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Failure of allopurinol and a spin trapping agent N-t-butyl-α-phenyl nitrone to modify significantly ischaemia and reperfusion-induced arrhythmias

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1 The possible role of free radicals in the genesis of occlusion and reperfusion-induced arrhythmias was studied by determining the effects of the xanthine oxidase inhibitor allopurinol (400 mg p.o. 24 h before experimentation + 25 mg kg^{-1} i.v.) and the free radical scavenger N-t-butyl-α-phenyl nitrone (PBN; 50 mg kg^{-1} i.v.) on these arrhythmias in chloralose anaesthetized greyhounds.

2 Neither of the drugs had any major effects on haemodynamic variables, although allopurinol caused a significant increase in heart rate.

3 The mean number of extrasystoles observed during ischaemia in dogs given allopurinol or PBN was not significantly different from those seen in controls. Further, the incidence of ventricular fibrillation during either occlusion or reperfusion was unchanged by either drug and there was thus no improvement in survival.

4 These results suggest that, in this model of myocardial ischaemia and reperfusion, free radicals may not play a major role in the genesis of life-threatening arrhythmias.

Introduction

Coronary artery occlusion and reperfusion result in damage to the myocardium, an important consequence of which is the development of life-threatening ventricular arrhythmias (Corr & Witkowski, 1984). It is widely believed that agents which modify mechanisms underlying tissue injury may also alter the severity and extent of arrhythmias. Free radicals have been identified as metabolic intermediates during acute myocardial infarction, and electron spin resonance studies have suggested that they appear in coronary venous blood within 5 min of coronary artery occlusion (Rao *et al.*, 1983). In the same study, raised levels of malondialdehyde after 45 min of occlusion suggest that there is increased lipid peroxidation, possibly initiated by free radical reactions. Uncontrolled peroxidation of biomembranes may lead to profound effects on membrane structure and function sufficient to cause cell death, suggesting that rapid production of free radicals by ischaemic myocardium may trigger later secondary events such as lipid peroxidation and even, perhaps, depletion of protective antioxidants (e.g. superoxide dismutase, catalase). However, it has been proposed that tissue damage occurs not so much during the period of ischaemia as after reperfusion has been established (Haglund & Lundgren, 1978) and a major source of pathological free radical production due to ischaemia or hypoxia may result from reoxygenation.

One source of superoxide production has been associated with the conversion of hypoxanthine to xanthine by xanthine oxidase with the reintroduction of oxygen (Roy & McCord, 1983). Several studies performed *in vivo* have provided evidence that myocardial damage may to some extent be reduced by intervention with antioxidants such as superoxide dismutase plus catalase (Jolly *et al.*, 1984; Myers *et al.*, 1985; Gross *et al.*, 1986) or ascorbate or vitamin E (Gauduel & Duvelleroy, 1984). In further studies (Akizuki *et al.*, 1984; Werns *et al.*, 1985) inhibition of xanthine oxidase with allopurinol has been shown to protect against reperfusion induced damage.

While the evidence builds up for the involvement of free radicals in the production of cellular damage, little attention has been paid to the arrhythmias which accompany the ischaemia-reperfusion insult. Studies using rat isolated perfused hearts subjected to coronary artery ligation have shown a decrease in the incidence of ventricular fibrillation (VF) induced by ischaemia (Woodward & Zakaria, 1985), and in reperfusion-induced VF (Bernier *et al.*, 1986), when

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antioxidants are present in the perfusate. *In vivo* studies in rats have shown that inhibition of xanthine oxidase with allopurinol protects against reperfusion-induced arrhythmias (Manning *et al.*, 1984). The aim of the present study was to determine the effects of removing oxygen-derived free radicals, by inhibition of xanthine oxidase with allopurinol and (as xanthine oxidase is not the only possible source of free radical generation during ischaemia and reperfusion) those produced by other mechanisms with a spin-trapping agent N-t-butyl-α-phenyl nitrone (PBN), on the arrhythmias resulting from ischaemia and subsequent reperfusion in anaesthetized greyhounds.

Methods

Animal preparation

Greyhounds of either sex (23–31 kg) were anaesthetized with sodium thiopentone (25 mg kg^{-1} i.v.) followed by chloralose (80–90 mg kg^{-1} i.v.) and prepared for coronary artery occlusion as described in detail by Marshall *et al.* (1974). The dogs were ventilated with a Palmer respiration pump and the stroke volume and oxygen content of inspired air were adjusted to maintain arterial CO_2 and O_2 tensions of 40 mmHg and 100 mmHg respectively. Pancuronium bromide (0.1–0.15 mg kg^{-1} i.v.) was administered to prevent reflex muscular movement. Catheters were placed in the descending aorta and vena cava, via the femoral vessels, for measurement of arterial blood pressure and administration of drugs, respectively. Intracardiac catheters were placed in the coronary sinus and pulmonary artery, via the left jugular vein, and the left ventricular cavity, via the left carotid artery, under fluoroscopic control (Siemens Image Intensifier).

