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Repeated Cardiac Pacing Extends the Time During Which Canine Hearts are Protected Against Ischaemia-induced Arrhythmias: Role of Nitric Oxide

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A. Kis, Á. Végh, J. G. Papp and J. R. Parratt. Repeated Cardiac Pacing Extends the Time During Which Canine Hearts are Protected Against Ischaemia-induced Arrhythmias: Role of Nitric Oxide. *Journal of Molecular and Cellular Cardiology* (1999) 31, 1229–1241. Right ventricular pacing in lightly anaesthetized dogs (4 × 5 min periods at a pacing rate of 220 beats/min) protects against the consequences of coronary artery occlusion when this is initiated 24 h after the pacing stimulus. The main purpose of the present experiments was to determine whether repeating the pacing stimulus, at a time when protection from the initial stimulus had faded (48 h), prolonged the protection afforded against ischaemia-induced ventricular arrhythmias and other ischaemic changes (epicardial ST-segment mapping; changes in the degree of electrical inhomogeneity in the ischaemic region). Dogs were paced on two occasions, with a 48 h period between and, at different times (48, 72 and 96 h) after the second pacing stimulus, were re-anaesthetized and subjected to occlusion of the left anterior descending coronary artery. There was a marked reduction in the severity of ischaemia-induced arrhythmias 48 and 72 h after the second pacing stimulus (reduction in occlusion-induced and reperfusion-induced ventricular fibrillation, e.g. at 72 h 0/11 during occlusion and only 3/11 following reperfusion, compared to 7/21 and 10/21 respectively in the controls; $P<0.05$). The protection had disappeared 96 h following the second pacing stimulus. Changes in ST-segment elevation and in the degree of inhomogeneity largely followed these changes in the severity of ventricular arrhythmias. The results suggest the possibility of maintaining protection against life-threatening arrhythmias following coronary occlusion by repeating a preconditioning pacing stimulus. We also demonstrate that this prolonged protection afforded by repeated cardiac pacing is mediated by nitric oxide, since the marked antiarrhythmic effect observed, e.g. 72 h after the second pacing stimulus, was abolished when S-(2-aminoethyl)-isothiourea (AEST), a particularly selective inhibitor of iNOS, had been administered before coronary artery occlusion.

Key Words: Cardiac pacing; Ischaemia; Arrhythmias; Nitric oxide; Aminoethylisothiourea.

Introduction

One of the consequences of regular exercise is a reduction in the relative risk of sudden cardiac death and of non-fatal myocardial infarctions which result from coronary artery occlusion (Mittleman *et al.*, 1993; Tofler *et al.*, 1996). However, the conclusion that "the protective effects of exercise requires continued exertion" implies that the duration of the protection is short-lived (Lee *et al.*, 1995). In the experimental situation, both exercise training in conscious dogs (Hull *et al.*, 1994) and right ventricular pacing in anaesthetized dogs (Végh *et al.*, 1991, 1994; Kaszala *et al.*, 1996) and in conscious

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rabbits (Szekeres *et al.*, 1993; Szilvássy *et al.*, 1994, 1997) markedly reduce the consequences of ischaemia as assessed by haemodynamic and endocardial ST-segment changes and by the severity of ventricular arrhythmias. This protection is both acute, occurring within minutes of the pacing stimulus, and delayed, returning around 20–24 h after the stimulus. This pacing-induced acute and delayed cardioprotection is similar to that achieved by short and complete occlusions of a coronary artery, a phenomenon known as ischaemic preconditioning (Murry *et al.*, 1986; Kuzuya *et al.*, 1993; Marber *et al.*, 1993; Baxter *et al.*, 1994 and recently reviewed by Parratt and Szekeres, 1995 and by Ferdinandy *et al.*, 1998).

Little attention has been paid to the time course of this delayed protection although, like the early protection afforded by classical preconditioning, it is relatively short-lived. For example, in a recent study of pacing-induced cardioprotection, Kaszala *et al.* (1996) showed that protection against ventricular arrhythmias following coronary artery occlusion was present 20–24 h after a pacing stimulus but was lost by 48 and 72 h. The primary aim of the present investigation was to determine if, by repeating the preconditioning stimulus at a time when the delayed protection afforded by the initial stimulus had faded, it is possible to prolong the time period during which the heart was relatively resistant to acute myocardial ischaemia. We have also determined, in view of our previous work implicating nitric oxide (NO) in the acute (Végh *et al.*, 1992a) and delayed (Végh *et al.*, 1994) antiarrhythmic effects of ischaemic preconditioning, whether prolonged protection by cardiac pacing is mediated by NO. For this we used S-(2-aminoethyl)-isothiourea (AEST) which is a particularly selective inhibitor of NO production by induced nitric oxide synthase (Southan *et al.*, 1995; Wolfárd *et al.*, 1997). In brief, we find that repeating the stimulus prolongs the period of protection, and that this protection is abrogated by a selective inducible nitric oxide synthase (iNOS) inhibitor.

Materials and Methods

We used mongrel dogs of both sexes with a body weight in excess of 17 kg, since severe ventricular arrhythmias resulting from coronary artery occlusion are relatively infrequent in small dogs (Végh *et al.*, 1992b). The dogs were lightly anaesthetized by the intravenous administration of sodium pentobarbitone and were allowed to breathe spontaneously. A Cordat F4 bipolar pacing electrode

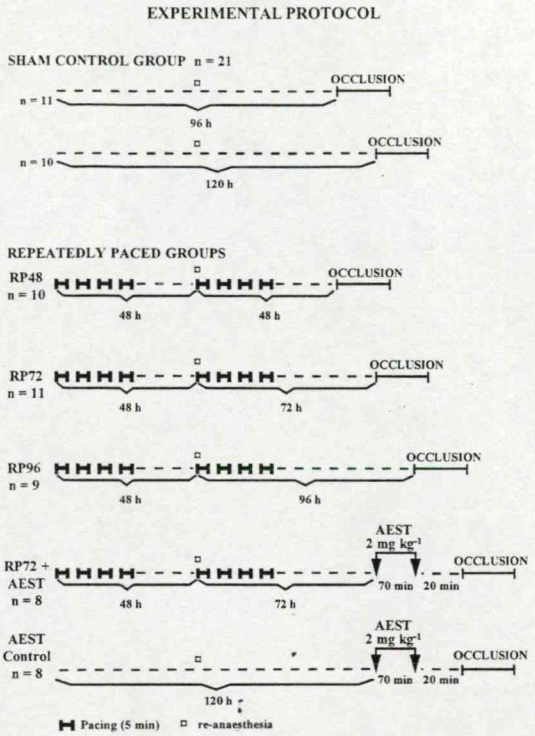


Figure 1 Experimental protocol for the repeat paced dogs. The interval between the two periods of pacing was 48 h and the interval between the end of the second pacing period and the occlusion was either 48, 72 or 96 h. Sham control dogs also had the pacing electrode inserted in the right ventricle, either for a period of 96 h ($n=11$) or 120 h ($n=10$). In a separate group of dogs subjected to either repeat pacing 72 h before occlusion ($n=8$) or sham operation without pacing ($n=8$), S-(2-aminoethyl)-isothiourea (AEST) was administered in a total dose of 2 mg/kg over a period of 70 min by intravenous infusion, terminating 20 min prior to ischaemia.

was introduced, by way of the right jugular vein, into the right ventricle such that it made contact with the ventricular endocardium. The correct position of this electrode was confirmed by recording the endocardial electrocardiogram. Blood pressure was monitored from the left carotid artery. The dogs were then paced, at a rate of 220 beats/min for four 5-min periods, with 5-min rest periods between the pacing stimuli.

The dogs were allowed to recover from the anaesthetic with the pacing catheter remaining *in situ* and 48 h later [a time when protection for a single pacing stimulus is no longer apparent (Kaszala *et al.*, 1996)]: the dogs were again anaesthetized and, as described above, subjected to a repeat of the same pacing stimulus (i.e., 220 beats/min for four periods of 5 min). Then, at various times (48, 72 and 96 h; Fig. 1) after this second

pacing stimulus the dogs were re-anaesthetized with a mixture of chloralose and urethane (60 and 200 mg/kg respectively), thoracotomized and subjected to a 25-min occlusion of the left anterior descending coronary artery (LAD) as described previously (Végh *et al.*, 1992b). The effects of coronary artery occlusion were compared with those in dogs 24, 48 and 72 h after a single pacing stimulus (4 × 5 min at 220 beats/min) the results of which have been described previously (Kaszala *et al.*, 1996).

The controls for these paced dogs were those in which the pacing electrode was positioned in the right ventricle, as described above, but these dogs were not subjected to pacing (Fig. 1). Forty-eight hours later these control dogs were again anaesthetized with sodium pentobarbitone but again these dogs were not paced. Then, a further 48 or 72 h later (i.e., 96 and 120 h, respectively, after the initial insertion of the catheter) these dogs were re-anaesthetized with chloralose and urethane as described above, thoracotomized and also subjected to occlusion of the left anterior descending coronary artery. Since there was no difference between the response of these two control groups to the effects of coronary artery occlusion these two groups were combined.

In order to determine the role of nitric oxide generation from iNOS in the protection afforded by repeated pacing we investigated, in eight repeat-paced dogs (i.e., dogs paced for a second time 72 h later) and in eight control (sham-operated, non-paced) dogs, the effects of the selective iNOS inhibitor S-(2-aminoethyl)-isothiourea (AEST; Southan *et al.*, 1995; Wolfárd *et al.*, 1997a, b). This was given in a total dose of 2 mg/kg by intravenous infusion over a 70-min period. It has been shown that this dose of AEST, given in dogs in which endotoxaemia was induced by *Escherichia coli* endotoxin, markedly inhibited the elevated iNOS activity (Wolfárd *et al.*, 1997a,b). Since there is some evidence that AEST, at physiological pH, undergoes intramolecular rearrangement, resulting in mercaptoethylguanidine (MEG) which, together with AEST, inhibits iNOS activity (Southan *et al.*, 1996), the infusion was terminated 20 min prior to coronary artery occlusion in order to provide adequate time for this chemical conversion (Fig. 1).

In all dogs, prior to coronary artery occlusion, catheters were inserted into the right femoral artery for monitoring arterial blood pressure, into the left ventricle for the measurement of left ventricular pressure and dP/dt and into the right femoral vein for drug and anaesthetic administration. A composite electrode was sutured to the left ventricular wall in the area distal to the proposed site of the coronary artery occlusion and was used to measure epicardial ST-segment elevation and the degree of inhomogeneity of electrical activation as described previously (Williams *et al.*, 1974; Végh *et al.*, 1992b). The composite electrode gives a summarized recording of R-waves from 30 epicardial measuring sites. In the normal, adequately perfused and oxygenated left ventricular wall, all sites are activated almost simultaneously resulting in a single large spike. However, during ischaemia widening and fractionation of the summarized R-waves occurs indicating that adjacent fibres have not been activated simultaneously because of inhomogeneity of conduction. We expressed inhomogeneity and delay of conduction as the greatest delay in activation (in ms) within the ischaemic area. All the parameters, together with a standard limb lead electrocardiogram, were recorded on a Graphtec Thermal Array Recorder (Hugo Sachs, Germany).

Ventricular arrhythmias during ischaemia resulting from coronary artery occlusion and subsequent reperfusion were analysed as previously described (Végh *et al.*, 1992b; Kaszala *et al.*, 1996). This analysis is based on suggestions made at the "Lambeth Conventions" (Walker *et al.*, 1988) except that no distinction was made between couplets and salvos, which were included as single ventricular ectopic (premature) beats (VPBs), and that we defined ventricular tachycardia (VT) as a run of four or more ectopic beats at a rate faster than the resting sinus rate. We also estimated the number of episodes of ventricular tachycardia which occurred in each dog, as well as the incidences of ventricular tachycardia (VT) and ventricular fibrillation (VF) both during occlusion and on reperfusion at the end of the 25 min occlusion period. Survival indicates those dogs that were predominantly in sinus rhythm 5 min after reperfusion.

At the end of the experiment the area at risk was assessed by infusing patent blue V dye into the occluded artery at a pressure equivalent to that of mean arterial pressure. This area at risk was expressed as a percentage of the left ventricular wall, together with the septum.

The data were expressed as means ± SEM and differences between means were compared by analysis of variance or by the Student's *t*-test. A one-way analysis of variance (ANOVA) was used to determine haemodynamic differences between the groups. For arrhythmias we used the Mann–Whitney U-test and for comparison of the incidences of VT and VF, and survival from the combined ischaemia–reperfusion insult, we used the Fisher

Exact test. Differences between groups were considered significant when $P<0.05$.

Although these experiments were carried out in Szeged, the protocol complied with UK Home Office Regulations (Project Licence No. 60/00307).

Preliminary accounts of these studies have been given to various meetings of the International Society for Heart Research (Kis *et al.*, 1996) and to the Physiological Society in Cambridge in December 1997 (Kis *et al.*, 1998b).

Results

Haemodynamic and electrocardiographic effects of cardiac pacing and of iNOS inhibition

The initial pacing stimulus, which increased the heart rate from a mean of 169 ± 14 beats/min to 220 beats/min, decreased mean arterial blood pressure from 141 ± 4 mmHg to 112 ± 6 mmHg ($P<0.01$) immediately after commencing the pacing stimulus. These changes in arterial pressure were similar when the pacing stimulus was repeated 48 h later. The mean arterial pressure prior to the second period of pacing was 110 ± 4 mmHg, a value significantly ($P<0.05$) less than the pressure prior to the initial pacing stimulus; again, it was reduced by pacing (to 82 ± 4 mmHg; $P<0.05$). Pacing also resulted in significant increases in the ST-segment recorded from the endocardial (pacing) electrode; these were similar after each of the four 5-min pacing stimuli (from 1.1 ± 0.5 mV to 3.2 ± 0.6 mV after the first 5-min pacing period, to 3.3 ± 0.8 mV after the second and third stimuli and to 3.3 ± 0.5 mV after the fourth 5-min pacing period. All these values were significantly higher than the ST-segment immediately prior to pacing ($P<0.01$). When the pacing stimulus was repeated 48 h later ("repeat pacing") the changes in the endocardial ST-segment were similar, but slightly more marked (from 1.6 ± 0.4 mV to 4.5 ± 1.0 mV; 4.2 ± 1.2 mV; 4.9 ± 1.5 mV; and 4.9 ± 1.5 mV after the pacing periods 1–4, respectively).

