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AH23848, a thromboxane antagonist, suppresses ischaemia and reperfusion-induced arrhythmias in anaesthetized greyhounds

Susan J. Coker[1] & James R. Parratt

Department of Physiology and Pharmacology, University of Strathclyde, 204 George Street, Glasgow G1 1XW and Department of Surgery, University of Glasgow (Western Infirmary)

1 The effects of the thromboxane antagonist AH23848 (1 mg kg^{-1} i.v.) were examined in anaesthetized greyhounds prepared for occlusion of the left anterior descending coronary artery with subsequent reperfusion after 40 min of myocardial ischaemia.

2 Pretreatment with AH23848 30 min before coronary artery occlusion reduced the number of ischaemia-induced extrasystoles to 339 ± 111 compared with 736 ± 153 in the control group. The incidence of ventricular fibrillation following reperfusion was also markedly reduced; 25% compared with 88% in the controls.

3 Late intervention with AH23848, 25 min after the onset of myocardial ischaemia did not significantly alter the incidence of reperfusion-induced ventricular fibrillation; 70% of the control group died and 60% of those that received AH23848.

4 The ischaemia-induced release of thromboxane A_2 and prostacyclin was not altered by pretreatment with AH23848.

5 The results suggest that blockade of thromboxane receptors before myocardial ischaemia is an effective antiarrhythmic strategy in this model.

Introduction

Previous studies have led us to suggest that locally released thromboxane A_2 may be arrhythmogenic during acute canine myocardial ischaemia (Coker *et al.*, 1981a). Pretreatment with drugs that inhibit thromboxane synthesis such as low dose aspirin, dazoxiben or dazmegrel has been shown to be effective against both ischaemia-induced and reperfusion-induced arrhythmias (Coker *et al.*, 1981b; Coker & Parratt, 1983a; Coker, 1984). There is also evidence, however, which suggests that prostacyclin is antiarrhythmic. Increasing prostacyclin concentrations in the coronary circulation reduced arrhythmias in anaesthetized greyhounds subject to coronary artery occlusion and reperfusion (Coker & Parratt, 1983b; 1984a). Since inhibition of thromboxane synthetase could cause rediversion of prostaglandin endoperoxides to prostacyclin we decided to investigate the effects of a thromboxane receptor blocking drug to determine whether thromboxane A_2 *per se*, is arrhythmogenic. The present study describes the effects of a specific thromboxane antagonist AH23484 ([1α(Z),2β,5α]-(±)-7-[5-[[(1,1-biphenyl)-4-yl]methoxy]-2-(4-morpholinyl)-3-oxocyclopentyl] -4-hepatonoic acid) (Brittain *et al.*, 1984), on arrhythmias induced by coronary artery occlusion and reperfusion in anaesthetized greyhounds. Preliminary results from this study have been presented to the British Pharmacological Society (Coker & Parratt, 1984b).

[1]Present address, and to whom correspondence should be addressed: Department of Pharmacology & Therapeutics, University of Liverpool, P.O. Box 147, Liverpool L69 3BX.

Methods

Greyhounds of either sex (19–35 kg) were anaesthetized with chloralose (80–90 mg kg^{-1} i.v.) after induction with sodium thiopentone (25 mg kg^{-1} i.v.) and prepared for coronary artery occlusion as described in detail previously (Coker & Parratt, 1983c). The dogs were ventilated with oxygen using a Palmer respiration pump, the stroke volume of which was adjusted to give an arterial CO_2 tension of approximately 38–40 mmHg. Pancuronium (0.15–0.20 mg kg^{-1} i.v.) was administered to prevent reflex muscular

movement. Catheters were placed in the aorta and vena cava via the femoral vessels and intra-cardiac catheters were positioned under fluoroscopic control (Siemens image intensifier) in the coronary sinus and in the pulmonary artery (via the left jugular vein) and in the left ventricle (via the left carotid artery).

Following a left thoracotomy the pericardium was incised and a ligature was placed around the left anterior descending coronary artery (LAD) approximately 15 to 40 mm distal from the tip of the left atrial appendage. A 6 inch Longdwel teflon catheter (20 G) was placed in a coronary vein adjacent to the LAD and the tip advanced so that it lay within the area rendered ischaemic by coronary artery occlusion. After completion of the surgical preparation heparin, 100 u kg^{-1}, was administered.