A left thoracotomy was performed at the level of the sixth rib and the heart suspended in a pericardial cradle. A silk ligature was placed around the left anterior descending coronary artery (LAD) approximately 20–40 mm distal to the left atrial appendage. A 15 cm Longdwel teflon catheter was placed in a coronary vein adjacent to the LAD and the tip advanced to a position within the area rendered ischaemic by occlusion of the LAD. On completion of the surgery 100 u kg^{-1} of heparin was administered.

Pressures were recorded with Gould transducers and displayed on a Mingograf 82 ink jet recorder (Siemens Elema) and the electrocardiogram (ECG) was recorded from Lead II. Left ventricular end-diastolic pressure (LVEDP) was recorded from the full left ventricular pressure trace at high gain. Cardiac output was measured by thermodilution using a Devices cardiac output monitor. Two ml blood samples were taken, without exposure to air, at regular intervals from the aorta, pulmonary artery, coronary sinus and local coronary vein so that P_{O_2}, P_{CO_2} and pH could be measured with an IL 213 blood gas analyser. Oxygen content was calculated from the haemoglobin and corrected blood gas levels using a Hewlett Packard desk-top computer.

Lactate measurement

Blood lactate (mmol l^{-1}) was determined in 1 ml samples obtained from the aorta before occlusion and 30 min after the onset of ischaemia. Further samples were obtained from the local coronary vein draining the ischaemic area before occlusion and at 2, 7,15 and 30 min post-occlusion and 1 min post-reperfusion. Samples were immediately deproteinized by mixing with 0.8% perchloric acid and centrifuged. The supernatant was stored at −23°C (for not more than three weeks) until assayed spectrophotometrically using standard lactate assay kits (Boehringer).

Experimental protocol

Dogs receiving the allopurinol treatment were given an oral dose of 400 mg 24 h before anaesthesia. Once the cardiovascular and blood gas parameters had stabilized following the surgical procedure, allopurinol (25 mg kg^{-1} i.v.) or PBN (50 mg kg^{-1} i.v.) was administered. These doses were chosen on the basis of previous studies where allopurinol was shown to protect the canine myocardium against ischaemic damage (Akizuki *et al.*, 1984) and PBN to improve survival in a model of endotoxin shock in rats (McKechnie *et al.*, 1986). Two groups of 10 control dogs were given the solvent vehicle for each drug (0.1 N NaOH for allopurinol and saline for PBN). Fifteen min after drug (or vehicle) administration, the haemodynamic parameters were again recorded and blood samples taken for blood gas and pH measurements. Twenty min after drug administration the LAD was occluded, following which coronary sinus and local coronary venous blood samples were withdrawn from all animals for immediate determination of blood gases at 2, 7, 15 and 30 min post-occlusion. The occlusion around the LAD was released after 40 min of ischaemia and blood gases were measured 1 min after reperfusion.

The area of occluded tissue was estimated at the end of each experiment by removing the heart and injecting a small volume of Comassie Blue dye into the coronary artery at the site of occlusion. The occluded zone was then dissected free and expressed as a percentage (by weight) of the free left ventricular wall.

Statistics

All values are expressed as mean ± s.e.mean of n experiments. Changes within each group were com-

pared using a paired t test or Wilcoxon rank sum test, whereas differences between groups of dogs were assessed using an independent t test or Mann Whitney U-test. Changes in the incidences of events were analysed by Fischer's Exact Probability test. Results were considered to be statistically significant at $P < 0.05$.

Results

Haemodynamic effects of allopurinol and N-t-butyl-α-phenyl nitrone

The haemodynamics in the dogs receiving the oral dose of allopurinol 24 h before experimentation were not significantly different from the haemodynamics of the NaOH controls before intravenous administration of either drug or vehicle (Table 1, column 1). The intravenous administration of allopurinol (25 mg kg^{-1}; Table 1) or PBN (50 mg kg^{-1}; Table 2) had no immediate haemodynamic effects. Fifteen min after drug administration (just before coronary occlusion) the dogs given allopurinol had a significantly raised heart rate compared to the pre-drug values (Table 1) whereas neither drug significantly affected systemic arterial pressure, left ventricular end-diastolic pressure (LVEDP) or dP/dt_{max} (Tables 1 and 2). Pulmonary arterial pressure was also unchanged by both allopurinol (23 ± 2/13 ± 1; mean 16 ± 1 mmHg) and PBN (27 ± 2/13 ± 2; mean 19 ± 2 mmHg) compared to pre-drug values (24 ± 2/16 ± 1; mean 20 ± 1 mmHg and 25 ± 2/12 ± 1; mean 18 ± 2 mmHg, respectively).