There were no differences in any haemodynamic parameters between the paced dogs 48 or 72 h after the second period of pacing. These parameters were also similar to those in control, sham-paced dogs (Table 1). At 96 h after the repeat period of pacing the arterial pressure was somewhat lower [i.e., 118 ± 4 mmHg (systolic), 76 ± 5 mmHg (diastolic) and 90 ± 4 mmHg (mean)], values significantly lower ($P<0.05$) than those found in dogs either 48 or 72 h after the second pacing stimulus (Table 1).

AEST itself had no significant effect on any haemodynamic parameters measured either during or after cessation of the infusion (Table 2).

Haemodynamic effects of coronary artery occlusion

These are also summarized in Table 1. Coronary artery occlusion resulted in transient decreases in systemic arterial pressure, heart rate and left ventricular dP/dt and significant increases in left ventricular end-diastolic pressure (LVEDP). These changes were similar whether the dogs had been paced or not (Table 1), and were not modified by AEST (Table 2).

Ventricular arrhythmias resulting from coronary artery occlusion and their modification by pacing

In the 21 control dogs, coronary artery occlusion resulted in marked ventricular ectopic activity; the distribution of these arrhythmias is illustrated in Figure 2. Periods of ventricular tachycardia (VT) occurred in most of these dogs and in seven this progressed to ventricular fibrillation (VF) mainly in the later (1b) phase of the occlusion period (i.e., after 10–15 min of arrhythmia). Only four of the 21 control dogs (19%) survived reperfusion. Because there was no significant difference in the responses of coronary artery occlusion of these sham-control dogs, whether a catheter had been inserted for 96 or 120 h (data not shown), these two groups were combined.

When the coronary artery occlusion had been preceded 48 or 72 h earlier by a second period of cardiac pacing there was marked suppression of ventricular ectopic beats (from a total of 215 ± 58 in the controls to 87 ± 31 and 101 ± 49 respectively). The distribution of these arrhythmias is also shown in Figure 2. Ventricular tachycardia (VT) occurred in only four of these 21 repeat-paced dogs (10 of the 48 h and 11 of the 72 h groups); and there were only a few episodes of VT per dog (0.6 ± 0.4 and 1.6 ± 1.5 compared to 4.9 ± 1.6 in the controls; $P<0.05$). None of these dogs fibrillated during the occlusion period ($P<0.05$ compared to controls). On reperfusion, ventricular fibrillation occurred in five out of 10 of the dogs subjected to repacing 48 h previously and in three out of 11 in those repaced 72 h earlier. The survival from the combined arrhythmia–reperfusion insult was thus 50% and 73% ($P<0.05$ compared to controls) respectively in those dogs repaced 48 and 72 h previously. The protection had disappeared when the

Table 1 Maximum haemodynamic changes induced by occlusion of the left anterior descending coronary artery in anaesthetized dogs at different times after repeated pacing

	Sham control ($n=21$)		RP48 ($n=10$)		RP72 ($n=11$)		RP96 ($n=9$)	
	Baseline	Change	Baseline	Change	Baseline	Change	Baseline	Change
SABP (mmHg)	131±6	−10±3*	140±5	−2±3	149±9	−11±4*	118±4	−7±4
DABP (mmHg)	89±4	−7±3*	92±4	−2±2	88±7	−5±3	76±5	−9±3*
MABP (mmHg)	103±4	−8±3*	108±4	−2±1	109±7	−7±3	90±4	−8±2*
LVSP (mmHg)	128±7	−11±4*	133±6	0±2	131±11	−7±3*	112±5	−5±5
LVEDP (mmHg)	9.0±2.0	3.4±2.0	7.0±1.0	6.0±1.0	7.0±1.0	4.0±1*	8.0±1.5	5.0±1*
LV $-dP/dt$ (mmHg/s)	2237±214	−360±219	2576±171	−299±98	2936±265	−355±304	2820±6.9	−563±467
LV $+dP/dt$ (mmHg/s)	3004±267	−326±107*	3592±275	−341±122	3295±191	−533±216*	2850±170	−343±162
HR (beats/min)	148±5	5.4±1.5*	164±9	4.2±1.4	146±10	0.6±1	149±5	1±1

Values are the means±SEM.
* $P<0.05$ *vs* baseline.

Table 2 Maximum haemodynamic changes following AEST (2 mg/kg total dose) administration and after coronary artery occlusion in anaesthetized dogs subjected to repeat cardiac pacing 72 h previously

	AEST ($n=8$)			Occlusion ($n=8$)		
	Initial value	End of infusion	Change	Initial value	3–5 min	Change
SABP (mmHg)	143±11	139±11	−4±6	132±11	114±8	−18±5*
DABP (mmHg)	96±8	91±9	−5±5	91±10	76±8	−15±6*
MABP (mmHg)	111±9	107±9	−5±5	105±11	89±8	−16±6*
LVSP (mmHg)	124±11	120±11	−3±7	120±11	101±9	−19±8*
LVEDP (mmHg)	4.1±0.4	4.4±0.4	0.3±0.3	4.3±0.4	8±1.0	3.7±0.8*
LV −dP/dt (mmHg/s)	2857±417	2845±390	−11±249	2398±239	1829±177	−569±162*
LV +dP/dt (mmHg/s)	4488±657	4286±682	−186±300	3737±485	2607±302	−1130±269*
HR (beats/min)	164±5	160±6	−4±3	154±5	154±3	0

Values are the means±SEM.
* $P<0.05$ *vs* initial value.

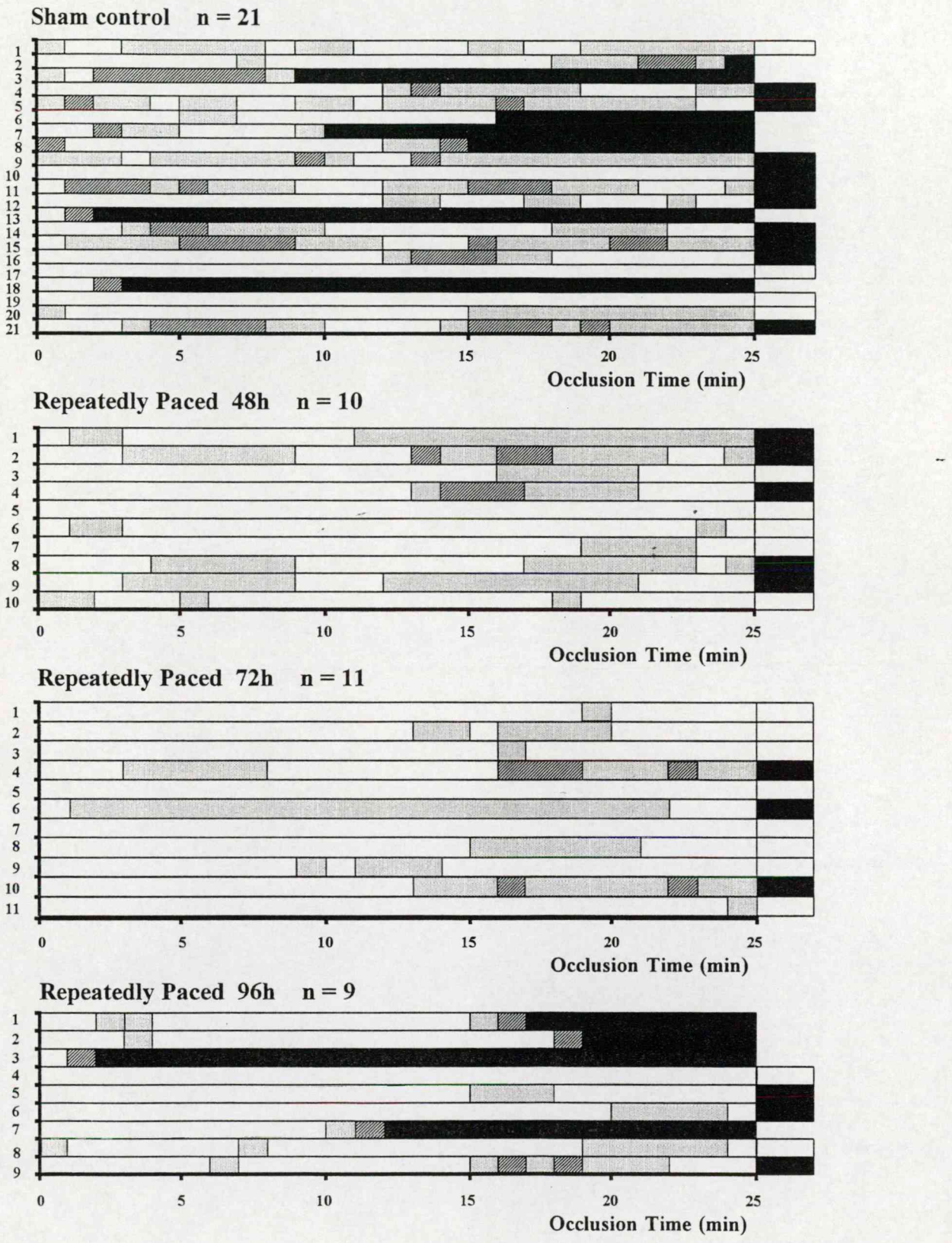
interval between the second period of pacing and the coronary artery occlusion was extended to 96 h (Fig. 2). At this time periods of VT occurred in five out of nine dogs and in four of these it progressed to VF. Only 2/9 (22%) of these dogs survived reperfusion, a figure not significantly different to that in the sham controls (19%).

This protection against ventricular arrhythmias resulting from acute coronary artery occlusion following repeat cardiac pacing persisted for a longer period than in dogs subjected to only a single period of pacing. This is illustrated in Figure 3 which compares the incidence of VF and the number of survivors from the combined arrhythmia–reperfusion insult in dogs subjected to a single pacing stimulus (data taken from Kaszala *et al.*, 1996) and to a double pacing stimulus when the coronary artery was occluded 48, 72 and 96 h later. This difference between single and repeat pacing is also illustrated in Figure 4, which compares the distribution of arrhythmias 72 h after pacing. These two figures (Figs 3 and 4) show that 48 and 72 h after a single period of pacing protection against VF, evident at 24 h, had disappeared, whereas at these times after a repeat period of pacing dogs were still markedly protected against occlusion and reperfusion-induced VF. Thus, repeating the pacing stimulus prolongs the period of protection during which the heart is resistant to both ischaemia and reperfusion-induced arrhythmias. This protection afforded by repeat pacing was not apparent when the coronary artery was occluded 96 h later (Fig. 3).

Changes in indices of ischaemia severity

The changes in epicardial ST-segment elevation and in the degree of inhomogeneity of electrical activation during coronary occlusion in dogs subjected to two periods of pacing and in control dogs is illustrated respectively in Figures 5 and 6. Epicardial ST-segment elevation was evident within the first minutes of occlusion and, in the sham-operated controls, peaked around 5 min and was maintained for the remainder of the occlusion period. In those dogs subjected to two periods of pacing, and in which the coronary occlusion was carried out 48, 72 and 96 h later, there was a marked and significant ($P<0.05$) reduction in ST-segment elevation from 3 min into the occlusion

Figure 2 The distribution of ventricular arrhythmias following coronary artery occlusion and reperfusion in 21 control dogs and in dogs that were subjected to repeated pacing 48, 72 or 96 h previously. The filled columns are the times during which the dog was in ventricular fibrillation, the shaded columns show periods of ventricular tachycardia and the lightly stippled columns are periods during which ventricular premature beats were evident. VF on reperfusion is also shown by the black horizontal columns and this was the only arrhythmia assessed during the reperfusion phase. The results show that in the controls ventricular fibrillation occured in 7/21 dogs and that a further 10 dogs fibrillated on reperfusion. There were thus only four survivors from the combined ischaemia–reperfusion insult. When the coronary artery was occluded 48 and 72 h after a repeated period of pacing (see experimental protocol), no dog fibrillating during the occlusion period, and there were 5/10 and 8/11 survivors respectively from the combined ischaemia–reperfusion. The protection was not apparent in dogs paced 96 h previously. See results section for detailed analysis.


Sham control n = 21
1
2
3
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5
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7
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9
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11
12
13
14
15
16
17
18
19
20
21
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5
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15
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25
Occlusion Time (min)
Repeatedly Paced 48h n = 10
1
2
3
4
5
6
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8
9
10
0
5
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15
20
25
Occlusion Time (min)
Repeatedly Paced 72h n = 11
1
2
3
4
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6
7
8
9
10
11
0
5
10
15
20
25
Occlusion Time (min)
Repeatedly Paced 96h n = 9
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Occlusion Time (min)

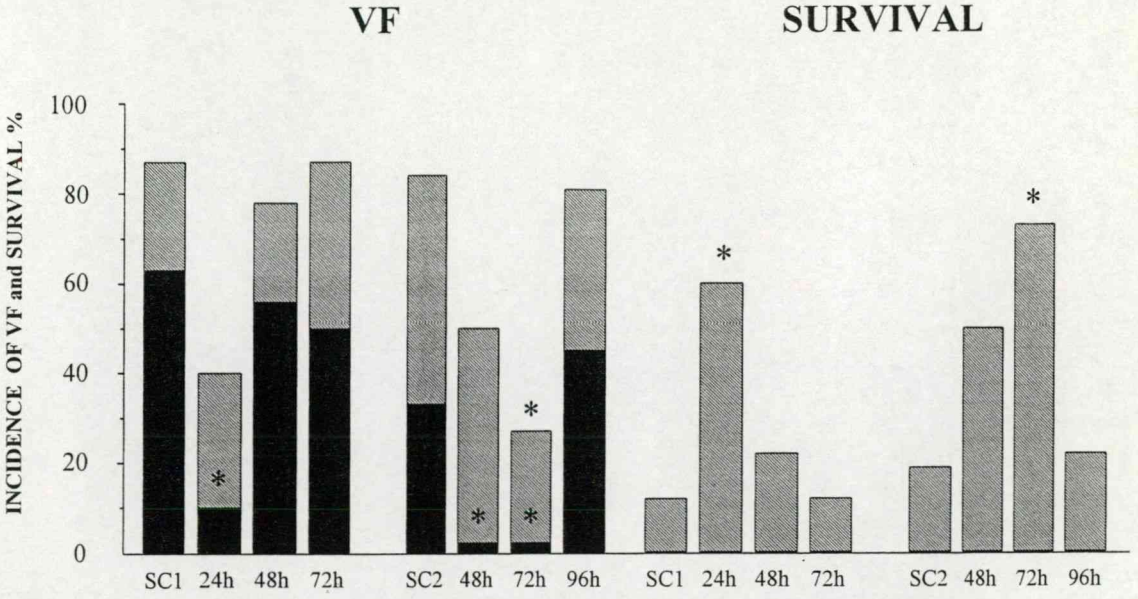

Figure 3 The incidence of ventricular fibrillation (VF) and survival from the combined ischaemia–reperfusion insult in control dogs (SC1: data taken from Kaszala *et al.*, 1996, and SC2: present experiments $n=21$), in dogs subjected to a single pacing stimulus (left hand columns: data taken from Kaszala *et al.*, 1996) or to a repeat pacing stimulus, and then subjected to coronary artery occlusion either 24, 48, 72 or 96 h after the end of the pacing stimulus. The results show that a single period of pacing protects dogs 24 h after the stimulus but that this protection is lost after 48 h. However, repeat pacing prolongs the protection for at least 72 h. The filled columns show VF during occlusion and the shaded columns VF during reperfusion. * $P<0.05$ *vs* controls.

period. Changes in the degree of electrical inhomogeneity during the occlusion (Fig. 6) in general followed the epicardial ST-segment changes. In dogs subjected to repeat pacing either 48, 72 and 96 h previously, inhomogeneity of activation was less pronounced than in the controls and again this was maintained throughout the entire occlusion period.

