Wang, Q.D., Li, X.S., Lundberg, J.M., Pernow, J., 1995b. Protective effects of non-peptide endothelin receptor antagonist bosentan on myocardial ischaemia and reperfusion injury in the pig. Cardiovasc. Res. 29, 805–812.

Winslow, E., 1984. Methods for the detection and assessment of antiarrhythmic activity. Pharmacol. Ther. 24, 401–433.

Wolk, R., Cobbe, S.M., Hicks, M.N., Kane, K.A., 1999. Functional, structural, and dynamic basis of electrical heterogeneity in healthy and diseased cardiac muscle: implications for arrhythmogenesis and anti-arrhythmic drug therapy. Pharmacol. Ther. 84, 207–231.

Yorikane, R., Koike, H., 1990. The arrhythmogenic action of endothelin in rats. Jpn. J. Pharmacol. 53, 259–263.