Intravascular pressures were recorded with Elcomatic 751A transducers and displayed on a Siemens-Elema Mingograf 82 ink-jet recorder along with a Lead II electrocardiogram (ECG). Cardiac output was measured by thermodilution using a Devices cardiac output monitor. Simultaneous anaerobic blood samples were taken at regular intervals from the aorta, coronary sinus, local coronary vein and the pulmonary artery, and P_{O_2}, P_{CO_2} and pH were measured with Instrumentation Laboratories 213 blood gas analyser. Oxygen content was calculated by the method of Douglas *et al.* (1975).

In some dogs blood samples were also taken at various times from the aorta, coronary sinus and the local coronary vein and analysed for thromboxane B_2 and 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) (stable breakdown products of thromboxane A_2 and prostacyclin respectively), using radioimmunoassay techniques which have been described in detail previously (Coker *et al.*, 1982). The samples were placed in tubes containing indomethacin, to prevent *ex vivo* generation of prostanoids, and EDTA as an anticoagulant. Samples were acidified and the prostanoids extracted with ethyl acetate. Extracts were subject to radioimmunoassay using specific antibodies (Pasteur Institute) and a dextran-charcoal separation procedure. The detection limit was 20 pg ml^{-1} for thromboxane B_2 and 100 pg ml^{-1} for 6-keto $PGF_{1\alpha}$.

At the end of each experiment the heart was excised and a small volume of blue dye was injected slowly into the LAD distal to the point of occlusion. The area outlined in this manner was then cut out and weighed; the 'area at risk' was expressed as a percentage of the free left ventricular wall.

Drug administration

AH23848b was dissolved in ethanol and 0.9% w/v NaCl solution (saline) such that the final ethanol concentration was 6% v/v and the final volume was 10 ml. The drug was administered as an intravenous bolus either 30 min before, or 25 min after, coronary artery occlusion. Control dogs received 10 ml of 6% v/v ethanol in saline at the appropriate time.

Statistics

Results are expressed as the mean ± s.e.mean of n experiments. Data were subject to analysis of variance and where appropriate the significance of differences was determined using a modified t test (Wallenstein *et al.*, 1980). The incidence of events was compared with Fisher's exact test. Since the prostanoid data may not be normally distributed these were analysed with a Kruskal-Wallis test and the significance determined with a Mann-Whitney U-test.

Table 1 Local coronary venous blood gases, pH and oxygen content in control dogs and in those which received AH23848 30 min before coronary artery occlusion (at time 0)

	Po_2 (mmHg)	Pco_2 (mmHg)	pH (units)	O_2 *content* (ml 100 ml^{-1})
Controls				
− 35 min	33 ± 1	62 ± 3	7.30 ± 0.01	12.0 ± 0.6
− 10 min	31 ± 1	60 ± 3	7.29 ± 0.01	10.9 ± 0.7
7 min	27 ± 1*	72 ± 3*	7.20 ± 0.03*	8.2 ± 0.8*
15 min	26 ± 1	74 ± 3**	7.18 ± 0.03**	7.6 ± 0.7**
30 min	27 ± 1	68 ± 3*	7.22 ± 0.02	8.4 ± 0.8*
AH23848				
− 35 min	31 ± 1	64 ± 2	7.27 ± 0.01	10.2 ± 0.7
− 10 min	29 ± 1	64 ± 2	7.26 ± 0.01	10.0 ± 0.5
7 min	26 ± 1	70 ± 3	7.19 ± 0.02**	7.5 ± 0.4**
15 min	26 ± 2	72 ± 2	7.20 ± 0.02*	7.0 ± 0.5**
30 min	26 ± 1	70 ± 1	7.20 ± 0.01*	7.9 ± 0.5*

Each value is the mean ± s.e.mean, $n = 9$. * $P < 0.05$, ** $P < 0.01$, t test.

Results

The effects of AH23848 on haemodynamics, blood gases and prostanoids.

The slow intravenous injection of AH23848 1 mg kg^{-1} 30 min before coronary artery occlusion caused a mild transient increase in arterial blood pressure, left ventricular pressure and left ventricular *dP/dt max*. These effects were short lasting and 15 min after administration of the drug the measured haemodynamic parameters were not significantly different from control values. When AH23848 was administered 25 min after coronary artery occlusion similar transient alterations in haemodynamics were observed.