The severity of ventricular arrhythmias following inhibition of inducible nitric oxide synthase

In eight dogs subjected to repeat cardiac pacing, AEST, a selective inhibitor of iNOS activity was infused 72 h after the second pacing stimulus, but prior to coronary artery occlusion. The results are summarized in Figure 7, where the effects are compared with dogs in which the coronary artery was also occluded 72 h after the second pacing stimulus but without iNOS inhibition. The controls were dogs which had not been paced but in which the pacing catheter was present in the lumen of the right ventricle for 120 h (see protocol Fig. 1). Inhibition of iNOS prior to coronary artery occlusion abolished the protection associated with two periods of cardiac pacing. To determine whether AEST itself modified arrhythmia severity, eight dogs, that had not been paced, were given the drug before the 25 min occlusion of the LAD (see Fig. 1). Compared to the sham-operated controls, AEST did not modify arrhythmia severity occurring during coronary artery occlusion and following reperfusion (Fig. 7).

Discussion

Rapid ventricular (overdrive) pacing induces a form of cardioprotection similar to that achieved by brief periods of complete coronary artery occlusion (preconditioning) and, as demonstrated in both the dog and rabbit models (Szekeres *et al.*, 1993; Kaszala *et al.*, 1996), this pacing-induced protection follows the same characteristic time course as that achieved by brief periods of coronary artery occlusion. There is an immediate protection, evident within a few minutes of the cessation of the pacing stimulus (Végh *et al.*, 1991; Koning *et al.*, 1996) and akin to the "classical" preconditioning first described by Murry *et al.* (1986), and a second, delayed protection evident 20–24 h later (Szekeres *et al.*, 1993; Végh *et al.*, 1994). This delayed protection is also

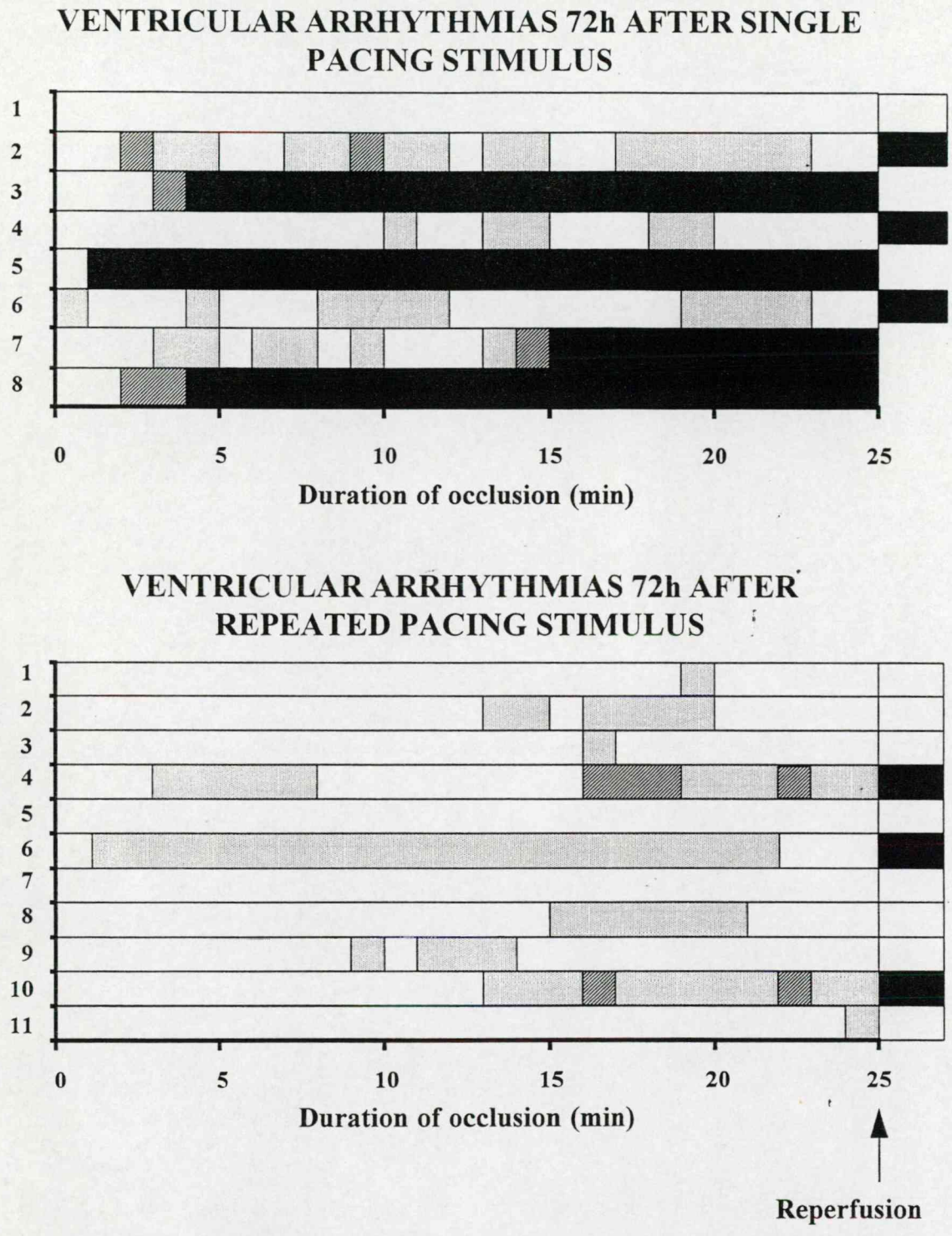

Figure 4 A comparison of ventricular arrhythmias following coronary artery occlusion 72 h after a single pacing stimulus (above: data adapted from Kaszala *et al.*, 1996) or a repeated pacing stimulus (below). The symbols are similar to those in Figure 2. Protection during occlusion (and reperfusion) are evident 72 h after the repeat pacing stimulus but not 72 h after a single pacing stimulus.

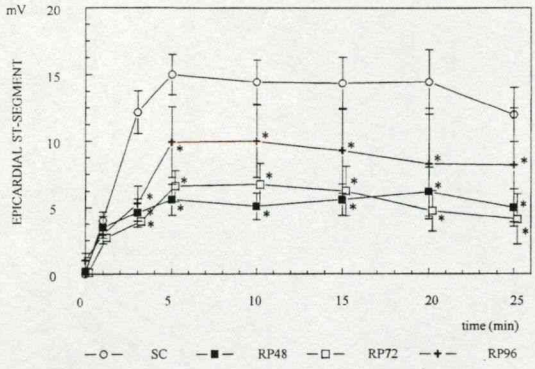

Figure 5 Changes in the epicardial ST-segment during a 25-min occlusion of the left anterior descending coronary artery in dogs subjected to two periods of rapid pacing 48 h (filled squares), 72 h (open squares) and 96 h (crosses) after the pacing stimulus. The degree of epicardial ST-segment elevation is markedly reduced in dogs subjected to two periods of cardiac pacing compared to controls (shown by the open circles). * $P<0.05$ compared to controls. All the changes are significant ($P<0.001$) compared to the pre-occlusion value.

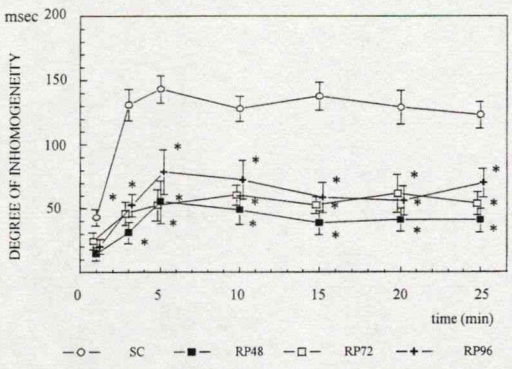

Figure 6 Changes in the degree of inhomogeneity of electrical activation within the ischaemic area (ms) during a 25-min occlusion of the left anterior descending coronary artery in dogs subjected to two periods of rapid pacing 48 h (filled squares), 72 h (open squares) and 96 h (crosses) after the pacing stimulus. The degree of inhomogeneity is markedly reduced in dogs subjected to two periods of cardiac pacing compared to control, sham-paced dogs (open circles). * $P<0.05$ compared to controls; all changes are significant ($P<0.01$) compared to pre-occlusion values.

seen if preconditioning is induced by brief periods of coronary artery occlusion (Yamashita *et al.*, 1992; Kuzuya *et al.*, 1993; Marber *et al.*, 1993; Baxter *et al.*, 1994, 1995; Yang *et al.*, 1996). The possible mechanisms of this delayed protection have been recently reviewed (Parratt and Szekeres, 1995; Yellon and Baxter, 1995). This delayed protection, like classical ischaemic preconditioning, is also transient. In the rapid pacing canine model described here the protection disappears 48 h after the preconditioning stimulus (Kaszala *et al.*, 1996); and this time course for the fading of the protection is similar to that observed following overdrive pacing in conscious rabbits (Szekeres *et al.*, 1993) or when preconditioning results from brief periods of coronary artery occlusion (Yellon and Baxter, 1995).

The purpose of the present experiments was to examine whether it is possible to extend the period of protection by repeating the preconditioning stimulus. That this is possible is clearly shown by the results. If a second, and similar, pacing stimulus is instigated 48 h after the first, that is at a time when the protection afforded by the initial preconditioning pacing stimulus has faded (Kaszala *et al.*, 1996), then the period of protection is extended such that even 3 days later there is still marked protection against ischaemia and reperfusion-induced ventricular arrhythmias (Figs 3 and 4). This protection had disappeared by 4 days after the second pacing stimulus but clearly the duration of the protection is greatly extended when compared to that resulting from a single preconditioning, pacing stimulus. We have yet to examine whether the particular degree of pacing we have used (220 beats/min, for a total period of 40 min) is optimum or whether one can prolong the protection still more by pacing at a higher frequency.

What are the mechanisms by which repeating the preconditioning pacing stimulus extends the duration of protection? One contributory factor must be that the severity of ischaemia during the 25 min coronary artery occlusion is decreased in dogs in which the pacing stimulus has been repeated. As in the single pacing studies (Kaszala *et al.*, 1996), there is some relation between reduction in arrhythmia severity and changes in the two indices we have used to assess ischaemia severity, i.e., epicardial ST-segment elevation and inhomogeneity of activation within the ischaemic area. As one might expect, this relationship between arrhythmias and these assessments of the degree of ischaemia is not precise but a comparison of ST-segment changes in single (Kaszala *et al.*, 1996; Fig. 7) and repeat paced dogs (Fig. 5 of the present paper) shows that ST-segment elevation following coronary artery occlusion is less in the repeat paced dogs and that this is maintained for a longer period of time. For example, in dogs paced only once coronary occlusion-induced epicardial ST-segment elevation 72 h after the pacing stimulus is no different to that seen in dogs that have not been paced; in contrast, 72 h after a second pacing stimulus ST-segment elevation is significantly less than that in the controls (Fig. 5). A similar conclusion can be

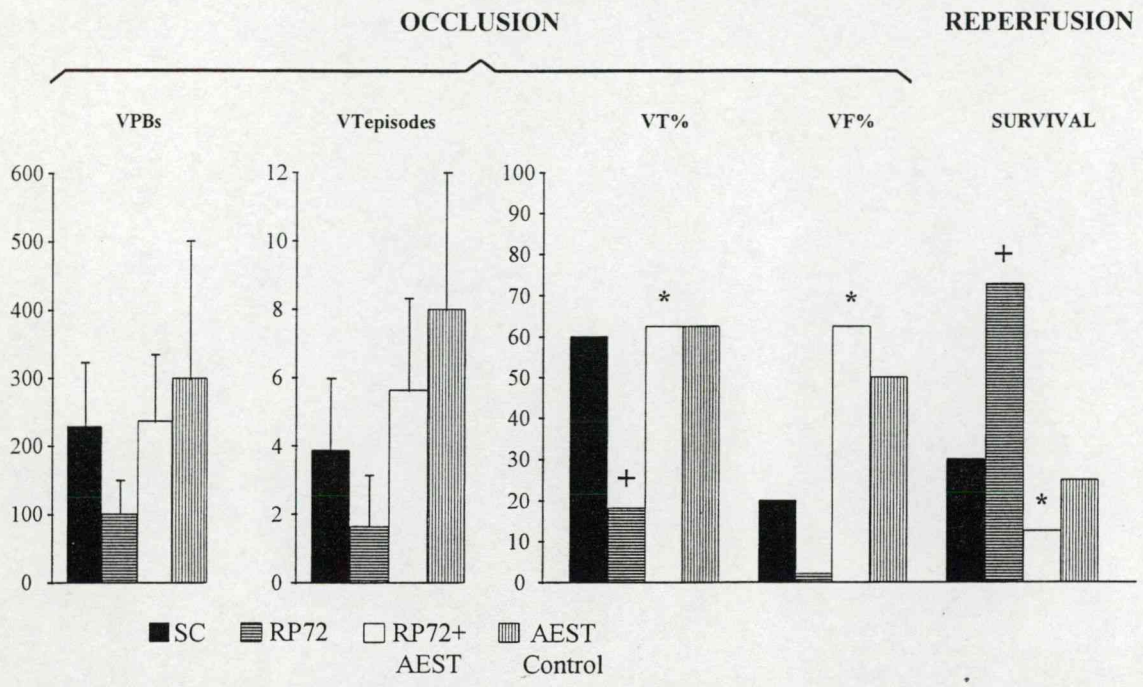

Figure 7 The number of ventricular premature beats (VPBs), the incidence (%) and number of episodes of ventricular tachycardia (VT), the incidence (%) of ventricular fibrillation (VF) during ischaemia and following reperfusion, and survival (%) from the combined coronary artery occlusion–reperfusion insult in sham control dogs in which the pacing catheter was left *in situ* for 120 h (closed histograms; $n=10$), in dogs subjected to repeated pacing 72 h earlier (cross-hedged histograms; $n=11$), in dogs also subjected to pacing but given the iNOS inhibitor S-(2-aminoethyl)-isothiourea (AEST) prior to the occlusion (open histograms; $n=8$), and in unpaced control dogs but given AEST before the occlusion (vertically striped histograms; $n=8$). * $P<0.05$ *vs* 72 h paced group; + $P<0.05$ compared to controls. NO inhibition abolishes the protection afforded by pacing 72 h previously.

reached when inhomogeneity of electrical activation within the area supplied by the occluded artery is used as an index of ischaemia severity. However, other factors must surely contribute to arrhythmia severity; at 96 h after a second preconditioning stimulus these two indices of ischaemia remained depressed yet the severity of ischaemia-induced arrhythmias is similar to that seen in control dogs.