Haemodynamic effects of coronary artery occlusion

The haemodynamic changes observed following 30 min of coronary artery occlusion were similar in both control groups (Tables 1 and 2). The only changes were slight reductions in dP/dt_{max}, cardiac output and stroke volume, none of which were statistically significant. In the saline-treated controls there was a significant increase in LVEDP 30 min after occlusion (Table 2), although this was not observed in the controls receiving NaOH (Table 1) which may be due to the different control vehicles used. In dogs treated with allopurinol (Table 1) the heart rate increase induced by the drug was still evident after the 30 min occlusion period (i.e. 50 min after drug administration). The small reductions in dP/dt_{max}, cardiac output and stroke volume were similar to those seen in the NaOH controls and there was a particularly marked increase in LVEDP. The haemodynamic changes following 30 min ischaemia in dogs given

Table 1 The haemodynamic effects of coronary artery occlusion in control (NaOH) dogs and in dogs given allopurinol 16 min before occlusion

	5 min pre-drug	*15 min post-vehicle/drug*	*30 min post-occlusion*
Control	($n = 10$)	($n = 10$)	($n = 8$)
Heart rate (beats min^{-1})	162 ± 8	160 ± 7	169 ± 10
Arterial pressure (mmHg)			
Systolic	182 ± 8	184 ± 11	173 ± 11
Diastolic	135 ± 8	129 ± 8	129 ± 9
Mean	154 ± 7	148 ± 8	146 ± 10
LVEDP (mmHg)	11.6 ± 1.3	10.6 ± 1.5	10.5 ± 1.6
dP/dt_{max} (mmHg s^{-1})	2680 ± 267	2630 ± 258	2444 ± 230
Cardiac output (1 min^{-1})	3.91 ± 0.36	3.86 ± 0.42	3.21 ± 0.49
Stroke volume (ml)	22.0 ± 2.2	25.5 ± 4.9	18.7 ± 3.2
Allopurinol	($n = 10$)	($n = 10$)	($n = 8$)
Heart rate (beats min^{-1})	170 ± 10	193 ± 12**	191 ± 16†
Arterial pressure (mmHg)			
Systolic	192 ± 9	182 ± 12	167 ± 12
Diastolic	139 ± 8	126 ± 9	121 ± 10
Mean	158 ± 8	145 ± 10	141 ± 10
LVEDP (mmHg)	10.1 ± 1.6	9.5 ± 1.5	11.7 ± 1.9†
dP/dt_{max} (mmHg s^{-1})	2730 ± 235	3133 ± 278	2663 ± 264
Cardiac output (1 min^{-1})	3.71 ± 0.26	3.68 ± 0.25	2.81 ± 0.23
Stroke volume (ml)	22.4 ± 1.7	19.8 ± 1.4	15.8 ± 1.9

Values are mean s.e.mean of n observations †$P < 0.05$ compared to 15 min post-drug (1 min before occlusion). **$P < 0.01$ compared to pre-drug values.

Table 2 The haemodynamic effects of coronary artery occlusion in control (saline) dogs and in dogs given PBN 16 min before occlusion

	5 min pre-drug	*15 min post-vehicle/drug*	*30 min post-occlusion*
Control	($n = 10$)	($n = 10$)	($n = 8$)
Heart rate (beats min^{-1})	155 ± 6	153 ± 5	158 ± 5
Arterial pressure (mmHg)			
Systolic	209 ± 12	206 ± 13	215 ± 13
Diastolic	157 ± 9	150 ± 9	162 ± 11
Mean	176 ± 10	171 ± 11	181 ± 12
LVEDP (mmHg)	11.9 ± 1.6	11.5 ± 1.5	13.1 ± 1.3††
dp/dt_{max} (mmHg $^{-1}$)	2789 ± 312	2656 ± 330	2414 ± 251
Cardiac output (1 min^{-1})	3.50 ± 0.37	3.50 ± 0.41	3.29 ± 0.52
Stroke volume (ml)	22.6 ± 2.0	22.9 ± 2.4	20.6 ± 2.9
PBN	($n = 10$)	($n = 10$)	($n = 8$)
Heart rate (beats min^{-1})	159 ± 7	169 ± 8	179 ± 11
Arterial pressure (mmHg)			
Systolic	224 ± 15	226 ± 16	215 ± 14
Diastolic	151 ± 9	153 ± 10	154 ± 10
Mean	176 ± 10	177 ± 11	171 ± 11
LVEDP (mmHg)	8.2 ± 1.2	8.1 ± 1.4	8,7 ± 2.0
dp/dt_{max} (mmHg s^{-1})	2811 ± 397	2878 ± 405	2700 ± 421
Cardiac output (1 min^{-1})	3.47 ± 0.25	3.55 ± 0.28	3.00 ± 0.32††
Stroke volume (ml)	21.9 ± 1.4	20.9 ± 1.5	16.9 ± 1.8††

All values are mean s.e.mean of n †$P < 0.05$, ††$P < 0.01$ compared to 15 min post-drug (1 min pre-occlusion values.)

PBN (Table 2) were similar to those observed in the saline controls, apart from a moderately increased peripheral vascular resistance.

Changes in blood gases and pH in dogs subjected to coronary artery occlusion

The blood gas and pH data for blood sampled at various times from the local coronary vein (draining predominantly from the ischaemic area) in both groups of controls and dogs treated with allopurinol or PBN are shown in Tables 3 and 4. Neither allopurinol (Table 3) nor PBN (Table 4) had any significant effects on arterial, pulmonary arterial, coronary venous (or sinus) blood gases, pH or oxygen content before occlusion. In both groups of controls, coronary artery occlusion resulted in changes in blood sampled from the coronary vein consisting of small, but significant, reductions in P_{O_2}, oxygen content and pH and a rise in P_{CO_2}. Similar but less marked changes were also observed in blood sampled from the coronary sinus (draining the essentially normal myocardium). In the group of dogs treated with allopurinol the fall in P_{O_2} and oxygen content was slightly greater, but not significantly so, than that in the control group (Table 5) whereas the changes in the dogs given PBN were of a similar magnitude to those seen in the corresponding controls.