The oxygen and carbon dioxide tensions, pH and oxygen content of blood sampled from the aorta, coronary sinus, local coronary vein and pulmonary artery were not altered by the administration of AH23848. Plasma prostanoid concentrations were measured in the nine dogs that received AH23848 before coronary artery occlusion. The concentrations of thromboxane B_2 were not significantly different in either the aorta or the coronary sinus after administration of AH23848 (54 ± 17 to 62 ± 18 pg ml^{-1} and 104 ± 28 to 83 ± 15 pg ml^{-1} respectively). The concentrations of 6-keto $PGF_{1\alpha}$ were also unchanged after administration of AH23848 (584 ± 87 to 513 ± 99 pg ml^{-1} in the aorta and 536 ± 122 to 553 ± 94 pg ml^{-1} in the coronary sinus).

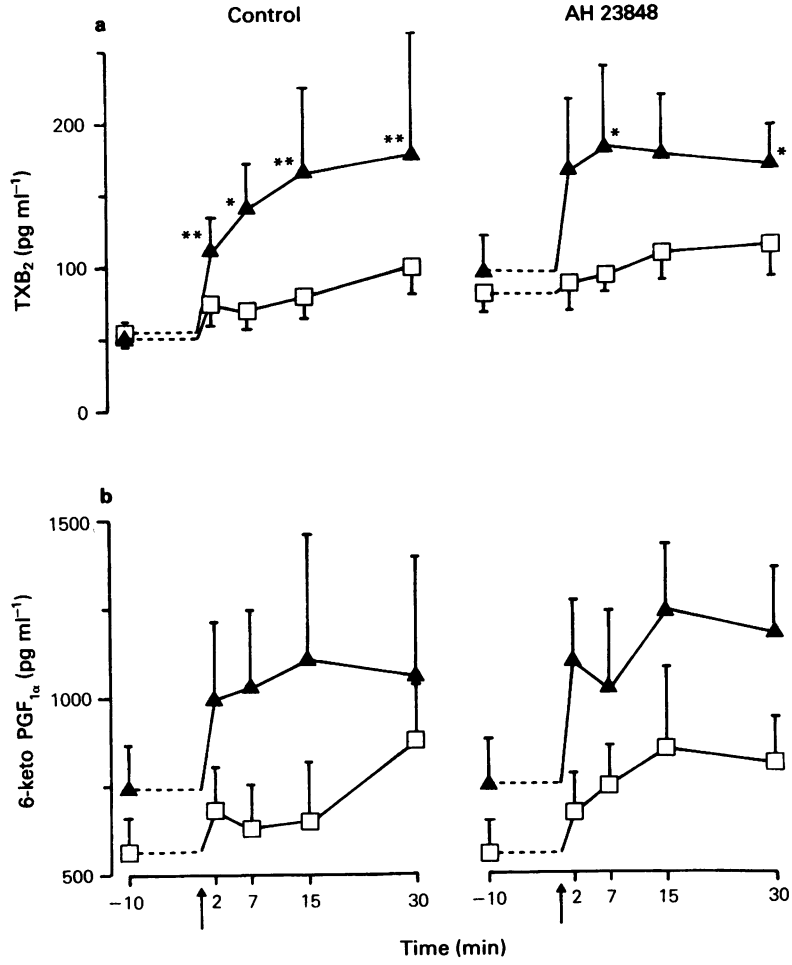

Figure 1 Plasma concentrations of (a) thromboxane B_2 (TxB_2) and (b) 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) in the coronary sinus (□) and the local coronary vein (▲) of greyhounds subject to coronary artery occlusion (at the arrow) in controls and in those pretreated with AH23848. Each value is the mean and vertical lines show s.e.mean, $n = 9$. * $P < 0.05$. ** $P < 0.01$ compared with corresponding pre-occlusion value, Mann Whitney U-test.

The effects of pretreatment with AH23848 on the metabolic changes induced by coronary artery occlusion

Occlusion of the LAD in control dogs caused an increase in P_{CO_2} and reductions in P_{O_2}, O_2 content and pH in local coronary venous blood (Table 1). These alterations were confined to blood draining from the acutely ischaemic region of the myocardium, no changes were observed in coronary sinus blood draining from the essentially normal region of the left ventricle. Similar alterations in blood gases were observed in the dogs that received AH23848 30 min before coronary artery occlusion although the magnitude of the increase in P_{CO_2} appeared to be smaller.

During coronary artery occlusion, blood samples were taken from the coronary sinus and the local coronary vein for prostanoid analysis. Figure 1 shows the plasma concentrations of thromboxane B_2 and 6-keto $PGF_{1\alpha}$ at these sites in the control dogs and in those pretreated with AH23848. Both the time course and the magnitude of the changes induced by coronary artery occlusion were similar in both groups of dogs, indicating that AH23848 did not alter the release of thromboxane or prostacyclin from the acutely ischaemic myocardium.