Whatever the relationship is between arrhythmia severity and these imprecise measurements of the severity of ischaemia, a major factor in the protection afforded by repeated pacing seems to be NO production. This is most probably derived from induced NO synthase (iNOS) since the protection is abolished by a selective inhibitor of this enzyme. There is evidence that both AEST and mercaptoethylguanidine, which is formed from AEST following chemical conversion at physiological pH, selectively inhibit endotoxin-induced iNOS activity in rats (Southan *et al.*, 1996). There is other support for this conclusion that NO is involved in delayed protection. For example, both exercise (Matsumoto *et al.*, 1994) and pacing-induced tachycardia (Egashira *et al.*, 1996) increases coronary vascular NO formation and, more recently, Bolli *et al.* (1997) have shown that the enhanced recovery of contractile function, which is one of the consequences of delayed preconditioning, is not seen following inhibition of NO formation. Our own studies demonstrated that the protection against arrhythmias afforded by single periods of cardiac pacing is lost following dexamethasone administration (Végh *et al.*, 1994) which, among many other actions, inhibits the formation of NO through the induced enzyme (iNOS). Further, aminoguanidine, which is a relatively selective inhibitor of iNOS, also markedly attenuates this protection (Kis *et al.*, 1998a). Nitric oxide thus seems to be an important protective mediator in delayed preconditioning, as it is in classical preconditioning against arrhythmias (Végh *et al.*, 1992a and reviewed by Parratt and Végh, 1997b). Further evidence for a protective role for NO derived from iNOS in other forms of delayed

cardioprotection comes from studies with bacterial endotoxin (Wu *et al.*, 1994, 1996), which is a potent inducer of iNOS (Julou-Schaffer *et al.*, 1990) and with monophosphoryl lipid A, a relative non-toxic derivative of the lipid A component of the endotoxin molecule (Wu *et al.*, 1998). In animals so treated there is a greatly reduced incidence and severity of ventricular arrhythmias that arise when a coronary artery is abruptly occluded.

Similarities have been drawn (Parratt and Végh, 1997a) between the delayed protection afforded by cardiac pacing and that of exercise. There is prospective evidence that the relative risk of sudden cardiac death, presumably due to ventricular fibrillation, as well as of non-fatal myocardial infarctions is reduced in individuals who exercise regularly (Mittleman *et al.*, 1993; Tofler *et al.*, 1996), although the intensity of exercise required to induce this protection as well as its time course, are subjects of ongoing debate (Lee *et al.*, 1995; Tofler *et al.*, 1996). On the other hand, there is evidence that strenuous exercise may trigger a cardiac event in the immediate post-exercise period (Mittleman *et al.*, 1993; Willich *et al.*, 1993) and that men with a lifelong history of regular, and very strenuous, exercise have a higher incidence of complex arrhythmias, and perhaps a higher risk of sudden cardiac death, later in life. The conclusion from some of these studies that the protective effect of exercise against the consequences of acute myocardial ischaemia requires continued exertion (Tofler *et al.*, 1996) implies that the duration of the protection afforded by exercise is relatively short-lived. This is borne out by the fact that among sedentary individuals the relative risk of infarction decreases as the number of episodes of exercise undertaken per week increases (Tofler *et al.*, 1996). This phenomenon might be related to the present findings; increasing the number of preconditioning (pacing) stimuli (akin to exercise?) decreases the risk from ventricular fibrillation when a coronary artery is occluded. Whether increasing the number of pacing periods beyond two extends the protection even longer remains to be clarified.

In summary, we show that it is possible to prolong significantly, to at least 72 h, the ability of the heart to resist the effects of acute coronary artery occlusion by repeating a preconditioning pacing stimulus. This antiarrhythmic effect of pacing is probably the result of less severe ischaemic changes during coronary occlusion, as shown by less marked ST-segment elevation recorded from epicardial electrodes over the ischaemic area and by reduced inhomogeneity of electrical activation within this area in dogs that have been subjected to two periods of right ventricular pacing. Further, we show that this protection is mediated mainly through NO generation formed by iNOS.

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Pacing-induced delayed protection against arrhythmias is attenuated by aminoguanidine, an inhibitor of nitric oxide synthase

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1 Cardiac pacing, in anaesthetized dogs, protects against ischaemia and reperfusion-induced ventricular arrhythmias when this is initiated 24 h after the pacing stimulus. Now we have examined whether this delayed cardioprotection afforded by cardiac pacing is mediated through nitric oxide.

2 Twenty-two dogs were paced (4 × 5 min periods at 220 beats min^{-1}) by way of the right ventricle, 24 h prior to a 25 min period of coronary artery occlusion. Nine of these dogs were given the inhibitor of induced nitric oxide synthase, aminoguanidine (50 mg kg^{-1} i.v.), 0.5 h prior to coronary artery occlusion. Sham-operated non-paced dogs with and without aminoguanidine treatment served as controls.

3 Pacing markedly ($P<0.05$) reduced arrhythmia severity (ventricular fibrillation, VF, during occlusion 15%; survival from the combined ischaemia-reperfusion insult 62%) compared to control, sham-operated, unpaced dogs (VF during occlusion 58%; survival 17%). This protection was attenuated by the administration of aminoguanidine prior to coronary artery occlusion (survival from the combined ischaemia-reperfusion insult 11%, which was significantly ($P<0.05$) less than in the paced dogs not given aminoguanidine and similar to the controls). Aminoguanidine had no significant effects on coronary artery occlusion when given to dogs that had not been paced. In the dose used aminoguanadine transiently elevated systemic arterial pressure by a mean of 20 mmHg and reduced heart rate by a mean of 22 beats min^{-1}.

4 These results suggest that nitric oxide, probably derived from induced nitric oxide synthase, contributes significantly to the delayed cardioprotection afforded by cardiac pacing.

Keywords: Cardiac pacing; ischaemic preconditioning; nitric oxide; aminoguanidine; delayed cardioprotection; nitric oxide synthase

Abbreviations: AG, aminoguanidine; NO, nitric oxide; cNOS, constitutive nitric oxide synthase; iNOS, inducible nitric oxide synthase; LAD, left anterior descending coronary artery; VPBs, ventricular premature beats; VT, ventricular tachycardia; VF, ventricular fibrillation

Introduction

Rapid cardiac pacing in dogs results in both an immediate (Végh *et al.*, 1991) and delayed (Végh *et al.*, 1994; Kaszala *et al.*, 1996) protection against the early life-threatening arrhythmias that result from acute coronary artery occlusion. It has been argued (Kaszala *et al.*, 1996) that this is a form of ischemic preconditioning. The mechanisms of this delayed protection are unknown but there is some evidence that cardiac pacing (and exercise) may promote the formation of nitric oxide (NO) through nitric oxide synthase gene expression in endothelial cells (Zhao *et al.*, 1997) and in cardiac myocytes (reviewed by Parratt & Végh, 1997). Further, there is recent evidence in rabbits that when the preconditioning stimulus consists of brief periods of coronary artery occlusion there is delayed protection against myocardial stunning and that this is also triggered by the generation of NO (Bolli *et al.*, 1997a, b). Under these conditions there is also enhanced NO formation by the canine myocardium, as demonstrated by elevated nitrite and nitrate levels in coronary sinus blood 24 h after a 10 min preconditioning coronary artery occlusion (Kim *et al.*, 1997).

Although there is a good deal of evidence that, at least in dogs, NO is a key mediator in the antiarrhythmic effects of the early ('classical') phase of ischaemic preconditioning (Végh *et al.*, 1992a), there have been no studies concerned with the possible role of NO in mediating the delayed antiarrhythmic effects of ischaemic preconditioning, or of cardiac pacing. The purpose of the present study was therefore to examine whether the induced form of nitric oxide synthase (iNOS) is involved in the delayed antiarrhythmic effects of cardiac pacing by examining the effects of aminoguanidine, a reasonably selective inhibitor of iNOS (Corbett *et al.*, 1992; Griffiths *et al.*, 1993; Misko *et al.*, 1993; Kengatharan *et al.*, 1996) in a canine model in which the preconditioning stimulus was right ventricular pacing 24 h prior to coronary artery occlusion (Végh *et al.*, 1994; Kaszala *et al.*, 1996).

Methods

Animals and pacing procedure

Forty-two mongrel dogs, of either sex and with a mean body weight in excess of 17 kg, were anaesthetized by the intravenous administration of sodium pentobarbitone and were allowed to breathe spontaneously. A Cordat F4 bipolar pacing electrode was inserted, *via* the right jugular vein, into the right ventricle; the correct placing of this electrode was confirmed by recording the endocardial electrocardiogram. Blood pressure was monitored from the left carotid artery. Thirteen of these dogs were then paced for four 5 min periods, at a rate of 220 beats min^{-1} with 5 min resting intervals

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between the pacing stimuli (Végh *et al.*, 1994; Kaszala *et al.*, 1996). The twelve control (sham operated) dogs were those in which the pacing electrode was introduced into the right ventricle, left for the same period of time but these dogs were not paced. The remaining nine dogs, which were also paced as described above, were then given aminoguanidine, as the hemisulphate salt (Sigma; 50 mg kg^{-1} i.v.) 0.5 h prior to the coronary artery occlusion i.e. 24 h after the pacing stimulus. A further eight dogs were not paced but were given aminoguanidine at the same dose, 0.5 h prior to coronary occlusion. The dose of aminoguanidine has been selected on the basis of those studies performed in dogs which aimed to investigate the effect of aminoguanidine on the inducible nitric oxide synthase (Tárnoky *et al.*, 1996; Numata *et al.*, 1998). The experimental protocol is shown in Figure 1.

There were no significant differences between the four groups in respect to body weight (sham controls, 24±3 kg; paced dogs, 23±2 kg; paced dogs given aminoguanidine, 27±1 kg; and non-paced dogs given the drug, 25±4 kg).

Haemodynamic measurements

Those dogs subjected to pacing, as well as the sham-operated controls, were allowed to recover from the anaesthetic and 20–24 h later were re-anaesthetized with a mixture of chloralose and urethane (60 and 200 mg kg^{-1} i.v., respectively), ventilated with room air, thoracotomized and subjected to a 25 min occlusion of the left anterior descending (LAD) coronary artery as previously described (Végh *et al.*, 1992b; Kaszala *et al.*, 1996). Temperature was recorded from the mid-oesphagus and maintained at 37±0.5°C by means of a heating pad. Catheters were inserted into the right femoral artery (for monitoring blood pressure), the left ventricle (for the measurement of left ventricular pressure and dP/dt) and the right femoral vein for drug and anaesthetic administration. Epicardial ST-segment elevation and the degree of inhomogeneity of electrical activation were measured in the area supplied by the LAD coronary artery by means of a composite electrode (Végh *et al.*, 1992b). This electrode gives a summarized recording of R-waves from 30 epicardial measuring points. In the normal, well perfused and oxygenated, myocardium there is a single large spike because all sites are activated almost simultaneously. Following coronary occlusion, however, widening and fractionation of this summarized R-wave occurs since, because of the inhomogeneity of conduction in the ischaemic myocardium, fibres are not simultaneously activated. This inhomogeneity of conduction is expressed in the greatest delay in activation (in ms) within the ischaemic area underlying the composite electrode. All these parameters, together with a standard limb lead electrocardiogram, were recorded on a Graphtec Thermal Array Recorder (Hugo Sachs Electronics, Germany).

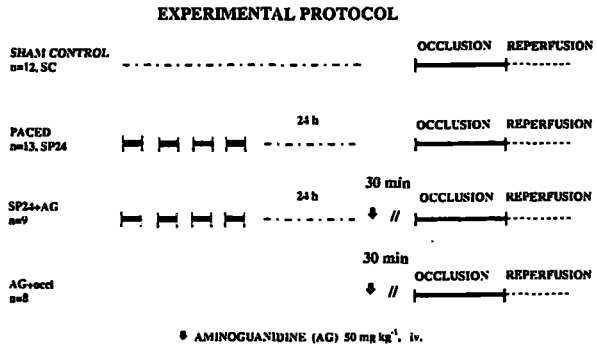

Figure 1 The experimental protocol outlining the procedures observed for the four groups of anaesthetized dogs.

Assessment of arrhythmias and area at risk

Ventricular arrhythmias during coronary artery occlusion and following reperfusion were assessed as previously described (Végh *et al.*, 1992b; Kaszala *et al.*, 1996). In brief, the total number of ventricular premature beats (VPBs), the incidence and number of episodes of ventricular tachycardia (VT; defined as a run of four or more ventricular premature beats at a rate faster than the resting heart rate), and the incidence of ventricular fibrillation (VF) were assessed. At the end of the 25 min occlusion period the myocardium was rapidly reperfused. Following reperfusion, ventricular arrhythmias were not assessed in detail but VF occurred in almost all the control, unpaced dogs; those dogs were pronounced as survivors if they were still alive, and predominantly in sinus rhythm, 10 min after reperfusion.