Reperfusion in controls resulted in a marked rise in P_{O_2} and oxygen content and a further decrease in pH within 1 min of release of the ligature. In dogs given allopurinol, reperfusion resulted in a significantly greater increase in P_{O_2} and oxygen content than in controls (Table 5).

Arrhythmias

Effects of allopurinol In control dogs there was marked ventricular ectopic activity (single extrasystoles, bigeminal rhythm and ventricular tachycarida) within seconds of coronary occlusion and which lasted for 4–7 min (Phase la arrhythmias). These were followed 8–12 min later by a further phase of more serious arrhythmias (Phase 1b) which terminated within 30 min of occluding the LAD. Examples of these arrhythmias are illustrated in Figure 1. The total number of extrasystoles in the 9 NaOH-treated control dogs which were still alive at the end of the 30 min occlusion period was 884 ± 163. Further 6 of these 9 dogs (67%) experienced ventricular tachycardia (VT)

Table 3 The effects of allopurinol (given at −20 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	Po_2 (mmHg)	Pco_2 (mmHg)	*pH* (units)	O_2 *content* (ml 100ml^{-1})
Control					
−35	10	23 ± 1	53 ± 3	7.28 ± 0.03	7.2 ± 0.9
−15	10	23 ± 2	53 ± 3	7.28 ± 0.03	7.5 ± 1.0
7	9	22 ± 1	56 ± 3	7.23 ± 0.03*	6.3 ± 1.0
15	9	22 ± 1	60 ± 5	7.21 ± 0.05*	5.6 ± 0.7
30	9	21 ± 1	58 ± 6	7.22 ± 0.05	5.5 ± 0.8*
41	9	27 ± 2†	68 ± 11	7.14 ± 0.06†	8.2 ± 1.9†
Allopurinol					
−35	10	23 ± 1	55 ± 3	7.26 ± 0.03	6.3 ± 0.7
−15	10	23 ± 1	55 ± 3	7.29 ± 0.03	6.4 ± 0.6
7	9	17 ± 1**	61 ± 3*	7.23 ± 0.02	3.8 ± 0.3**
15	9	17 ± 1**	61 ± 3*	7.24 ± 0.02	4.0 ± 0.3**
30	8	18 ± 1*	57 ± 2	7.25 ± 0.02	4.1 ± 0.4*
41	8	39 ± 3††	43 ± 4	7.33 ± 0.04	16.1 ± 1.5††

Values are mean ± s.e.mean of *n* observations.
*$P<0.05$, **$P<0.01$ compared to −15 pre-occlusion value; †$P<0.05$, ††$P<0.01$ compared to 30 min post-occlusion value.

Table 4 The effects of PBN (given at −20 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	Po_2 (mmHg)	Pco_2 (mmHg)	*pH* (units)	O_2 *content* (ml 100ml^{-1})
Control					
−35	10	22 ± 2	52 ± 1	7.29 ± 0.01	6.7 ± 0.9
−15	10	22 ± 1	54 ± 2	7.28 ± 0.02	6.6 ± 0.9
7	9	21 ± 2	61 ± 3	7.20 ± 0.02**	4.4 ± 0.5*
15	9	19 ± 1*	65 ± 4*	7.18 ± 0.02**	4.1 ± 0.4**
30	8	20 ± 2*	59 ± 3	7.20 ± 0.02**	4.8 ± 0.6*
41	8	29 ± 2††	61 ± 7	7.18 ± 0.04†	8.6 ± 0.6††
PBN					
−35	10	26 ± 2	52 ± 2	7.25 ± 0.03	7.5 ± 1.0
−15	10	25 ± 2	50 ± 1	7.29 ± 0.01	7.6 ± 0.9
7	10	21 ± 1**	59 ± 3**	7.21 ± 0.02**	4.9 ± 0.6**
15	9	21 ± 2**	57 ± 2*	7.23 ± 0.01**	5.0 ± 0.6**
30	8	23 ± 2*	57 ± 3	7.24 ± 0.01*	5.7 ± 0.6**
41	8	37 ± 3††	55 ± 4	7.18 ± 0.2†	11.7 ± 1.4††

Values are mean ± s.e.mean of *n* observations.
*$P<0.05$, **$P<0.01$ compared to −15 pre-occlusion value; †$P<0.05$, ††$P<0.01$ compared to 30 min post-occlusion value.

Table 5 Changes in coronary venous P_{O_2}, P_{CO_2}, pH and oxygen content during coronary artery occlusion and reperfusion in control dogs and dogs administered allopurinol or PBN.

