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The effects of timolol on arrhythmias and prostanoid release during canine myocardial ischaemia and reperfusion

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1 Timolol ($50\,\mu g\,kg^{-1}$), administered intravenously to chloralose-anaesthetized open-chest greyhounds 30 min prior to occlusion of the left anterior descending coronary artery, reduced heart rate and mean arterial blood pressure. This dose caused a 20 fold increase in the dose of isoprenaline required to increase heart rate by 25 beats min^{-1}.

2 During the first 30 min of myocardial ischaemia the number of extrasystoles in the timolol-treated dogs (327 ± 179) was less than in the control group (888 ± 168) and none of the dogs that received timolol fibrillated.

3 The haemodynamic changes induced by coronary artery occlusion (decreased cardiac output and stroke volume, increased peripheral vascular resistance) were similar in both control and timolol-treated dogs as were the increases in PCO_2 and decreases in PO_2 and pH in blood draining from the ischaemic myocardium.

4 Timolol did not alter the release during myocardial ischaemia, of either thromboxane B_2 or prostacyclin (measured as 6-keto $PGF_{1\alpha}$).

5 Reperfusion-induced venticular fibrillation occurred in 7 out of 8 control dogs and in 5 out of 10 timolol-treated dogs. The overall survival following occlusion and reperfusion was improved by 10% to 50% by timolol.

Introduction

Previous studies by the present authors have demonstrated that both thromboxane and prostacyclin are released from the ischaemic myocardium of anaesthetized greyhounds during coronary artery occlusion (Coker *et al.*, 1981). A balance of prostanoid release in favour of thromboxane was associated with a higher incidence of very early (phase 1a) ischaemic arrhythmias, whilst studies with the thromboxane synthetase inhibitor, dazoxiben (Coker *et al.*, 1982) support the concept that thromboxane is also a particularly important causative factor in ventricular fibrillation induced by subsequent reperfusion of the ischaemic myocardium.

Several experimental and clinical studies (recently reviewed by Fitzgerald, 1982) have demonstrated that β-adrenoceptor blocking agents have antiarrhythmic activity in the early stages of acute myocardial ischaemia. Much less is known about the effect of these drugs on arrhythmias resulting from myocardial reperfusion, although propranolol has been reported to be ineffective under these conditions (Sheridan *et al.*, 1980; Williams *et al.*, 1982), However, some β-adrenoceptor blocking drugs (e.g. propranolol and metoprolol) inhibit platelet aggregation, reduce the generation of thromboxane A_2 by platelets (Campbell *et al.*, 1981) and decrease plasma thromboxane B_2 concentrations (Graham *et al.*, 1982). It is thus conceivable that the antiarrhythmic activity of some β-adrenoceptor blocking drugs might, to some extent, be mediated through inhibition of thromboxane synthesis. The present study was designed to investigate this possibility by examining the effects of the non-selective β-adrenoceptor blocking drug, timolol, both on arrhythmias and prostanoid release during acute myocardial ischaemia and on the serious arrhythmias that result from subsequent myocardial reperfusion.

Methods

Animal preparation

Twenty greyhounds of either sex (19–31 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 (Marshall *et al.*, 1974; Coker & Parratt, 1983a). The dogs were ventilated with oxygen by means of a Palmer respiration pump, the stroke volume of which was adjusted to give an arterial CO_2 tension of approximately 40 mmHg (5.3 kPa). Pancuronium (0.15 to 0.20 mg kg^{-1}, i.v.) was administered to prevent reflex muscular movement. Catheters were placed in the aorta and the 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 heart was suspended in a pericardial cradle and a ligature was placed around the left anterior descending coronary artery (LAD) approximately 10 to 40 mm distal from the tip of the left atrial appendage. A six inch Longwel 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 units 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 blood samples were taken, without exposure to air, at regular intervals from the aorta, coronary sinus, local coronary vein and pulmonary artery, and Po_2, Pco_2 and pH were measured with an IL 213 blood gas analyser. Oxygen content was calculated by the method of Douglas *et al.*, (1975).

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 ligation. The area outlined in this manner was then cut out and weighed; this 'occluded zone' was expressed as a percentage of the free left ventricular wall.

Prostanoid measurement

Blood samples were taken at various times from the aorta, coronary sinus and local coronary vein and analysed for thromboxane B_2 and 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). Blood samples were placed in tubes containing indomethacin to prevent *ex vivo* generation of prostanoids, and EDTA as anticoagulant. Plasma was stored at −20°C until assayed. 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 $PGF_{1\alpha}$.