The risk area following coronary artery occlusion was assessed in each dog at the end of the experiment by injecting patent blue V dye into the re-occluded coronary artery, and was expressed as a percentage of the left ventricular wall together with the septum (Kaszala *et al.*, 1996).

Statistical evaluation

All data are expressed as means±s.e.mean and the differences between means were compared by analysis of variance (ANOVA for repeated measures) or the Student's *t*-test as appropriate. A one-way ANOVA was undertaken to determine whether or not there were significant haemodynamic differences between the groups. Ventricular premature beats were compared by using the Mann-Whitney Rank Sum test, and the incidence of arrhythmias was compared using the Fisher Exact test. Differences between groups were considered significant when $P<0.05$.

Results

Haemodynamic effects of aminoguanidine

The administration of aminoguanidine, when given 0.5 h prior to coronary artery occlusion, resulted in transient increases in arterial pressure (maximal at 5 min) and a reduction in heart rate (Table 1). Both blood pressure and heart rate, however, had started to return to the initial levels (i.e. to those before injection) by the time the coronary artery was occluded.

Effects of coronary artery occlusion

The haemodynamic effects of coronary artery occlusion were similar in control (sham operated) and paced dogs and are summarized in Table 2. In each of the groups there were significant reductions in arterial pressure and in LVdP/dt and marked increases in LVEDP. These changes are similar to those previously reported for both preconditioned (paced) and control dogs (e.g. Kaszala *et al.*, 1996) and there was no marked difference between the haemodynamic effects of coronary artery occlusion in the controls, in the paced dogs and in the dogs given aminoguanidine 0.5 h prior to coronary occlusion (Table 2).

Table 1 Haemodynamic effects of aminoguanidine (50 mg kg^{-1} i.v.) in dogs paced 24 h previously and in unpaced dogs

	Baseline	*Time after injection* (min) *1*	*5*	*15*	*30*
Systolic arterial blood pressure (mmHg)					
Paced	139±6	141±9	157±8[b]	151±7	147±9
Unpaced	136±7	140±7	176±5[c]	162±7[c]	156±10[b]
Diastolic arterial blood pressure (mmHg)					
Paced	92±5	91±7	113±5[c]	102±5	99±6
Unpaced	95±3	99±4	129±6[c]	114±3[c]	106±5[b]
Mean arterial blood pressure (mmHg)					
Paced	107±5	107±7	128±6[c]	119±6	115±7
Unpaced	109±4	113±5	145±6[c]	130±4[c]	123±6[b]
Heart rate (beats min^{-1})					
Paced	146±9	139±8[a]	134±7[b]	143±9	143±8
Unpaced	144±5	144±6	132±7[c]	145±2	148±8

The results are given as means±s.e.mean of 12 (paced) or nine (unpaced) observations. Baseline values are those immediately prior to the injection of aminoguanidine (AG). There was no significant difference between the two groups in the effect of aminoguanidine. [a]$P<0.05$ *vs* baseline; [b]$P<0.01$ *vs* baseline; [c]$P<0.001$ *vs* baseline.

Table 2 Haemodynamic parameters immediately prior to coronary artery occlusion and when the maximal changes occurred 3–5 min afterwards

	Sham controls		*Paced*		*Paced+AG*		*AG*	
	pre-occlusion	*occlusion*	*pre-occlusion*	*occlusion*	*pre-occlusion*	*occlusion*	*pre-occlusion*	*occlusion*
Arterial blood pressure								
systolic (mmHg)	134±4	−12±3[b]	141±6	−10±2[c]	147±9	−19±5[b]	156±13	−13±3[b]
diastolic (mmHg)	87±3	−7±3[a]	86±5	−8±2[b]	99±6	−13±3[b]	107±4	−7±2[a]
mean (mmHg)	103±3	−8±3	104±5	−9±2[c]	115±7	−15±3[b]	123±5	−9±2[b]
LVSP (mmHg)	140±6	−11±3[b]	141±7	−10±2[c]	131±7	−14±4[b]	134±6	−14±3[b]
LVEDP (mmHg)	4.9±0.2	+15.7±2.4[c]	5.1±0.1	+16.8±2.8[c]	5.6±0.6	+16.3±1.1[c]	7.3±0.7	+6.5±0.9[c]
$LVdP/dt_{max}$ (+ve;mmHg s^{-1})	2913±302	−386±101[b]	2979±183	−584±131[c]	2854±526	−608±150[b]	3248±350	−593±132[b]
$LVdP/dt_{max}$ (−ve;mmHg s^{-1})	2370±115	−402±151[a]	2758±236	−426±154[a]	4011±521	−949±280[a]	4170±422	−987±320[a]
Heart rate (beats min^{-1})	162±5	5±2[a]	155±7	1±1	143±7	2±1	148±7	−2.5±2

The results are given as means±s.e.mean of 8–13 experiments in each of the four groups. [a]$P<0.05$ *vs* baseline; [b]$P<0.01$ *vs* baseline; [c]$P<0.001$ *vs* baseline; LV=left ventricular systolic; S=systolic; EDP=end-diastolic pressures; AG=aminoguanidine.

Ventricular arrhythmias during coronary artery occlusion

In control (sham-operated) dogs coronary artery occlusion led to marked ventricular ectopic activity; the distribution of these ventricular premature beats is shown in Figure 2. There were 330±110 ventricular premature beats over the 25 min occlusion period, and a mean of 6.4±3.6 episodes of VT per dog (Figure 3). All but three of the 12 control dogs exhibited VT at sometime during the occlusion period and seven of the 12 dogs fibrillated during occlusion (Figure 2). Three of the remaining five dogs fibrillated almost immediately on reperfusion. There were thus two survivors from the combined ischaemia-reperfusion insult in these 12 sham-operated, control dogs (i.e. a survival of 17%).

The pacing stimulus markedly reduced ventricular ectopic activity when the coronary artery was occluded 24 h later (Figure 3). There was a mean of only 70±28 ventricular premature beats during the occlusion period with only seven of the 13 dogs exhibiting VT (with a mean of 2.2±1.9 episodes per dog). Only two dogs fibrillated during occlusion (15%; $P<0.05$ compared to controls) and eight of the remaining dogs also survived reperfusion (Figure 2), giving a survival rate of 62% ($P<0.05$ compared to controls).

In the paced dogs that were given aminoguanidine prior to coronary artery occlusion there were 285±120 ventricular ectopic beats and there were 5.0±3.2 episodes of VT (Figure 3). The incidences of VT (56%) and of VF (33%) during the occlusion period were not significantly different to the sham controls (75 and 58%, respectively) and somewhat higher than in the paced dogs that had not been given aminoguanidine (incidences of VT and VF 46 and 15%, respectively). Ventricular fibrillation resulting from ischaemia and reperfusion was 89% in the aminoguanidine-treated dogs (Figure 4); this was significantly higher than in the paced dogs (38%; $P<0.05$) and not different to that in the controls (83%). Thus, 1/9 of the paced dogs given aminoguanidine survived the occlusion-reperfusion insult in contrast to 8/13 of the paced dogs and 2/12 of the sham controls (Figure 4). To determine whether aminoguanidine itself modified arrhythmia severity, eight sham-operated dogs, that had not been paced, were given the drug 0.5 h prior to the coronary artery occlusion. The number of ventricular premature beats (139±68) was somewhat less than in the controls and 3/8 of these dogs fibrillated during occlusion (38%). Four of the remaining five dogs survived reperfusion, giving a survival rate of 50% from the combined occlusion-reperfusion insult (Figure 4).

There was no significant difference in the area at risk between the paced dogs given aminoguanidine (37±1%), the sham controls (37±3%), the paced dogs without aminoguanidine (43±1%) and the non-paced dogs given aminoguanidine (37±3%).

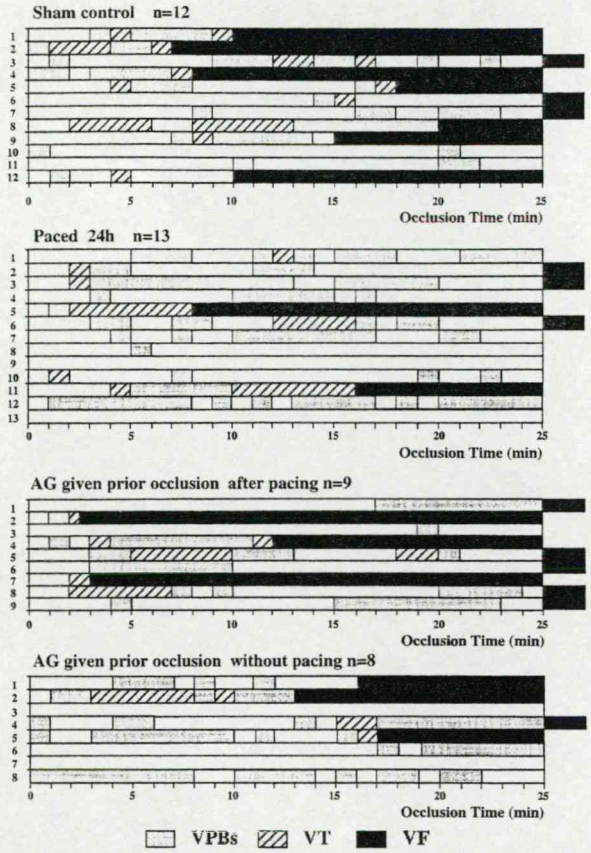

Figure 2 The distribution of ventricular arrhythmias; ventricular premature beats (VPBs), ventricular tachycardia (VT) and ventricular fibrillation (VF) during a 25 min occlusion of the left anterior descending coronary artery in anaesthetized dogs; this was followed by rapid reperfusion. Seven of the 12 sham-operated controls fibrillated during the occlusion and all but three had periods of ventricular tachycardia. Only two of these controls survived the combined ischaemia-reperfusion insult. These arrhythmias were markedly suppressed in the paced 24 h group; thus only two dogs fibrillated during the occlusion period and there was a 62% survival from the ischaemia-reperfusion insult. In the paced dogs given aminoguanidine (AG) only one of the nine dogs (i.e. 11%) survived ischaemia and reperfusion. There was no evidence that AG given prior to occlusion in unpaced dogs was pro-arrhythmic.

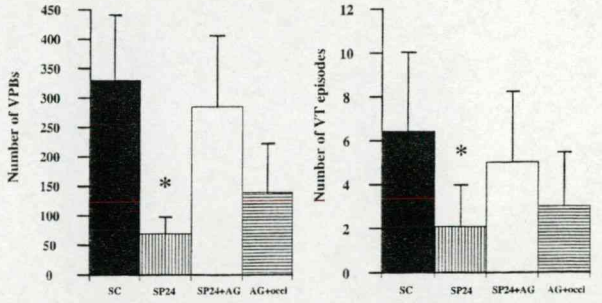

Figure 3 The total number of ventricular premature beats (VPBs) and the number of episodes of ventricular tachycardia (VT) during coronary artery occlusion in sham control dogs (SC), in dogs paced 24 h previously (SP24), in paced dogs given aminoguanidine (AG) prior to occlusion (SP24+AG) and in unpaced dogs also given AG (AG+occl.). $*P<0.05$ compared to sham controls.

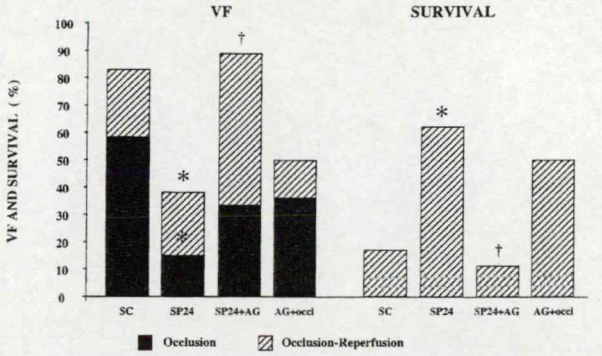

Figure 4 The incidence of ventricular fibrillation during coronary artery occlusion and following reperfusion at the end of the 25 min occlusion in control dogs (SC), in dogs subjected to right ventricular pacing 24 h prior to the occlusion (SP24) and in paced (SP24+AG) and non-paced (AG+occl.) dogs given aminoguanidine (AG) 0.5 h prior to coronary artery occlusion. Also shown in the right hand panels is survival from the combined ischaemia-reperfusion insult. The marked protection against ventricular fibrillation during both occlusion and reperfusion which results from cardiac pacing is markedly attenuated by the prior administration of aminoguanidine. $*P<0.05$ compared to sham controls; $\dagger P<0.05$ compared to paced dogs given aminoguanidine.

Changes in ischaemia severity following coronary artery occlusion

This was assessed in two ways; by epicardial ST-segment mapping and by changes in the degree of electrical inhomogeneity within the ischaemic area. The changes following coronary artery occlusion are illustrated in Figure 5 (for ST-segment changes) and Figure 6 (for changes in the degree of electrical inhomogeneity). They demonstrate that in the paced dogs ST-segment changes were less than in the controls and that this was largely reversed in those paced dogs given aminoguanidine prior to occlusion. Changes in the degree of electrical inhomogeneity were also less in paced dogs compared to controls and, again, this was reversed by aminoguanidine.

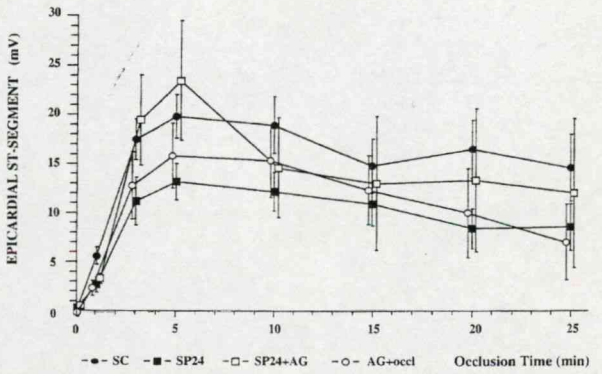

Figure 5 Changes in ST-segment elevation recorded from epicardial electrodes during a 25 min occlusion of the left anterior descending coronary artery in control dogs, dogs paced 24 h previously, and in dogs given aminoguanidine (50 mg kg^{-1}) prior to the coronary artery occlusion with or without pacing. Pacing decreased ST-segment elevation recorded over the ischaemic area; this was markedly attenuated by the prior administration of aminoguanidine.