Time (min)	n	P_{O_2}	P_{CO_2}	*pH*	*O_2 content*
Control					
+ 7	9	−0.9 ± 1.3	+2.9 ± 1.8	−0.05 ± 0.02	−1.1 ± 0.8
+30	9	−2.8 ± 1.1	+5.8 ± 4.2	−0.06 ± 0.02	−2.0 ± 0.5
+41	9	+7.0 ± 2.3	+1.8 ± 7.0	−0.09 ± 0.03	+3.8 ± 1.7
Allopurinol					
+ 7	9	−4.3 ± 0.8	+10.7 ± 3,8*	−0.08 ± 0.03	−3.0 ± 0.6
+30	8	−4.7 ± 1.4	+5.2 ± 3.2	−0.06 ± 0.01	−2.7 ± 0.7
+41	8	+22.3 ± 2.7**	−10.7 ± 4.3*	−0.06 ± 0.04	+11.4 ± 1.6**
Control					
+ 7	9	−1.6 ± 0.9	+4.4 ± 3.0	−0.06 ± 0.03	−1.5 ± 1.7
+30	8	−2.6 ± 0.9	+4.4 ± 3.0	−0.07 ± 0.03	−1.7 ± 0.5
+41	8	+9.0 ± 1.7	−1.0 ± 1.0	−0.09 ± 0.01	−4.2 ± 0.6
PBN					
+ 7	10	−4.0 ± 1.0	+8.9 ± 2.6	−0.08 ± 0.02	−2.6 ± 0.5
+30	8	−3.7 ± 1.0	+7.4 ± 3.5	−0.05 ± 0.02	−2.3 ± 0.6
+41	8	+15.7 ± 2.8	−3.3 ± 2.7	−0.05 ± 0.02	+6.5 ± 1.4

Values are mean ± s.e.mean. *$P < 0.05$; **$P < 0.01$ compared to change seen in corresponding control group.

during ischaemia and one dog developed VF. Of the 10 dogs receiving allopurinol, 2 died from VF during occlusion and the number of extrasystoles in the 8 remaining dogs was slightly reduced (406 ± 189; $P = 0.074$). However, the distribution of extrasystoles at 1 min intervals (illustrated in Figure 2) shows that 4 of these dogs experienced fewer than 100 extrasystoles whereas none of the control group had as few arrhythmias as this. Furthermore, only 2 of the 8 dogs treated with allopurinol experienced VT (25%; $P > 0.1$).

Release of the occlusion around the LAD 40 min after the onset of ischaemia resulted in reperfusion

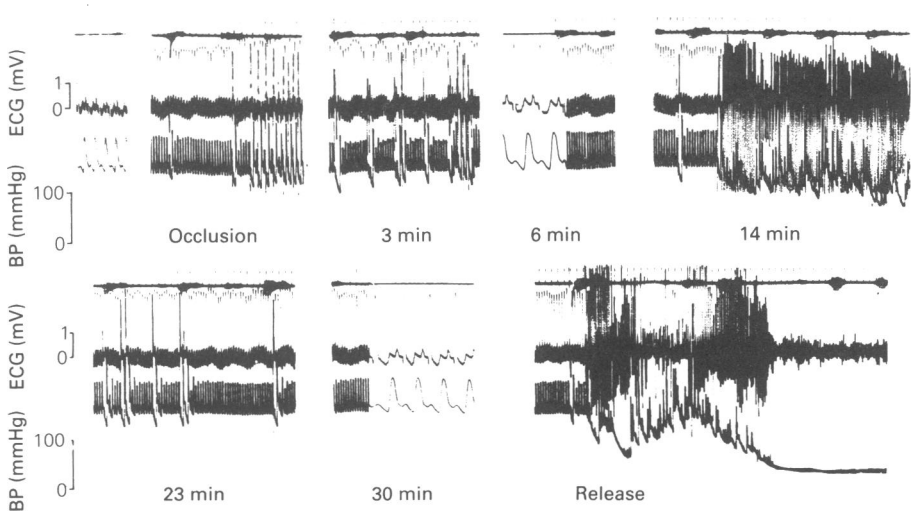

Figure 1 Original tracing illustrating the events following coronary artery occlusion and reperfusion in a control anaesthetized greyhound. The upper panel is the ECG (lead II) and the lower trace the arterial pressure (BP). The time tracing at the top of the figure represents 1 s intervals. The coronary artery was occluded at time zero and reperfused after 40 min of occlusion (release).