Statistics

All values have been expressed as the mean ± s.e. mean of n experiments. Changes within each group of dogs were assessed using a paired t test or a Wilcoxon paired rank sum test, while differences between the groups of dogs were compared with an independent t test or a Mann-Whitney U-test (Eason *et al.*, 1980). Results were considered to be statistically significant at $P < 0.05$. The incidence of events was analysed by Fisher's exact test.

Experimental protocol

After ensuring that control values for various cardiovascular and blood gas parameters were stable, a dose-response curve to isoprenaline was obtained. When haemodynamic parameters had returned to control values, blood samples were taken from the aorta, coronary sinus and the local coronary vein for prostanoid analysis. Timolol maleate 50 μg kg^{-1} (dissolved in isotonic saline to give a 1 ml kg^{-1} solution) was then administered intravenously. This dose of timolol maleate is equivalent to 1.16×10^{-7} mol kg^{-1} of timolol. Fifteen minutes later the haemodynamic parameters were measured and samples taken for blood gases and for the assessment of prostanoid levels. A second dose-response curve to isoprenaline was then obtained. The LAD was occluded 30 min after the administration of timolol and coronary sinus and local coronary venous blood samples were obtained at 2, 7, 15 and 30 min post-occlusion for prostanoid analysis; blood gases and pH were also determined at 7, 15 and 30 min post-occlusion. After 40 min of ischaemia the ligature around the LAD was released and blood gases and prostanoids were measured at 1, 5, and 15 min post-reperfusion.

Results

The intravenous administration of timolol maleate 50 μg kg^{-1} caused significant reductions in heart rate,

Table 1 The effects of timolol and coronary artery acclusion and reperfusion on haemodynamics in anaesthetized greyhounds

			Control	*15 min post-timolol*	*30 min post-occlusion*	*15 min post-reperfusion*
			$n=10$	$n=10$	$n=10$	$n=6$
Heart rate		(beats. min^{-1})	150±5	122±5†††	122±6	123±8
Arterial blood pressure:	Systolic	(mmHg)	178±9	158±8†††	151±7	140±7
	Diastolic	(mmHg)	124±6	106±6††	109±6	101±6
	Mean	(mmHg)	141±6	125±7†††	121±6	111±6
Pulmonary artery pressure:	Systolic	(mmHg)	23±1	24±2	24±2	21±1
	Diastolic	(mmHg)	12±1	12±1	14±1	12±1
	Mean	(mmHg)	16±1	17±1	18±1	15±1
Left ventricular end-diastolic pressure		(mmHg)	8.4±0.7	11.3±1.6	16.1±2.7**	9.4±1.3
Left ventricular dP/dt_{max}		(mmHg s^{-1})	1990±140	1580±160†	1320±100*	1370±190
Cardiac output		(litre min^{-1})	2.61±0.09	2.36±0.15	1.92±0.12**	2.15±0.12
Stroke volume		(ml)	17.6±0.9	19.8±1.3	16.3±1.5**	18.4±1.5
Total peripheral vascular resistance		(kPa litre^{-1}min)	7.3±0.4	7.5±0.8	8.8±0.8*	7.3±0.8

Each value is the mean ± s.e.mean. † $P<0.05$, †† $P<0.01$, ††† $P<0.001$ compared with control value; * $P<0.05$, ** $P<0.01$ compared with pre-occlusion value, paired test.

arterial blood pressure and left ventricular dP/dt_{max} (Table 1). Fifteen minutes after the administration of timolol the dose of isoprenaline required to elevate heart rate by 25 beats min^{-1} had to be increased approximately 20 times. Timolol had no significant effect on arterial, coronary venous or pulmonary arterial Po_2, Pco_2, oxygen content or pH. The P–R and Q–T intervals were increased by timolol (from 97±3 to 113±6 ms and 199±8 to 227±10 ms respectively: $P<0.001$, paired t test) but these changes were probably due to the bradycardia.