Discussion

These studies confirm our previous findings (Végh *et al.*, 1994; Kaszala *et al.*, 1996) that right ventricular pacing markedly

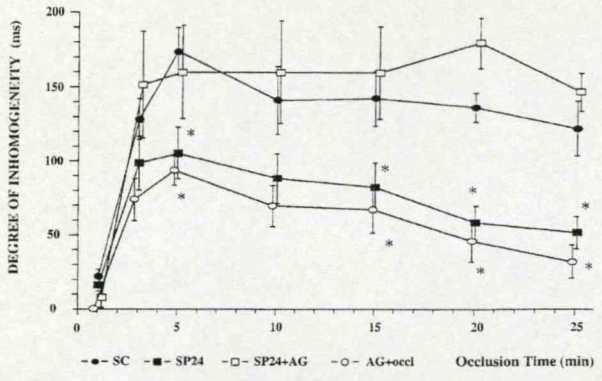

Figure 6 Changes in the inhomogeneity of electrical activation within the ischaemic area in control dogs, dogs paced 24 h previously, and in dogs given aminoguanidine (50 mg kg^{-1}) prior to the coronary artery occlusion with or without pacing. Cardiac pacing reduced the severity of the changes in inhomogeneity; this was not seen in those paced dogs given aminoguanidine. $*P<0.05$ compared to sham controls.

reduces the severity of arrhythmias that occur when a major coronary artery is occluded 24 h later. For example, only two of the paced dogs succumbed in ventricular fibrillation during the occlusion period; this incidence of VF during occlusion (15%) is similar to that observed by Kaszala *et al.* (1996) in a similar group of paced dogs. Other indices of ischaemia severity were also reduced by cardiac pacing, e.g. epicardial ST-segment changes mapped over the ischaemic area and delayed conduction, as assessed by changes in the degree of electrical inhomogeneity within the ischaemic area. The time course of this protection (absent 6 h after pacing, present 24 h after pacing but lost after 48 and 72 h; Kaszala *et al.*, 1996) suggests protein induction, and the fact that the protection at 24 h is not seen in dogs treated with dexamethasone (Végh *et al.*, 1994) suggests the involvement of induced nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), or both.

The present experiments suggest that NO is involved in this protection, since the administration of aminoguanidine attenuated the protection afforded by cardiac pacing. There were more ventricular premature beats and episodes of VT following coronary artery occlusion, and a higher incidence of VF and a significantly lower rate of survival following reperfusion at the end of the occlusion period. Further, the reduction in other indices of ischaemia severity (epicardial ST-segment elevation; changes in the degree of inhomogeneity of electrical activation within the ischaemic area), which are markedly reduced by cardiac pacing, were not seen in those paced dogs given aminoguanidine prior to coronary artery occlusion.

The attenuation of the protective effects of pacing by aminoguanidine could conceivably be due to a pro-arrhythmic effect of the drug, especially as this drug has some effect on cNOS, as evidenced by the studies of Laszlo *et al.* (1995) in rats. In fact, aminoguanidine did not modify significantly arrhythmia severity during coronary artery occlusion and indeed tended to increase, rather than decrease, survival from the combined ischaemia-reperfusion insult (Figure 4). This was perhaps as a result of the less severe ischaemic changes following coronary artery occlusion as suggested by the less marked changes in epicardial ST-segment (Figure 5) and inhomogeneity of electrical activation (Figure 6).

Most workers (Corbett *et al.*, 1992; Griffiths *et al.*, 1993; Misko *et al.*, 1993; Kengatharan *et al.*, 1996) have provided evidence that aminoguanidine is a rather selective inhibitor of iNOS. Aminoguanidine, although in larger doses than those used in the present study, can inhibit other enzymes, such as catalase (Ou & Wolff, 1993) and histaminase (Kusche *et al.*, 1977). In the present study aminoguanidine administration resulted in a short-lasting increase in systemic arterial blood pressure and a reduction in heart rate, presumably reflex in origin. This suggests that nitric oxide derived from the constitutive synthase (cNOS) might also have been inhibited. However, the blood pressure changes were not nearly as marked as those following administration of unselective inhibitors of the L-arginine nitric oxide pathway, such as N^G-nitro-L-arginine methyl ester. When such inhibitors are given under similar conditions to those described in the present study there are marked and sustained increases in systemic arterial blood pressure (Végh *et al.*, 1992a). It is not possible, therefore, to state categorically that it is NO derived from the induced enzyme which is responsible for the delayed protection; it could be derived from the enhanced synthesis of NO through cNOS in coronary vascular endothelium. In a recent study, although in a quite different model of ischaemic preconditioning (conscious rabbits; recovery of contractile function following a period of ischaemia and reperfusion), Bolli and his colleagues (1997b) found that three different NOS inhibitors (aminoguanidine and S-methylisothiourea sulphate, both of which are relatively selective for iNOS, and N^{ω}-nitro-L-arginine which is non-selective) all exacerbated stunning in the preconditioned but not in the normal myocardium. The authors suggest that nitric oxide has a dual role in late preconditioning acting both as a trigger and as a mediator. We had earlier suggested a similar hypothesis to account for the antiarrhythmic effects of preconditioning, induced by brief periods of coronary artery occlusion, during the first (classical) window of protection (Végh *et al.*, 1992a). This concept has been recently reviewed (Parratt & Végh, 1996). Our concept (Végh *et al.*, 1994; Végh & Parratt, 1998), that NO plays an important role in the delayed phase of protection has been confirmed by two, more recent studies, from other laboratories. Takano *et al.* (1998) and Imagawa *et al.* (1999) have found that in conscious rabbits, the induction of iNOS, following preconditioning by brief coronary artery occlusions, and the resultant formation of NO, play a role in the delayed cardioprotective (infarct size limiting) effect of preconditioning, since the protection was abolished by aminoguanidine.

These studies do not eliminate the possibility that other endogenous myocardial protective substances are also involved in the delayed protective effects of cardiac pacing and of ischaemic preconditioning induced by brief periods of coronary artery occlusion. This possibility is suggested by the fact that whereas aminoguanidine only attenuated the protective delayed effects of pacing, dexamethasone abolished this protection (Végh *et al.*, 1994). It remains a possibility that protective prostanoids, such as prostacyclin, which are also profoundly antiarrhythmic (Parratt, 1989), are also involved in the delayed antiarrhythmic effects of pacing-induced preconditioning. This possibility is at present under investigation.

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III.

Simultaneous blockade of the cyclooxygenase and L-arginine-nitric oxide pathways prevents the antiarrhythmic effects of classical preconditioning

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A Kis, A Vegh, JGy Papp, JR Parratt. Simultaneous blockade of the cyclooxygenase and L-arginine-nitric oxide pathways prevents the antiarrhythmic effects of classical preconditioning. Exp Clin Cardiol 1997;2(2):112-118.

OBJECTIVES: To determine the role of nitric oxide and prostanoids in the protection afforded by ischemic preconditioning against ventricular arrhythmias.
METHODS: Mongrel dogs anesthetized and thoracotomized were subjected to left coronary artery occlusion. Preconditioning was achieved by two 5 min occlusions of the artery followed 20 mins later by a 25 min occlusion. Control dogs were simply subjected to a 25 min occlusion. Ventricular arrhythmias and standard hemodynamics were recorded. Full blockade of the L-arginine-nitric oxide and cyclooxygenase pathways was achieved by the simultaneous use of N^G-nitro-L-arginine methyl ester (L-NAME) and sodium meclofenamate.
RESULTS: Preconditioning led to a marked reduction in arrhythmia severity (eg, premature ventricular complexes 79±20 versus 439±72 in controls, ventricular fibrillation 0% versus 43%, survival 63% versus 7%). It was impossible, except in two dogs, to precondition in the presence of L-NAME and sodium meclofenamate. None of the dogs survived preconditioning and prolonged occlusion. Ischemic changes were more pronounced in the presence of dual blockade.
CONCLUSIONS: Results suggest that both nitric oxide and a prostanoid (perhaps prostacyclin) are the key mediators of the antiarrhythmic effects of preconditioning in this model.

Key Words: *Cyclooxygenase pathway, Ischemic preconditioning, L-arginine-nitric oxide pathway*

It is well established that short periods of sublethal ischemic episodes protect the myocardium against the serious consequences of a subsequent prolonged ischemia-reperfusion insult. This form of cardiac adaptation is known as ischemic preconditioning (1), and is manifested by a delay in myocardial ischemic damage and cell necrosis (1), by enhanced recovery of contractile function during reperfusion (2), and perhaps, most important, by a marked reduction in ischemia and reperfusion-induced ventricular arrhythmias (3-5). Although the precise mechanisms of this antiarrhyth-

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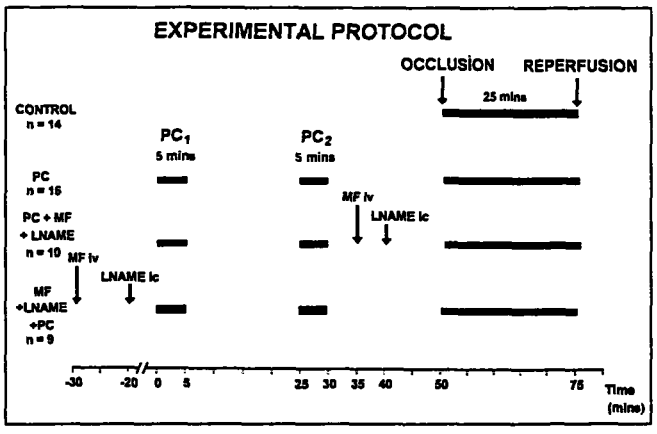

Figure 1 *Experimental protocol for studies designed to evaluate the respective roles of nitric oxide and prostanoids in protection against ventricular arrhythmias by ischemic preconditioning (PC). PC was induced by two 5 min coronary artery occlusions separated by a 20 min reperfusion period; the control occlusion, 25 mins in duration, was begun 20 mins after the end of the second PC occlusion or after PC but before the prolonged ischemic period. ic Intracoronary injection; iv Intravenous injection; L-NAME* N^G*-nitro-L-arginine methyl ester; MF Meclofenamate*

mic protection afforded by preconditioning are unclear, we have put forward the hypothesis (reviewed in 6-9) that the brief ischemic insults involved in preconditioning result in the release of 'endogenous myocardial protective substances' (10). These substances, through binding and stimulation of relevant receptors, initiate intracellular signal transduction mechanisms that ultimately lead to the protection of the myocardium (11,12).

There is some evidence that the marked antiarrhythmic effects of preconditioning involve the generation of nitric oxide (13) and bradykinin (14). Bradykinin is perhaps the initial trigger for nitric oxide release from endothelial cells (15) and from cardiac myocytes (16), and for the release of a cardioprotective prostanoid, probably prostacyclin (4,12). These mediators, generated during the preconditioning stimulus and acting in concert, are then responsible for the antiarrhythmic protection during a subsequent more prolonged ischemic insult (12,15). We have demonstrated an antiarrhythmic, nitric oxide-mediated effect of bradykinin in myocardial ischemia (17,18) and a role for it in ischemic preconditioning (14). Bradykinin has also been found to be involved in the protective effect of preconditioning in reducing infarct size in rabbits (19,20) and in enhanced postischemic myocardial recovery in isolated rat hearts (21). This latter effect was attributed to the release of both prostacyclin and nitric oxide.

Evidence that nitric oxide and prostacyclin might be involved in the antiarrhythmic protection of preconditioning came initially from our previous studies in which either inhibition of the L-arginine-nitric oxide pathway by N^G-nitro-L-arginine methyl ester (L-NAME) (13) or blockade of the cyclooxygenase pathway by sodium meclofenamate (4) markedly attenuated the antiarrhythmic effects of preconditioning. Although protection against arrhythmias was markedly attenuated after the separate inhibition of either of these pathways, it was not fully reversed. In the present study we therefore examined whether the early protective effects of preconditioning against arrhythmias are completely abolished if both the L-arginine-nitric oxide and the cyclooxygenase pathways are blocked simultaneously. A preliminary account of these results was presented at the 15th World Congress of the International Society for Heart Research in Prague, Czech Republic (22).

ANIMALS AND METHODS

Methods were similar to those previously described (4,5). In brief, mongrel dogs of both sexes and with a body weight in excess of 18 kg (mean 23±1.2 kg) were used. The dogs were anesthetized with a mixture of chloralose and urethane (60 and 200 mg/kg, respectively, given intravenously) and ventilated with room air by a Harvard Respirator (Harvard, Boston, Massachusetts, USA) at a rate and volume sufficient to maintain arterial blood gases and pH within normal limits (4). The body temperature was measured in the esophagus and maintained, by a heating pad, around 37±0.5°C.

The animals were thoracotomized at the fifth intercostal space and the left anterior descending (LAD) branch of the coronary artery prepared for occlusion just proximal to the first main diagonal branch. Epicardial ST segment changes and the degree of inhomogeneity of activation were measured from the left ventricular (LV) wall distal to the occlusion site with the use of a 'composite' electrode described previously (23,24). This gives a summarized recording of R waves from 30 epicardial measuring points. In the adequately perfused and oxygenated myocardium all sites are activated simultaneously, resulting in a single large spike. However, following occlusion, widening and fractionation of this summarized R wave occurs, indicating that the adjacent fibres are not simultaneously activated because of inhomogeneity of conduction. This was expressed as the greatest delay in activation (in milliseconds) within the ischemic area.

Arterial blood pressure, LV systolic and end-diastolic pressures, and LV dP/dt were measured by means of transducers (P23XL Statham, Hugo Sachs, March-Hugstetten, Germany) and connected to System-6 (Triton Technology, San Diego, California, United States). All these parameters, together with a limb lead electrocardiogram, were recorded on an eight-channel Medicor R81 recorder (Medicor, Esztergom, Hungary). At the end of the experiment patent blue V dye was infused into the occluded LAD coronary artery at the occlusion site to estimate the area at risk. This was expressed as a percentage of the LV free wall.

Ventricular arrhythmias during ischemia and reperfusion were assessed and analyzed as outlined previously (5), ie, total premature ventricular complexes (PVCs), the incidence and number of episodes of ventricular tachycardia (VT) and the incidence of ventricular fibrillation (VF). No distinction was made between couplets and salvos, which were included as single PVCs. VT was defined as a run of four or more ectopic beats at a rate faster than the resting sinus rate.