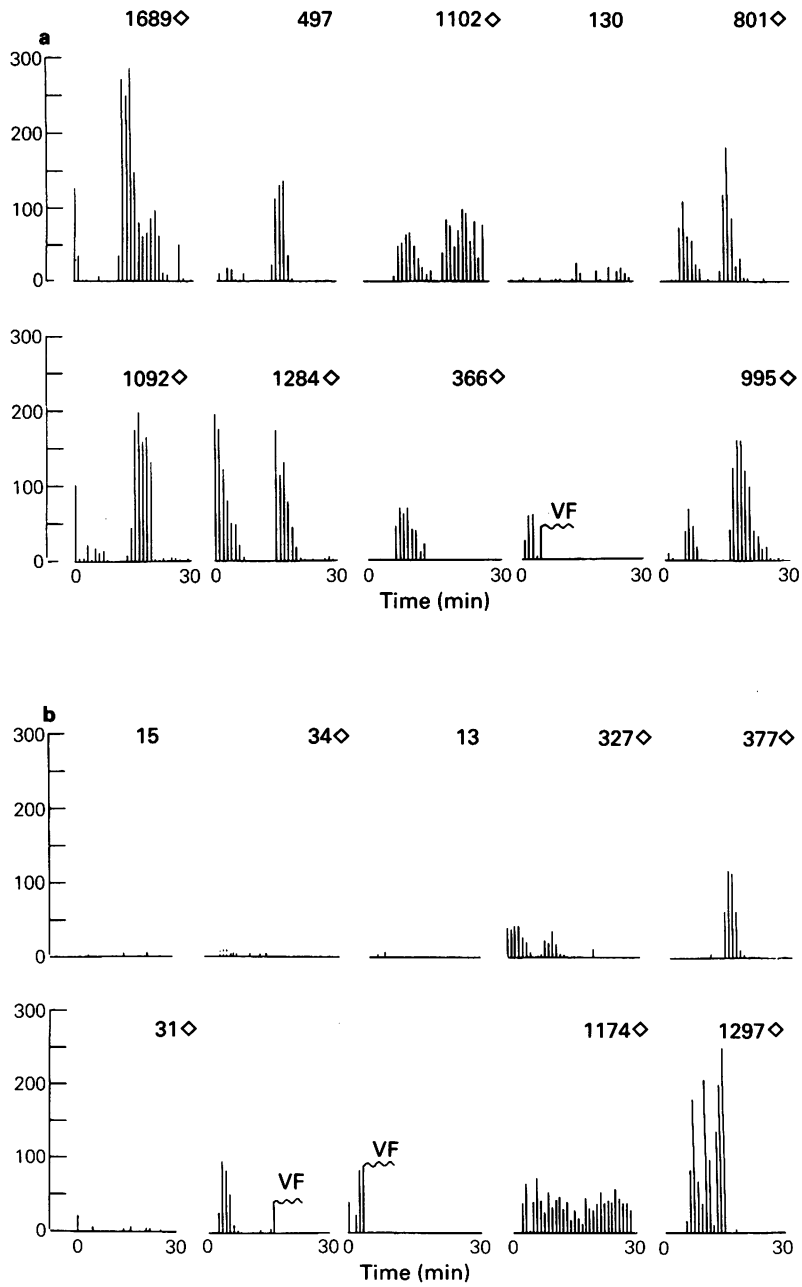

Figure 2 Distribution of extrasystoles over the 30 min occlusion period in 10 individual control dogs (a) and 10 dogs given allopurinol (b). The numbers represent the total number of extrasystoles in the 30 min occlusion period experienced by each dog and the open diamonds signify those dogs which died from ventricular fibrillation following reperfusion.