Cardiovascular and blood gas changes during coronary artery occlusion

Coronary artery occlusion resulted in changes in certain haemodynamic parameters (Tables 1 and 2). In both groups left ventricular end-diastolic pressure (LVEDP) and total peripheral vascular resistance were increased and there were reductions in cardiac output and stroke volume. Left ventricular dP/dt_{max} was also reduced following coronary artery occlusion in the timolol group. In both control and timolol-treated dogs significant and similar, increases in Pco_2 and decreases in Po_2, oxygen content and pH, were observed in blood sampled from the local coronary vein draining the area rendered ischaemic by occlusion of the LAD (Table 3). Less marked changes were observed in blood sampled from the coronary sinus which represents venous drainage from both ischaemic and essentially normal regions of the left ventricle.

After release of the coronary artery occlusion (reperfusion) in the dogs treated with timolol there were

Table 2 Haemodynamic effects of coronary artery occlusion in control dogs

			Control	*15 min post-vehicle*	*30 min post-occlusion*
			$n=10$	$n=10$	$n=8$
Heart rate		(beats min^{-1})	137±7	136±7	150±10
Arterial blood pressure:	Systolic	(mmHg)	166±14	161±15	173±16
	Diastolic	(mmHg)	118±8	114±10	125±10
	Mean	(mmHg)	136±9	130±11	143±12
Pulmonary artery pressure:	Systolic	(mmHg)	22±1	22±1	23±1
	Diastolic	(mmHg)	13±1	13±1	14±1
	Mean	(mmHg)	16±1	16±1	17±1
Left ventricular end-diastolic pressure		(mmHg)	7.6±0.7	7.8±0.7	9.8±0.9*
Left ventricular dp/dt_{max}		(mmHg s^{-1})	2070±150	2110±180	2150±190
Cardiac output		(litre min^{-1})	2.41±0.24	2.32±0.19	2.04±0.17*
Stroke volume		(ml)	17.8±1.7	17.2±2.3	13.9±1.4*
Total peripheral vascular resistance		(kPa litre^{-1} min)	7.9±0.7	8.1±0.9	9.9±1.3**

Each volume is the mean s.e.mean. * $P<0.05$; ** $P<0.01$ compared with pre-occlusion value, paired t test.

Table 3 The effects of timolol (given at −30 min), coronary artery occlusion (at 0 min) and reperfusion (at 40 min) on local coronary venous blood gases, pH and oxygen content

Time (min)	n	P_{O_2} (mmHg)	P_{CO_2} (mmHg)	*pH* (units)	*O_2 content* (ml 100ml^{-1})
Control					
−35	10	32±1	60±2	7.31±0.01	11.3±0.6
−15	10	30±2	60±2	7.30±0.01	10.6±0.8
7	10	26±2*	72±4**	7.20±0.02***	7.5±0.7**
15	9	26±1*	74±4**	7.19±0.03**	7.2±0.7**
30	8	26±1*	68±4*	7.24±0.02*	8.3±0.8
Timolol					
−35	10	31±1	58±1	7.31±0.01	10.5±0.4
−15	10	31±1	56±1	7.33±0.01	11.2±0.7
7	10	25±1***	63±1***	7.26±0.01***	7.1±0.7***
15	10	25±1***	65±2***	7.25±0.01***	7.1±0.7***
30	10	25±1***	63±1**	7.27±0.01**	8.2±0.6***
46	8	59±6‡‡	63±5	7.24±0.03	16.4±1.9‡‡‡
50	7	49±3‡‡‡	48±2‡‡‡	7.33±0.02‡‡	18.5±1.3‡‡‡
55	6	33±1‡‡‡	51±1**	7.33±0.01‡‡	13.5±0.7‡‡‡

Each value is the mean ± s.e.mean, $*P<0.05$ $**P<0.01$ $***P<0.001$ compared with pre-occlusion value, $‡P<0.05$ $‡‡P<0.01$ $‡‡‡P<0.001$ compared with 30 min post-occlusion value, paired *t* test.

marked increases in local coronary venous P_{O_2} and oxygen content (Table 3). Five minutes after the onset of reperfusion, local coronary venous pH (but not P_{CO_2}) had returned to pre-occlusion values (Table 3). Since all but one of the ten control dogs fibrillated soon after reperfusion we were unable to sample for blood gases (or prostanoids) in this particular group.

Arrhythmic activity during ischaemia and reperfusion

The total number of extrasystoles that occurred during the period of coronary artery occlusion in those control dogs that survived 30 min was 888±168. These arrhythmias included single ectopic beats and periods of bigeminy and ventricular tachycardia. Two of the ten control dogs died in ventricular fibrillation during coronary artery occlusion. Figure 1 illustrates the distribution of extrasystoles in each individual dog in the control group.