All the data were analyzed statistically as previously de-

TABLE 1
Hemodynamic effects of meclofenamate (2 mg/kg intravenously) and L-NAME (5 mg/kg intracoronary injection)

	Before meclofenamate	Meclofenamate	Change	Before L-NAME	L-NAME	Change
Arterial blood pressure (mmHg)						
Systolic	121±8	127±7	6±2	125±8	141±8	16±3*
Diastolic	78±4	82±5	4±3	80±5	105±6	26±2*
Mean	92±5	97±6	5±2	95±5	117±7	22±2*
LV systolic pressure (mmHg)	125±7	130±7	5±2	129±7	147±4	18±3*
LVEDP (mmHg)	6±1	6±1	0	7±1	8±1	1±1
Heart rate (beats/min)	151±9	153±10	2±1	153±10	145±9	−8±2*
LV dP/dt_{max} (mmHg/s)						
Positive	2746±162	2863±204	118±78	2817±204	3036±231	218±126
Negative	2560±114	2752±97	192±66	2674±100	2845±121	171±119

*$P<0.05$; $n=19$. *LV Left ventricle; L-NAME N^G-nitro-L-arginine methyl ester; LVEDP LV end-diastolic pressure*

scribed (5), ie, data were expressed as means ± SEM and differences between means were compared by Student's *t* test corrected for multiple comparisons or, for arrhythmias, by the Mann-Whitney U-test. For comparison of the incidences of arrhythmias the χ^2 test for independence in a 2x2 table was used. Differences between groups were considered significant at a level of $P<0.05$.

The protocol was as follows (Figure 1).

Group 1 (controls): Sixteen animals served as controls and were allowed to stabilize after surgery for 1 h. Twenty minutes before LAD occlusion, saline (0.5 mL/min) was infused into a small branch of the LAD proximal to the occlusion site, then the LAD was occluded for 25 mins followed by reperfusion.

Group 2 (preconditioned): Twenty-two animals were preconditioned as previously described (5) by occluding the LAD for two 5 min periods, with a 20 min reperfusion period in between. Twenty minutes later the artery was reoccluded for 25 mins, after which the ischemic area was reperfused.

Group 3 (meclofenamate plus L-NAME plus preconditioned): Nine dogs were given sodium meclofenamate (2 mg/kg intravenously) 30 mins before, and L-NAME (5 mg/kg, by slow local intracoronary injection) 20 mins before, the first preconditioning occlusion. This was to determine whether nitric oxide and prostaglandins are generated during the preconditioning procedure, and hence whether they are important trigger substances for preconditioning. The dogs were then preconditioned as described above. In this group, seven of the nine dogs died during the preconditioning procedure; only the two surviving dogs were then subjected to the long occlusion.

Group 4 (preconditioned plus meclofenamate plus L-NAME): Ten dogs were preconditioned in the same manner as the group 2 dogs but, 5 mins after the second preconditioning occlusion (ie, 15 mins before the prolonged [25 min] occlusion), sodium meclofenamate and, 5 mins later (ie, 10 mins before the 25 min occlusion), L-NAME were given by the same routes and in the same doses as described for the group 3 dogs. This was to determine whether inhibition of both the L-arginine-nitric oxide and the cyclooxygenase pathways after the preconditioning stimulus modifies the antiarrhythmic effects of preconditioning; that is, whether the generation of nitric oxide and prostaglandins is also important in mediating protection during the prolonged ischemic episode.

RESULTS

Hemodynamic effects of meclofenamate, L-NAME and coronary artery occlusion: The hemodynamic effects of meclofenamate and L-NAME are summarized in Table 1. Meclofenamate administration resulted in no sufficient changes in any hemodynamic parameter. However, infusion of L-NAME significantly increased arterial blood pressure and LV systolic pressure, and reduced heart rate, LV end-diastolic pressure, and both positive and negative LV dP/dt (Table 1). Following coronary artery occlusion, changes in diastolic and mean arterial blood pressures were somewhat more marked after this drug treatment. Reductions in positive and negative LV dP/dt were similar in both groups.

Ventricular arrhythmias during preconditioning occlusions in the absence and in the presence of meclofenamate and L-NAME: In normal dogs there are usually relatively few PVCs during a 5 min coronary artery occlusion. For example, in the present series of dogs subjected to preconditioning in the absence of meclofenamate and L-NAME (group 4), the mean number of PVCs during the first 5 min preconditioning occlusion was 12±8; three of the 10 dogs exhibited 12 episodes of VT (mean 1.2±0.6) and four dogs died in VF, during either occlusion or reperfusion of the ischemic myocardium. When, 20 mins later, the remaining dogs were subjected to the second 5 min preconditioning occlusion, there were only 1±1 PVCs, and no dog exhibited VT or VF. When meclofenamate and L-NAME were given before the preconditioning procedure (group 3), there was a mean of 82±43 PVCs (n=9) during the first 5 min preconditioning occlusion, and five of the nine dogs exhibited 37 episodes of VT (mean of the nine dogs was 4.1±2.3). Furthermore, two dogs fibrillated during the 5 min occlusion and three dogs died in VF following reperfusion

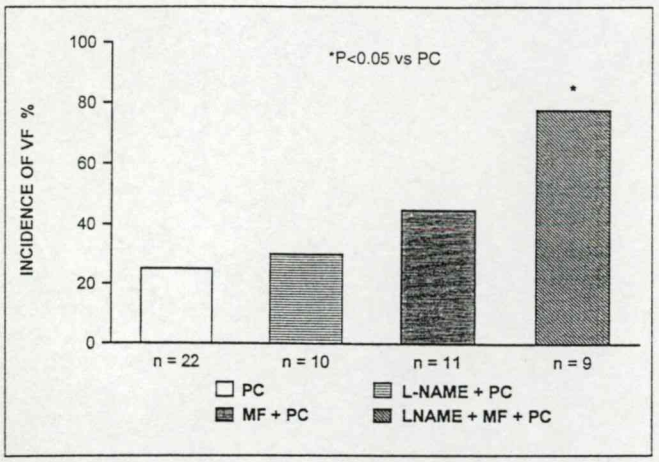

Figure 2 *Percentage incidence of ventricular fibrillation (VF) during the preconditioning (PC) procedure in dogs with no treatment (open columns), in dogs given sodium meclofenamate (MF) before PC, in dogs given N^G-nitro-L-arginine methyl ester (L-NAME) before PC and in dogs given both L-NAME and MF before PC. The combined blockade led to a very high incidence of VF during PC*

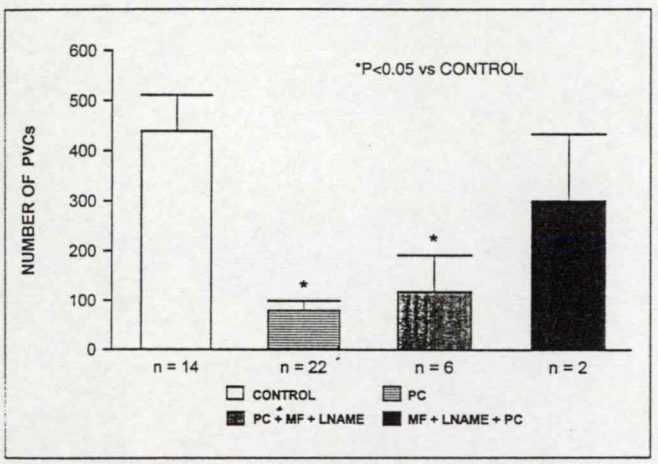

Figure 3 *Number of premature ventricular complexes (PVCs) occurring during a 25 min occlusion of the left anterior descending coronary artery in anesthetized dogs in controls (open columns; no preconditioning), in preconditioned (PC) dogs, in dogs given sodium meclofenamate (MF) and N^G-nitro-L-arginine methyl ester (L-NAME) after PC and in dogs given these inhibitors before preconditioning (filled columns)*

(Figure 2). When the remaining four dogs were subjected to the second preconditioning occlusion, there was still an increased number of PVCs (mean 52±38), and one of the four dogs exhibited two VT episodes. When the occlusion was released two additional dogs fibrillated during reperfusion. Thus, in this group, only two dogs survived the preconditioning procedure.

Effect of dual blockade of the L-arginine-nitric oxide pathway and the cyclooxygenase pathway on the incidence and severity of prolonged ischemia-reperfusion-induced ventricular arrhythmias and on survival: Figure 3 illustrates the number of PVCs during a 25 min coronary occlusion in control dogs, in preconditioned dogs and in dogs in which sodium meclofenamate and L-NAME were given either before or after the preconditioning occlusions. In confirmation of previous studies, preconditioning markedly reduced the severity of ischemia-induced arrhythmias during a prolonged occlusion of the LAD. When meclofenamate and L-NAME were given after the preconditioning procedure, but before the prolonged occlusion, the number of PVCs was similar to that in the preconditioned dogs. If meclofenamate and L-NAME were given together before the preconditioning procedure, the number of ectopic beats in the two dogs was similar to that in the controls.

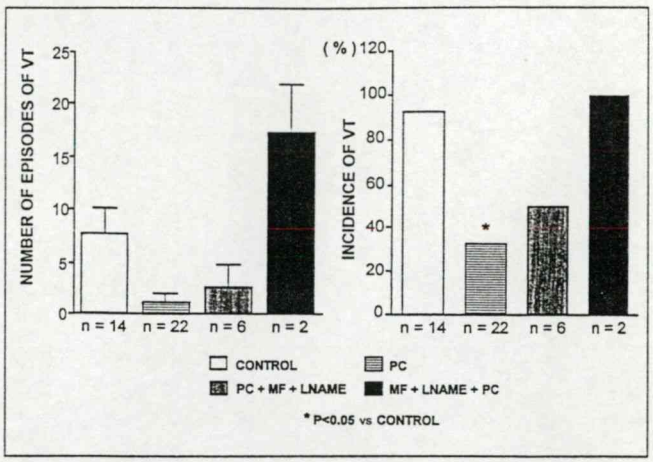

Figure 4 *Incidence and number of episodes of ventricular tachycardia (VT) during a 25 min occlusion of the left anterior descending coronary artery in anesthetized dogs that were preconditioned (PC), in dogs that were also PC but then given a combination of sodium meclofenamate (MF) and N^G-nitro-L-arginine methyl ester (L-NAME) before occlusion, in dogs given MF and L-NAME before PC and in control dogs not subjected to any treatment or to PC. PC markedly reduced the incidence of VT (and the number of episodes); this protection was completely lost when both pathways were blocked (MF + L-NAME + PC)*

Preconditioning also markedly reduced the incidence, during prolonged occlusion of the LAD, of the more malignant arrhythmias such as VT (Figure 4) and VF (Figure 5). Whereas in the control group 90% of the dogs exhibited VT and the number of episodes of VT per dog was 7.6±2.1, in the preconditioned group the incidence of VT was only 32% and the number of episodes of VT per dog was 1.1±0.5 episodes. The incidence and number of episodes of VT were still reduced when meclofenamate and L-NAME were given before the prolonged occlusion (incidence of VT 50%, number of episodes of VT 2.6±2.2) but they were markedly increased when the two inhibitors were administered before the preconditioning occlusions (incidence of VT 100%, number of episodes of VT 17.5±4.5; Figure 4).

In control dogs subjected to a 25 min occlusion, 42% of the dogs fibrillated during occlusion and all dogs died following reperfusion (Figure 5). In contrast, in preconditioned dogs, none died during the 25 min occlusion and 50% of the dogs survived reperfusion. In the two dogs in which L-NAME and meclofenamate were given before preconditioning (group 4) one dog died (VF) during occlusion and the other during reperfusion. Thus, no dog in this group survived.

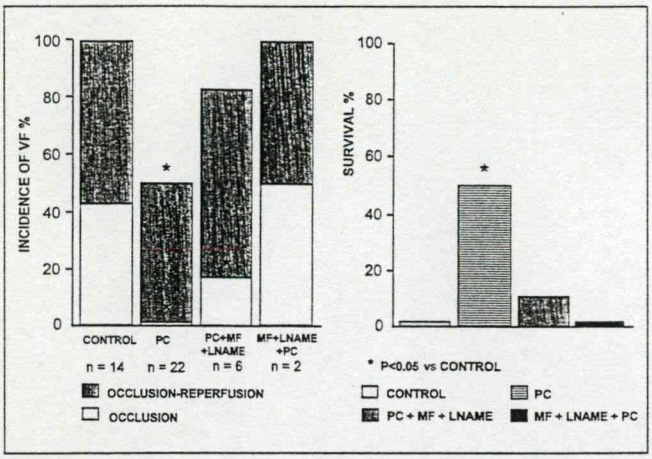

Figure 5 *Incidence of ventricular fibrillation (VF) during occlusion (open bars) and reperfusion (shaded bars) during and after a 25 min occlusion of the left anterior descending coronary artery in anesthetized dogs. There were no survivors in the control group, but a significant reduction in VF (especially during occlusion) and an increased survival in preconditioned (PC) dogs. This protection was markedly attenuated by inhibiting both the L-arginine-nitric oxide and the cyclooxygenase pathways. *P<0.05. MF Sodium meclofenamate; L-NAME N^G-nitro-L-arginine methyl ester*

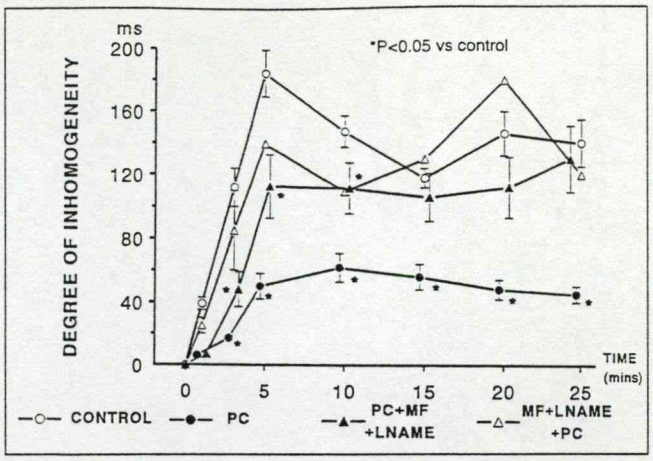

Figure 6 *Degree of inhomogeneity of electrical activation within the ischemic area during a 25 min coronary artery occlusion in control dogs (open circles), in preconditioned (PC) dogs (closed circles), in dogs that were PC but then given a combination of sodium meclofenamate (MF) and N^G-nitro-L-arginine methyl ester (L-NAME) (closed triangles) and in dogs given both inhibitors before PC (open triangles)*

When dogs were given meclofenamate and L-NAME after the preconditioning procedure, but before the prolonged occlusion (group 3), one of the six dogs (17%) died during occlusion and four dogs fibrillated on reperfusion. Thus, survival in this group was 10%.