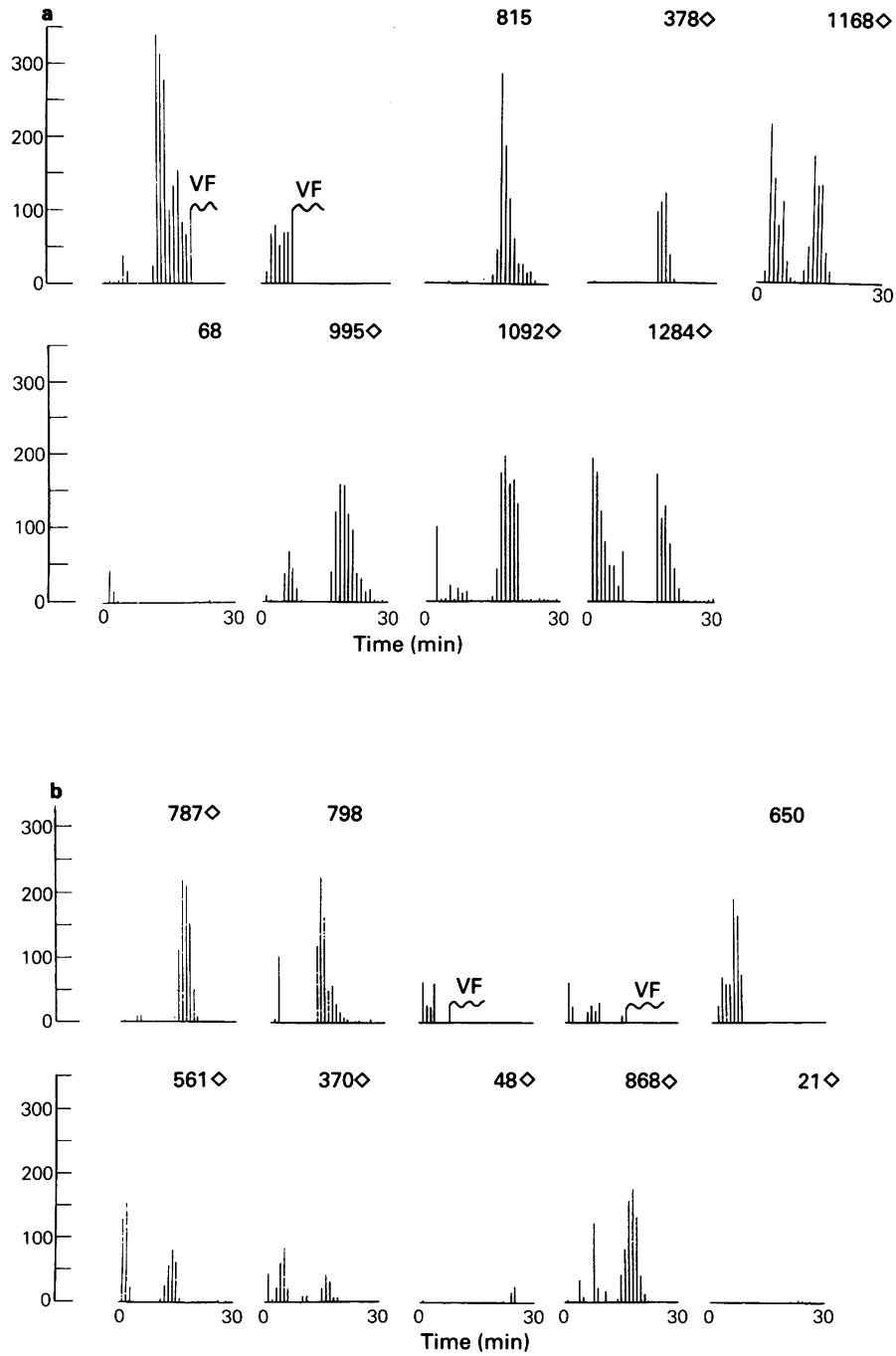

Figure 3 Distribution of extrasystoles over the 30 min occlusion period in 9 individual control dogs (a) and 10 dogs given N-t-butyl-α-phenyl nitrone (b). The numbers represent the total number of extrasystoles in the 30 min occlusion period experienced by each dog and the open diamonds signify those dogs which died from ventricular fibrillation following reperfusion.

arrhythmias commencing within seconds of perfusion to the ischaemic myocardium being restored. Reperfusion-induced arrhythmias were more severe than those seen during ischaemia and frequently consisted of multifocal tachycardia degenerating into ventricular fibrillation (Figure 1). In 6 of the remaining 9 control dogs, reperfusion resulted in VF within 1 min of release of the ligature. One other dog fibrillated 16 min after reperfusion and 2 survived the remainder of the experiment. Of the 8 remaining dogs which had received allopurinol, 3 died immediately on reperfusion, one after 7 min and one after 16 min giving 3 survivors (Figure 2). The overall survival from the combined occlusion-reperfusion insult was unchanged from 20% in controls to 30% in the allopurinol treated dogs.

Effects of N-t-butyl-α-phenyl nitrone In the second group of saline control dogs there was a similar pattern of arrhythmias. Two died in VF during occlusion and the mean number of extrasystoles in the 7 dogs still alive at the end of the occlusion period was 829 ± 169. Again, this was only slightly reduced to 513 ± 118 ($P = 0.14$) in the 8 remaining PBN dogs. The distribution of extrasystoles in these 2 groups of dogs is illustrated in Figure 3. Further, in the PBN treated dogs 5/8 (63%) experienced VT during occlusion compared to 1/7 (72%; $P > 0.7$) in the controls and two of the treated dogs fibrillated during occlusion. Following reperfusion, 4 of the controls developed VF within 1 min, 1 fibrillated at 15 min and 2 survived. A similar pattern was seen with PBN on reperfusion—3 of the dogs still alive at the end of the occlusion period fibrillated immediately on reperfusion and a further 3 developed VF 5–10 min later. The overall survival in controls was unchanged from 23% to 20% in the PBN group.

Lactate measurements

Table 6 illustrates the lactate levels in local coronary venous and aortic blood at various times during the occlusion-reperfusion procedure in both control dogs and in dogs given PBN. In controls there was a rise in lactate levels with time during occlusion and a very marked increased in levels in local coronary venous blood on reperfusion, presumably due to 'washout' of metabolic products. Changes, of a similar magnitude to those seen in controls, were also seen in dogs given PBN.

Table 6 Plasma lactate levels during coronary artery occlusion (at 0 min) and reperfusion (at 40 min) in control dogs and dogs administered PBN (50 mg kg^{-1})

	Plasma lactate concentration (mmol l^{-1})	
Time (min)	*Control*	*PBN*
Coronary venous		
−15	0.39 ± 0.12	0.69 ± 0.24
+ 2	1.05 ± 0.32	1.70 ± 0.48
+ 7	1.62 ± 0.39	1.97 ± 0.43
+15	2.07 ± 0.77	1.95 ± 0.47
+30	1.15 ± 0.24	1.62 ± 0.44
+41	3.65 ± 1.32	3.37 ± 0.65
Arterial		
−15	0.69 ± 0.17	0.73 ± 0.19
+30	0.77 ± 0.28	1.10 ± 0.37

Values are mean ± s.e.mean.