Pretreatment with timolol reduced the total number of extrasystoles to 327±179 ($P<0.05$) and none of these animals fibrillated during the occlusion period. Whereas none of the control dogs had less than 100 extrasystoles during the occlusion period, 5 out of 10 dogs which received timolol had very few extrasystoles (i.e. less than 20, Figure 2). The occluded zone (i.e. the anatomical area at risk) was similar in both groups; 39.1±1.4% of the free left ventricular wall in controls and 41.1±1.4% in the timolol group. The degree of the ST segment depression which had developed after 30 min of ischaemia was also similar in both groups (0.35±0.06 and 0.55±0.13 mV respectively).

Release of the ligature around the LAD after 40 min of ischaemia produced characteristic 'reperfusion' arrhythmias. These started as soon as direct perfusion of the formerly ischaemic region was restored, and were more severe than the arrhythmias that occurred during ischaemia. They are illustrated in Figure 3. In 7 out of 8 control dogs the rapid multifocal ventricular tachycardia induced by reperfusion progressed to ventricular fibrillation, usually within 2 min. Five of the 10 timolol treated dogs fibrillated (see Figure 3); 3 within 2 min, 1 at 12 min and one at 20 min after commencing reperfusion. Of the 5 survivors in the timolol group, 2 had multifocal ventricular tachycardia after the release of the occlusion whilst the other 3 dogs had less severe arrhythmias. Comparison of the incidence of reperfusion-induced ventricular fibrillation in the control (7/8) and timolol (5/10) groups using Fisher's exact test gives a probability value of $P=0.11$. When the overall survival following occlusion and reperfusion (i.e. 1/10 in controls and 5/10 in the timolol group) is compared the probability value is $P=0.067$. If the timolol-treated dogs are divided into those that survived both the occlusion and reperfusion periods and those that did not, then the dogs that died had a significantly greater ST-segment depression than the survivors (Table 4), although the size of the occluded zone was similar. It can also be seen from Figure 2 that the majority of the dogs which survived reperfusion had very little ectopic activity during ischaemia.