The effects of AH23848 on ischaemia and reperfusion-induced arrhythmias

Acute occlusion of the LAD in anaesthetized greyhounds results in a burst of arrhythmias varying from single premature beats, usually ventricular in origin, to ventricular tachycardia and in some cases ventricular fibrillation and death. One dog in each of the pretreatment groups (control and AH23848) died in ventricular fibrillation during coronary artery occlusion. Dogs which fibrillated during coronary artery occlusion were excluded from the late administration study. The number of extrasystoles including those occurring as ventricular tachycardia was counted in one minute intervals for the first 30 min of myocardial ischaemia. Pretreatment with AH23848 significantly reduced this number but, as expected, the late administration of AH23848 had no effect (Figure 2).

When the ligature around the LAD was released after 40 min of myocardial ischaemia, arrhythmias were observed as soon as direct perfusion of the formerly ischaemic area was restored. These arrhythmias were multifocal in origin and caused marked reductions in systemic arterial blood pressure. In the majority of control animals these arrhythmias quickly progressed to ventricular fibrillation, usually within 1 to 2 min of reperfusion. A different pattern was observed in the dogs that were pretreated with AH23848. The initial arrhythmias appeared to be similar but instead of progressing to ventricular fibrillation they often settled into a steady ventricular rhythm. Although ventricular in origin, the rate was only 5 to 10 beats min^{-1} faster than normal sinus rhythm and arterial blood pressure was fairly well maintained. This ventricular rhythm persisted for 2 to 20 min, after which time all the animals reverted to normal sinus rhythm. Only 2 out of 8 dogs that had received AH23848 30 min before coronary artery occlusion died in ventricular fibrillation following reperfusion. The late administration of AH23848 25 min after the onset of myocardial ischaemia failed to reduce the incidence of reperfusion-induced ventricular fibrillation (Figure 2).

The 'area at risk' was similar in both pretreatment groups; $35 \pm 1\%$ of the free left ventricular wall in controls and $33 \pm 2\%$ in the AH23848 group ($n = 9$). The magnitude of the ST-segment depression induced

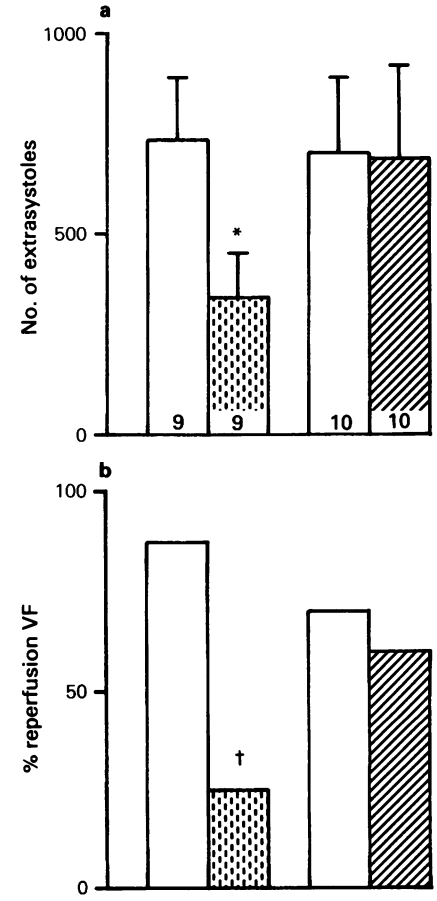

Figure 2 The effects of AH23848 (1 mg kg^{-1} i.v.), given 30 min before (stippled columns, $n = 9$) or 25 min after (hatched columns, $n = 10$) coronary artery occlusion, on (a) the number of ischaemia-induced extrasystoles and (b) the incidence of reperfusion-induced ventricular fibrillation (VF). Open columns represent control values obtained from untreated animals. * $P = 0.05$ Mann-Whitney U-test, † $P = 0.05$ Fisher's exact test.

by 30 min of ischaemia was also similar; 0.38 ± 0.07 mV and 0.33 ± 0.10 mV respectively ($n = 8$). In the late administration groups the areas at risk were $41 \pm 2\%$ and $42 \pm 2\%$ with ST-segment depression reaching 0.31 ± 0.03 mV and 0.41 ± 0.10 mV in the control and drug groups respectively ($n = 10$).