Discussion

While free radicals are strongly implicated in the production of myocardial reperfusion damage the evidence for the involvement of the xanthine oxidase system appears to be somewhat contradictory. Preliminary studies suggested that inhibition of the system with allopurinol is protective (Akizuki *et al.*, 1984; Werns *et al.*, 1985) whereas a recent study by Reimer & Jennings (1985) demonstrated no reduction in infarct size in a canine model of ischaemia-reperfusion damage. Further, while xanthine oxidase appears to play an important role in the reperfusion damage of other tissues (McCord, 1985), it has been argued that the activity of this enzyme in the canine heart is relatively low (Myers *et al.*, 1985). Although this may be so under normal conditions, the activity of the enzyme has been shown to be increased almost four fold in the canine myocardium during ischaemia (Downey *et al.*, 1984).

The results of this study have demonstrated that the inhibition of xanthine oxidase, and thus presumably the production of the superoxide radical as a by-product, was unable to protect against the serious life-threatening arrhythmias which occur during a combined occlusion-reperfusion insult in greyhound dogs, despite a mild, but insignificant, reduction in the number of ventricular extrasystoles and in the incidence of VT. These results with allopurinol are in contrast to the study by Manning *et al.* (1984) in which allopurinol, at a similar dose to that used in the present study, was shown to reduce markedly reperfusion-induced VF in anaesthetized rats subjected to coronary artery ligation. It is possible that these differences in findings could be explained by species differences, since the rat myocardium has high xanthine oxidase activity. However, it is also possible that xanthine oxidase may not be either partly or wholly responsible for the damage and arrthymias which occur during

ischaemia and reperfusion. This does not discount free radicals *per se* as there are several different sources of free radicals which may be associated with ischaemia. These are as follows:

(i) dissociation of the intramitochondrial transport system which may result in the generation of superoxide and hydrogen peroxide (Boveris & Chance, 1973). It has been suggested that this free radical activation is dependent on pH (Hess *et al.*, 1981) and occurs with prolonged periods of acidosis seen in the ischaemic myocardium. The result of this would be a breakdown of the excitation-coupling system which may result in conduction defects.

(ii) Free radicals are thought to be produced during the autoxidation of catecholamines (Cohen & Heikkila, 1974). Catecholamines have been associated with the development of myocardial necrosis for some time (Rona *et al.*, 1963). Therefore, it has been suggested that it is the oxidation products (e.g. free radicals and lipid peroxides), rather than the catecholamines themselves, which are responsible for the myocardial membrane changes occurring during ischaemia (Yates & Dhalla, 1975) which, in turn, result in structural damage and arrhythmias.

(iii) Lipid peroxidation resulting from free radical activation results in the release of products, such as arachidonic acid, into the extracellular space. The involvement of free radicals here is two fold; firstly the free radical-induced release of arachidonic acid and subsequent formation of prostaglandins and endoperoxides may further aggravate the injury process or generate arrhythmias (Coker *et al.*, 1981; 1982); secondly there is evidence that the conversion of prostaglandin G_2 (PGG_2) to PGH_2 is associated with the production of oxygen free radicals (Kuehl *et al.*, 1979).

(iv) Activated leukocytes, which may begin to infiltrate the myocardium within 1 h of the onset of ischaemia (Mullane *et al.*, 1984), release, among other things, oxygen radicals and products of the arachidonic acid cascade which results in the disruption of Ca^{2+} transport in the cardiac sarcoplasmic reticulum.

It was with these mechanisms in mind that the present study involved a determination of the effects of PBN, a spin trapping agent on arrhythmias. PBN has been demonstrated to 'trap' the radicals formed by *in vivo* metabolism of carbon tetrachloride (Poyer *et al.*, 1980), halothane (Payer & McCay, 1981) and 3-methylindole (Kubow *et al.*, 1984) at doses similar to those used in the present study. Further, it has been demonstrated to reduce mortality during endotoxin shock in rats (McKechnie *et al.*, 1986), a situation in which there is a great deal of evidence for the involvement of oxygen-derived free radicals. PBN is thus able to scavenge radicals produced by both intracellular (e.g. catecholamine metabolism) and extracellular (e.g. activated neutrophils) sources. The results presented here have shown that this did not reduce the incidence of reperfusion-induced VF. Other studies with 'anti-free radical' interventions such as superoxide dismutase, catalase and reduced glutathione (Woodward & Zakaria, 1985), methionine or desferrioxamine (Bernier *et al.*, 1986) have been performed in rat isolated heart preparations and have proved effective. A recent study with the antioxidant enzymes superoxide dismutase and catalase have suggested some protection *in vivo*, although only within a very narrow dose range (Riva *et al.*, 1986). As these positive results have been observed in rats it is perhaps an indication of species differences.

The evidence for a role of free radicals in the initiation of reperfusion injury to the myocardium is becoming more plentiful and more convincing, while that for the involvement of radicals in the genesis of arrhythmias is very scant. The results of this study suggest that, although radicals may play an important role in the development of damage, their part in causing arrhythmias, in this model at least, is probably relatively minor compared to other suggested factors such as catecholamines (Riemersma & Dart, 1985) and thromboxane A_2 (Coker *et al.*, 1982).

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