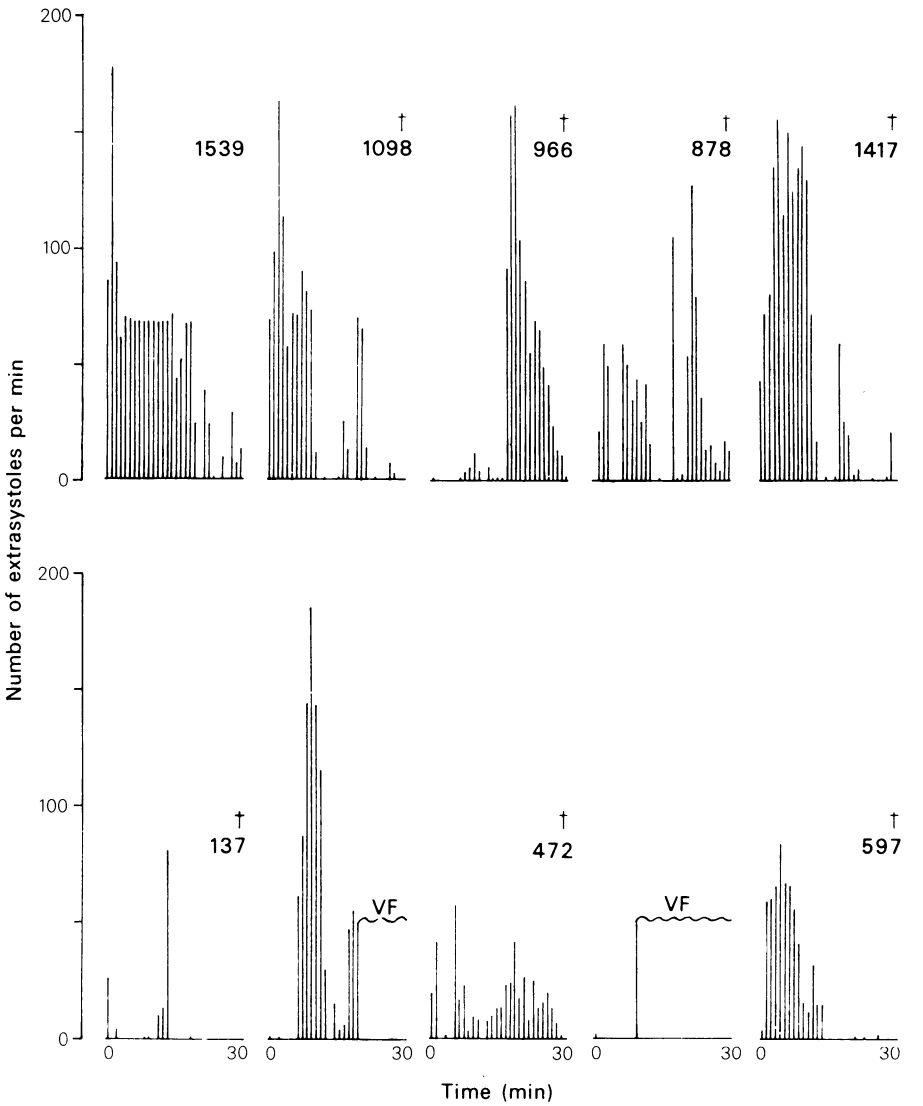

Figure 1 Arrhythmia profiles in the control dogs. Each panel represents one individual dog and illustrates the number of extrasystoles occurring in 1 min intervals during the first 30 min of coronary artery occlusion. The number at the upper right of each panel indicates the total number of extrasystoles that occurred in the 30 min period. † indicates an animal which fibrillated following reperfusion.

Table 4 Changes in local coronary venous P_{CO_2} and pH after 15 min of ischaemia, and in ST-segment depression after 30 min of ischaemia, in dogs pretreated with timolol

	n	δP_{CO_2} (mmHg)	*δpH* (units)	*δ ST* (mV)	*Occluded* zone (%)
Survivors	5	9.8 ± 2.9	0.076 ± 0.017	0.26 ± 0.15	40.4 ± 2.4
Non-survivors	5	8.0 ± 1.2	0.070 ± 0.012	0.84 ± 0.13*	41.8 ± 1.6

*$P < 0.05$, independent *t* test.

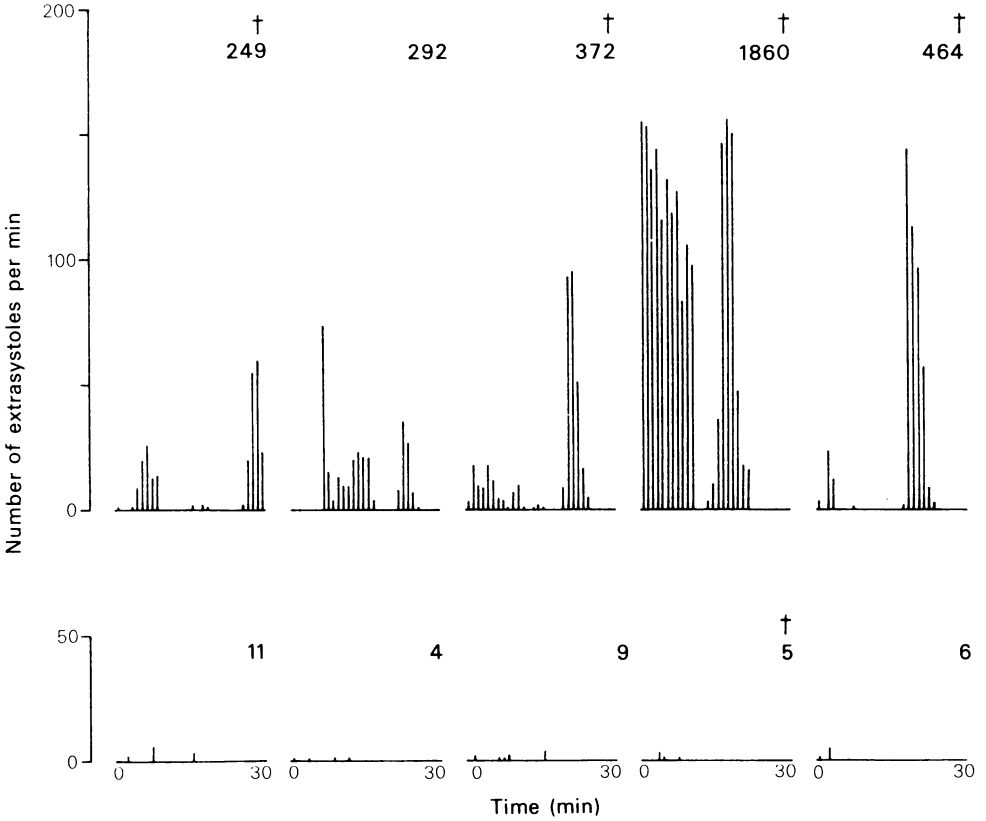

Figure 2 Arrhythmia profiles in the dogs which received timolol. Each panel represents one individual dog and illustrates the number of extrasystoles occurring in 1 min intervals during the first 30 min of coronary artery occlusion. The number at the upper right of each panel indicates the total number of extrasystoles that occurred in the 30 min period. † indicates an animal which fibrillated following reperfusion.