Discussion

The results presented above indicate that blockade of thromboxane receptors before and during acute myocardial ischaemia is an effective antiarrhythmic strategy. This suggests that thromboxane A_2 *per se* is arrhythmogenic. Since thromboxane A_2 is a particularly unstable compound (Hamberg *et al.*, 1975) which has not been chemically isolated it is extremely difficult to study the direct effects of thromboxane A_2 on cardiac rhythm. However, it is possible to generate thromboxane A_2 by adding PGH_2 to platelets. Within minutes of injecting thromboxane A_2 (generated in this way) into the coronary circulation of rabbits Morooka *et al.* (1977, 1979) observed ST-segment changes indicative of myocardial ischaemia. The endoperoxide analogue/thromboxane mimetic U46619 has actually been found to cause arrhythmias in anaesthetized dogs (Mehta *et al.*, 1982) and to exacerbate ischaemia-induced arrhythmias in anaesthetized rats (Wainwright, 1984).

The present study provides further evidence to support the hypothesis that thromboxane A_2 is arrhythmogenic at least in the setting of acute myocardial ischaemia. Unfortunately we still do not know the precise location of the site of action of thromboxane A_2. Receptors sensitive to thromboxane A_2 have been shown to be present on platelets and vascular smooth muscle from several sources (Jones *et al.*, 1982; Kennedy *et al.*, 1982) but no information is available for cardiac muscle. Thus it is possible that the arrhythmogenic activity of thromboxane A_2 may be an indirect effect. Coronary vasoconstriction or platelet aggregation induced by thromboxane A_2 would increase myocardial ischaemia and this may be the sole reason for the arrhythmogenic activity of thromboxane, although a direct action on the myocardial cell cannot be excluded. Since the pA_2 for AH23848 on vascular smooth muscle is similar to that on platelets (Humphrey & Lumley, 1984) this study cannot determine the relative contribution of coronary vasoconstriction versus platelet aggregation.

The inability of late administration of AH23848 to reduce reperfusion-induced ventricular fibrillation supports the suggestion that the arrhythmogenicity of thromboxane A_2 is an indirect action. We have shown previously that drugs (e.g. prostacyclin) do gain access to the ischaemic myocardium (Coker & Parratt, 1983a) so we have assumed that this would also be true for AH23848. Although thromboxane receptors were presumably blocked after late administration of AH23848, reperfusion of the ischaemic myocardium still caused ventricular fibrillation. Similar results were obtained with late administration of the thromboxane synthetase inhibitor dazmegrel (Coker & Parratt, 1985). This reduced coronary venous thromboxane B_2 concentrations but failed to prevent reperfusion-induced ventricular fibrillation. The inability of late intervention with dazmegrel or AH23848 to prevent reperfusion-induced ventricular fibrillation suggests that the occurrence of this type of arrhythmia is related to the release and actions of thromboxane A_2 (or some consequence of this) *during* ischaemia. It seems plausible to suggest that thromboxane A_2 released during ischaemia acts on receptors situated on platelets and/or vascular smooth muscle (perhaps myocardial cells?) triggering a train of events which subsequently result in ventricular fibrillation when the ischaemic myocardium is reperfused. In this model of ischaemia and reperfusion-induced arrhythmias we have found pretreatment with the thromboxane antagonist AH23848 and thromboxane synthesis inhibition to be equally effective. When thromboxane synthetase is inhibited, rediversion of endoperoxides to prostacyclin can occur (Vermylen & Deckmyn, 1983). This is not a detectable feature in our model because circulating prostacyclin levels are already elevated as a result of the surgical preparation and may mask local changes. Before thoracotomy, arterial and coronary sinus 6-keto $PGF_{1\alpha}$ values are below the detection limit of the assay. However, in the closed-chest situation without the influence of anaesthesia, rediversion of the endoperoxides to prostacyclin may be a significant factor. If this occurred locally in the ischaemic area it would probably be beneficial, but high systemic concentrations of prostacyclin can induce reflex tachycardia and exacerbate ischaemia-induced arrhythmias (Coker & Parratt, 1983a). With AH23848 the concentrations of endogenous prostanoids were not altered; it simply blocked the receptors thus preventing any detrimental action of thromboxane A_2. The direct effects of the endoperoxides, vasoconstriction and platelet aggregation (Moncada & Vane, 1978) would also be prevented by AH23848 since these actions of the endoperoxides are thought to result from stimulation of thromboxane receptors. This ability of AH23848 to block the effects of both thromboxane A_2 and the endoperoxides may have additional advantages.

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