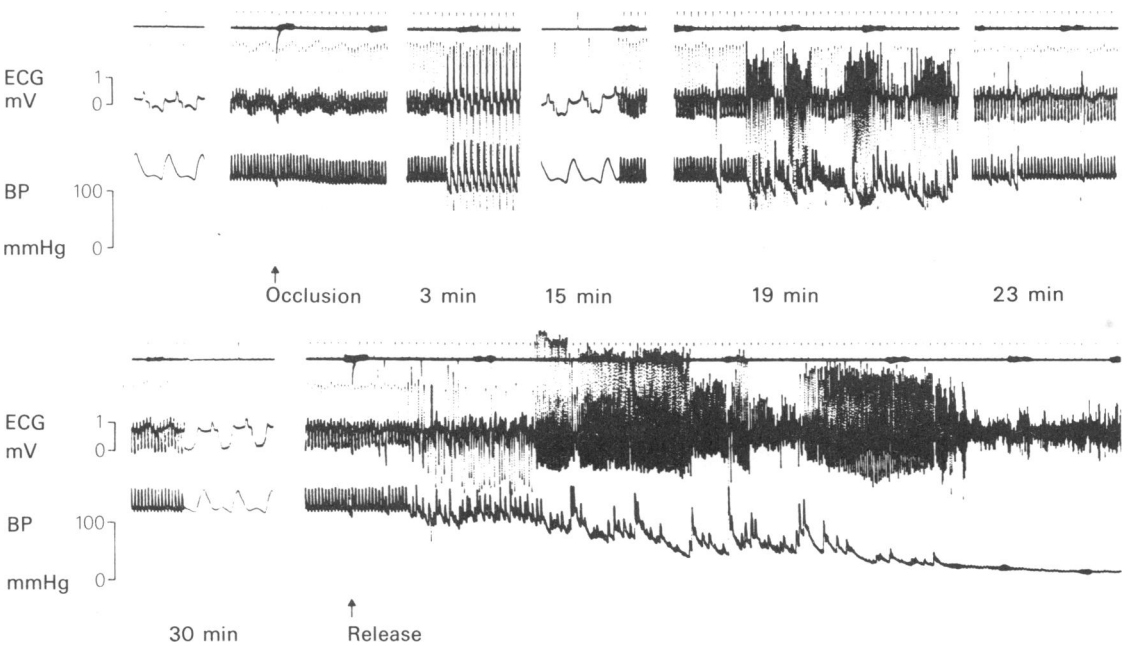

Figure 3 Characteristic occlusion and reperfusion-induced arrhythmias in a dog which received timolol (total number of extrasystoles during the first 30 min of occlusion = 464). The time marker at the top of each panel is in seconds.

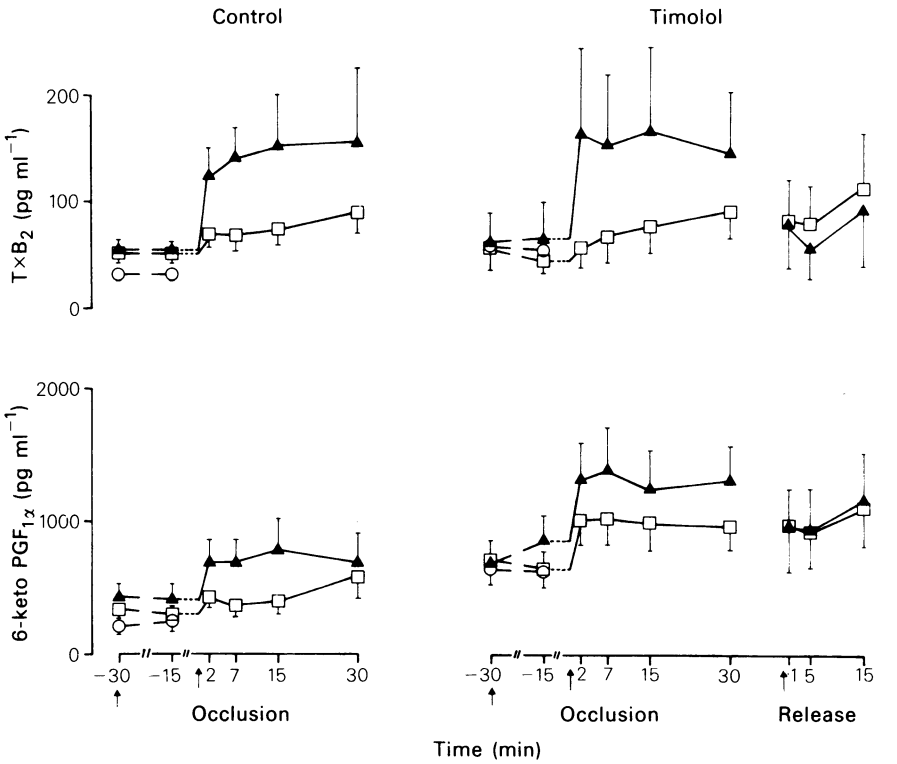

Figure 4 Plasma concentrations of thromboxane B_2 (TxB_2) and 6-keto $PGF_{1\alpha}$ in the aorta (○), the coronary sinus (□) and the local coronary vein (▲). Each value is the mean and vertical lines show s.e.mean. Statistical analysis was performed using Wilcoxon tests. At all times during occlusion the local coronary venous concentrations of TxB_2 and 6-keto $PGF_{1\alpha}$ were significantly ($P<0.01$) higher than the corresponding pre-occlusion values, in both groups. In the timolol group the coronary sinus concentrations of 6-keto $PGF_{1\alpha}$ were also significantly ($P<0.01$) elevated during occlusion. Five minutes after reperfusion the local coronary venous TxB_2 concentration was significantly ($P<0.01$) lower than the 30 min value and the 6-keto $PGF_{1\alpha}$ concentrations were significantly ($P<0.01$) lower at 1 and 5 min post-reperfusion.

Prostanoid release

Prior to occlusion of the LAD, timolol did not alter the concentrations of thromboxane B_2 or 6-keto $PGF_{1\alpha}$ in the aorta, the coronary sinus or the local coronary vein (Figure 4). During coronary artery occlusion both thromboxane B_2 and 6-keto $PGF_{1\alpha}$ were released into blood draining from the acutely ischaemic myocardium and the profiles of this release were similar in the control group and in the dogs

Table 5 Changes in the levels of prostanoids in local coronary venous plasma in timolol-treated dogs during coronary artery occlusion: the values given are percentage changes from pre-occlusion levels

		Time after occlusion:			
	n	2 min	7 min	15 min	30 min
Thromboxane B_2					
Survivors	5	27±13	81±25	59±17	35±18
Non-survivors	5	242±89**	243±44*	249±89	251±69*
6-keto $PGF_{1\alpha}$					
Survivors	5	56±15	82±41	61±31	67±30
Non-survivors	5	28±10	38±11	24±10	44±20

*$P<0.05$; **$P<0.01$; Mann-Whitney U-test.

pretreated with timolol (Figure 4). Although the range of thromboxane B_2 values in the drug-treated group was greater, thromboxane release into local coronary venous blood occurred in each individual dog. It is of interest that, within the timolol group, thromboxane B_2 release during ischaemia was greater in the dogs that fibrillated following reperfusion than in those that survived (Table 5).

Discussion

The results of this study demonstrate a clear antiarrhythmic effect of timolol when given prior to the onset of myocardial ischaemia. This is reflected both in the reduced incidence of ventricular ectopic beats during the early arrhythmic phase and in the prevention of ventricular fibrillation. These results with timolol are in accord with most other previously published reports involving β-adrenoceptor blocking drugs, studies which have been reviewed recently (Fitzgerald 1982). It can be concluded that this protection almost certainly results mainly from blockade of myocardial β-adrenoceptors, since timolol has minimal 'membrane stablising activity' (i.e. does not interfere with the rapid inward Na^+ current in cardiac muscle) and has negligible intrinsic sympathomimetic activity (Ulrych *et al.*, 1972). A similar conclusion has recently been reached from studies with a variety of β-adrenoceptor blocking drugs with varying ancillary properties (Campbell & Parratt, 1981; 1983). The results also lend further support to the contention that locally released noradrenaline plays a major role in initiating early ventricular ectopic activity during myocardial ischaemia (Abrahamsson *et al.*, 1982; Riemersma, 1982).

The effects of timolol on the serious arrhythmias that result from reperfusion of the previously ischaemic myocardium were less impressive. Five out of 10 timolol-treated dogs fibrillated on reperfusion; this is less than in the present control group (7 out of 8) but more than we have observed previously in this experimental model in dogs pretreated with the calcium antagonist, nifedipine (Coker & Parratt, 1983a) or with drugs that either inhibit the synthesis of thromboxane (such as dazoxiben; Coker *et al.*, 1982) or which 'promote' the release of prostacyclin (e.g. nafazatrom; Coker & Parratt, 1983b). Studies in other laboratories with propranolol have failed to demonstrate a protective effect either against reperfusion arrhythmias (Sheridan *et al.*, 1980; Williams *et al.*, 1982) or against the sudden reduction in the ventricular fibrillation threshold that follows immediately after the release of a coronary occlusion (Corbalan *et al.*, 1976).

In the present studies timolol afforded protection against a combined ischaemia-reperfusion insult in that survival was increased from only 10% (in the controls) to 50%. Further, the cardiovascular status of the animals that survived this procedure was good (Table 1) and coronary venous and arterial blood gases and pH were normal (Table 3). This result might imply that in the clinical situation of myocardial infarction, timolol would not only protect against ischaemic arrhythmias but might also reduce the severe consequences of 'reperfusion' in those patients who, for example, had a coronary artery obstructed by a platelet thrombus (or as a result of smooth muscle spasm) which then subsequently reopened. Such an effect might contribute to the ('secondary') protection afforded by timolol in those patients who had already suffered at least one myocardial infarction (Norwegian Multicentre Study Group, 1981), although it should be emphasized that results from acute experiments in healthy anaesthetized dogs may not be relevant to effects observed after long term treatment in conscious diseased humans.

The mechanism of this protection by timolol in the present study is unclear. However, there are at least two possibilities. First, timolol might increase survival in this model by reducing the severity of myocardial ischaemia resulting from coronary artery occlusion. There is some evidence for this from the present study since those dogs given timolol that ultimately survived reperfusion had fewer ischaemia-induced arrhythmias (Figure 2) and less marked ST-segment changes (Table 4) than those dogs that eventually succumbed. Others have also suggested that the severity of reperfusion arrhythmias depends upon the degree of the preceding myocardial ischaemia (Balke *et al.*, 1981) and we have ourselves shown (Coker & Parratt, 1983a) that where the area at risk is less than 30% of the free left ventricular wall little arrhythmic activity results either during ischaemia or reperfusion.

A second explanation for the protection afforded by timolol in the present study is that this drug can, on occasion, limit myocardial thromboxane release during ischaemia. There is no evidence for such an effect of timolol if all the treated dogs are grouped together (Figure 4). However, if the survivors and non-survivors are separated (Table 5) it is clear that there was substantially less thromboxane A_2 released during ischaemia in those dogs that eventually survived reperfusion. There are two possible explanations for this effect. The first is that the reduced thromboxane release in these particular dogs simply reflects a less severe ischaemia. The second possibility is that, on occasions, timolol can directly reduce thromboxane release from platelets. The possibility that timolol has a general inhibitory effect on platelet function has been raised by Thaulow *et al.*, (1981) in reference to the long-term protection this drug affords against

sudden death and re-infarction (Norwegian Multicenter Study Group, 1981). They found that the administration of a single oral dose of timolol (5 mg) to healthy men increased the ADP-threshold and reduced the platelet release reaction, as reflected by lower β-thromboglobulin levels. The primary aggregation rate was unchanged. Similar results were also observed with an equivalent β-adrenoceptor blocking dose of propranolol (40 mg). There is also evidence that some β-adrenoceptor blocking drugs (propranolol, metoprolol) reduce plasma thromboxane B_2 concentrations (Graham *et al.*, 1982). Whether this is due to β-adrenoceptor blockade *per se* is unclear; 'membrane stabilisation' is almost certainly involved in the platelet effects of propranolol since (+)-propranolol is just as effective as (±)-propranolol in inhibiting platelet aggregation (Campbell *et al.*, 1981). The results of the present study, however, suggest that timolol (at this particular dose) does not have any marked effects on thromboxane synthesis.

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