Changes induced by inhibition of the L-arginine-nitric oxide and cyclooxygenase pathways in the degree of inhomogeneity of activation within the ischemic area and in the epicardial electrocardiogram: As shown previously (5), the inhomogeneous conduction that occurs within an area of the LV wall supplied by the occluded coronary artery is markedly reduced by preconditioning. Inhomogeneity of electrical activation was still largely suppressed when L-NAME and meclofenamate were given after the preconditioning procedure, but was markedly increased when both drugs were administered before the preconditioning protocol (Figure 6).

Occlusion of a coronary artery also results in a rapidly developing elevation of the epicardial ST segment. This is most marked when the coronary artery is occluded for the first time, ie, during the first preconditioning occlusion (groups 2 and 4) or during the first 5 to 10 mins of the prolonged occlusion (control group). The second preconditioning occlusion and the prolonged occlusion in preconditioned dogs results in slower development of ST segment elevation and is less marked (Figure 7). However, in dogs in which meclofenamate and L-NAME were given before preconditioning, the increase in epicardial ST segment was significantly greater, during both the two preconditioning occlusions (there was now no difference between them) and the prolonged occlusion. In dogs in which the blockade was performed after the preconditioning procedure, the elevation in the epicardial ST segment, during both preconditioning and prolonged occlusion, was similar to that in the preconditioned dogs.

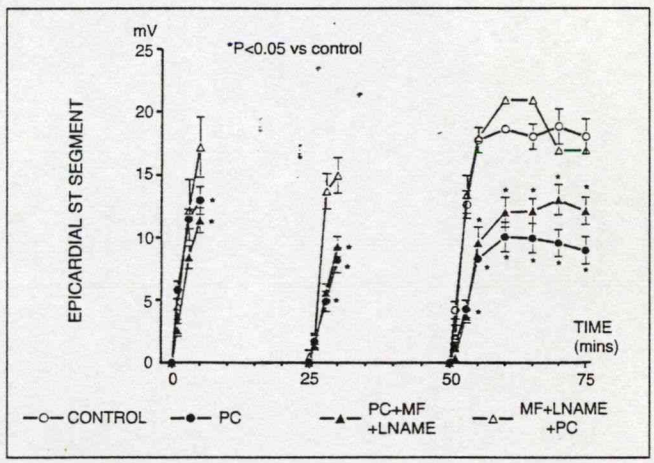

Figure 7 *Changes in epicardial ST segment elevation during two preconditioning (PC) occlusions (with a 20 min reperfusion period in between) and, 20 mins later, a 25 min occlusion of the same artery. Control data are represented by open circles (prolonged occlusion only), PC dogs by closed circles, PC dogs in the presence of a combination of meclofenamate (MF) and N^G-nitro-L-arginine methyl ester (L-NAME) by open triangles and PC dogs subsequently given a combination of MF and L-NAME before the prolonged occlusion by solid triangles. PC markedly reduced ST segment elevation during the prolonged occlusion, and this was unaffected if dual blockade was carried out before that occlusion. In contrast, dual blockade before PC (open triangles) intensified ischemia during both PC occlusions and the prolonged occlusion. *P<0.05*

DISCUSSION

As we expected, the simultaneous blockade of both the cyclooxygenase and L-arginine-nitric oxide pathways before preconditioning reduced the protection more markedly than that following blockade of either pathway alone. Usually, in dogs subjected to preconditioning by brief coronary artery occlusions, the incidence of VF occurring either during the first preconditioning occlusion or after its release is about

25% (Figure 2). In our previous studies (4,13) when the preconditioning procedure was performed in the presence of either meclofenamate or L-NAME, the incidence of VF during the preconditioning procedure was increased to 30% and 45%, respectively. When, in the present study, L-NAME and meclofenamate were administered together before the preconditioning occlusions, 78% of the dogs fibrillated during the preconditioning procedure. This result indicates that dual blockade of the cyclooxygenase and the L-arginine-nitric oxide pathways makes preconditioning almost impossible and lends support to the concept that *both* nitric oxide and prostacyclin, generated during the preconditioning stimulus (perhaps resulting from the rapid activation by bradykinin of B_2 receptors), are the mediators responsible for the protective antiarrhythmic effect of preconditioning in this species.

There is strong evidence that during the early phase of myocardial ischemia a substantial amount of prostacyclin is released and that this determines the severity of occlusion arrhythmias (25). Indeed, potentiation of prostacyclin formation, for example with nafazatrom (26) or administration of a stable prostacyclin analogue, 7-oxo-prostacyclin (27), results in a profound antiarrhythmic effect. On the basis of these previous studies it seemed likely that prostacyclin is also involved in the antiarrhythmic effect of ischemic preconditioning. Indeed, when the cyclooxygenase pathway was blocked with meclofenamate before preconditioning, antiarrhythmic protection was markedly attenuated (4). We presume that prevention of the formation of prostacyclin is one of the explanations for this loss of protection. Recently, Goulielmos and colleagues (28) demonstrated that inhibition of prostacyclin and nitric oxide release from endothelial cells during global ischemia in guinea-pig isolated hearts accentuated the ischemia-induced reduction in 95% action potential duration and reduced the time to onset of VT. They suggested that endothelium-derived nitric oxide and prostacyclin modify arrhythmogenesis during myocardial ischemia and that this is probably mediated through changes in platelet activation.

A variety of potentially protective mediators might be released under conditions of myocardial ischemia (10,12). These include adenosine, prostacyclin, bradykinin and nitric oxide; each of these may be considered as an endogenous cardioprotective substance (10). Indeed, there is good evidence that nitric oxide is released into the coronary circulation under both basal and stimulated conditions (29) and that, through the elevation of cGMP, it regulates the coronary circulation. The suggestion that nitric oxide might be involved in the antiarrhythmic effects of ischemic preconditioning in the canine came first from studies in which the generation of nitric oxide or its effect on soluble guanylyl cyclase were prevented by either L-NAME (13) or methylene blue (30), respectively. In contrast, L-NAME failed to reverse the antiarrhythmic effect of preconditioning in rats (31), and there is even some evidence that L-NAME reduces, rather than increases, infarct size in rabbit isolated hearts via an adenosine-dependent mechanism (32). Although we have no direct evidence for the hypothesis that nitric oxide plays a mediator role in preconditioning (nitric oxide levels were not measured in these particular experiments), a number of studies support the concept that nitric oxide is an important endogenous cardioprotective substance under conditions of ischemia and reperfusion. Recently, Pabla and Curtis (33) demonstrated that nitric oxide protected against VF in rat hearts during reperfusion following prolonged ischemia. One of the possible mechanisms of this antiarrhythmic protection is that nitric oxide may inactivate superoxide radicals during reperfusion (11,33). Similarly, inhibition of nitric oxide synthase with L-NAME augmented ischemia-reperfusion injury in rabbit hearts in vivo (34). Thus, nitric oxide appears to function as an endogenous cardioprotectant not only in the canine but also in rats and rabbits. Maulik and colleagues (11) demonstrated that nitric oxide plays an important role in transmembrane signalling in the ischemic myocardium. This signalling system seems to be transmitted via cGMP and opposes the effects of phosphodiesterase enzymes. A similar mechanism has been suggested to contribute to the protection seen after preconditioning (12,15).

In our previous experiments, the separate inhibition of the L-arginine-nitric oxide and the cyclooxygenase pathways with L-NAME (13) and meclofenamate (4), respectively, markedly attenuated, but did not completely reverse, the antiarrhythmic effects of 'classical' ischemic preconditioning. These studies provided indirect evidence of roles for nitric oxide and prostacyclin in the antiarrhythmic protection afforded by preconditioning in the canine heart. The present study, in which we blocked the generation of both nitric oxide and prostacyclin by simultaneously inhibiting their synthesis pathways before preconditioning, provides further evidence that both nitric oxide and prostacyclin are generated during the preconditioning stimulus and contribute to antiarrhythmic protection. Indeed, inhibition of the generation of these substances before short preconditioning occlusions made preconditioning almost impossible. However, when preconditioning was performed in the absence of L-NAME and meclofenamate, and thus the protection was allowed to develop, and when the two inhibitors were then administered only before the prolonged occlusion, antiarrhythmic protection was largely preserved.

The effects of complete reversal of the antiarrhythmic effect of preconditioning by the simultaneous inhibition of these two mediators were similar to those seen when the effects of bradykinin were inhibited with an antagonist (icatibant) at bradykinin (B_2) receptors (14). When icatibant was given before preconditioning, only 50% of the dogs survived the preconditioning procedure.

CONCLUSIONS

Simultaneous protection of the generation of both nitric oxide and prostacyclin in anesthetized dogs completely abolished the antiarrhythmic effects of preconditioning. The results suggest that both nitric oxide and prostacyclin are generated during the preconditioning procedure, probably as a result of early bradykinin release. Released nitric oxide and prostacyclin, generated in endothelial cells, may diffuse to

the cardiac myocytes (and platelets) and trigger signal transduction mechanisms that ultimately lead to antiarrhythmic protection.

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IV.

THE ISCHEMIC HEART

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EARLY AND DELAYED PROTECTION AGAINST VENTRICULAR ARRHYTHMIAS INDUCED BY PRECONDITIONING

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Summary. Ischemic preconditioning, induced by brief periods of coronary artery occlusion, results not only in a reduction in myocardial ischemic damage but also in suppression of those life-threatening ventricular arrhythmias that result from a subsequent, more prolonged ischemia–reperfusion insult. Although this protection is marked, the antiarrhythmic effect is transient and the protection wanes with time (e.g., 60 minutes after the preconditioning stimulus, the antiarrhythmic effect is almost lost). Protection against ventricular arrhythmias can also result from brief periods of cardiac pacing, which leads to both immediate and delayed protection, e.g., a marked reduction in the incidence of ischemia-induced ventricular fibrillation, five minutes after pacing and also 24 hours later. This delayed protection against arrhythmias is less marked 48 and 72 hours after the pacing stimulus but can be reinstated, and lasts for a more prolonged period, if dogs are repaced at a time when protection from the initial pacing stimulus begins to wane. Whether it is possible to protect the heart in the longer term by pacing is unknown but is under investigation. Although the precise mechanisms of both the early and delayed protection are not yet fully understood, there is some evidence that endogenous protective mediators derived from coronary vascular endothelium, such as bradykinin, nitric oxide, and prostanoids (most likely prostacyclin), are involved in both phases of this antiarrhythmic protection. These may then trigger the induction, during the late phase, of protective proteins.

INTRODUCTION

One of the most important consequences of the abrupt reduction in coronary blood flow that results from coronary artery occlusion, both in humans and in experimental animals, is the occurrence of those life-threatening ventricular arrhythmias that

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are, in the clinical situation, responsible for sudden cardiac death [1–3]. Severe myocardial ischemia, infarction, and sudden cardiac death are leading causes of cardiovascular mortality in modern societies. Protecting the myocardium from ischemic injury is thus a major aim in the reduction of morbidity and mortality from ischemic heart disease. Despite advances in drug therapy and interventional cardiology (e.g., early thrombolysis, percutaneous coronary angioplasty) and surgery (coronary bypass surgery), further research is required to explore new strategies that might ultimately provide practical therapeutic interventions.

There has been much recent interest in experimental cardiology in the observation that the heart (and indeed other organs) is able to protect itself against fatal, severe injury if it has been previously subjected to a similar, sublethal stress. Ischemic preconditioning is one form of such cardioprotective adaptation, first described by Murry and colleagues in 1986 [4]. Ischemic preconditioning can be defined as *the increased tolerance of the heart that can be induced by brief periods of ischemia and reperfusion.* There is evidence that ischemic preconditioning offers an extremely powerful protection against ischemic damage [4] and against occlusion and reperfusion-induced ventricular arrhythmias [5–7] and that it also enhances the recovery of contractile function during reperfusion of the ischemic myocardium [8]. The protection achieved by preconditioning exceeds the effectiveness of any known pharmacological intervention [9,10] and may represent an important clinically accessible component of myocardial protection. Although the precise mechanisms of this phenomenon are not fully understood, much of the evidence suggests that endogenous myocardial protective substances are involved [11].

The purpose of this chapter is to outline the evidence for the antiarrhythmic effects of preconditioning and to discuss the possible mechanisms of this protection with particular reference to studies made in a canine model of ischemia and reperfusion.

THE ANTIARRHYTHMIC EFFECTS OF ISCHEMIC PRECONDITIONING: HISTORICAL BACKGROUND

Before the "preconditioning era," there were several published observations demonstrating the reduction, by brief periods of ischemia, of the severity of ventricular arrhythmias resulting from a subsequent period of ischemia. For example, as early as 1950, Harris [12] showed that dogs are more likely to survive a coronary artery occlusion if this is performed in two stages. Much later, Gülker and his colleagues investigated the effect of multiple coronary artery occlusions on the ventricular fibrillation threshold [13]. They showed that the decrease in the ventricular fibrillation threshold became increasingly less marked, and the duration increasingly shorter, during repeated occlusions, and that the last (fifth) occlusion resulted in no significant decrease in fibrillation threshold at all. Later, Barber [14] not only confirmed that brief periods of ischemia result in less severe ischemic changes (i.e., epicardial ST-segment elevation) [15–17] but also reduced the number of ventricular premature beats that occurred during the second, and subsequent, occlusions of the same short duration. None of these studies was designed, however, to explore

the possibility that ventricular arrhythmias arising during a *prolonged* ischemic insult could be modified if that insult had been preceded by one or more shorter periods of ischemia—in other words, to test the hypothesis that ischemic preconditioning results not only in a reduction in myocardial ischemic damage but also in a suppression of ventricular arrhythmias.

The first report that ischemic preconditioning might protect the myocardium against reperfusion-induced ventricular arrhythmias came from the studies of Shiki and Hearse [5]. In anesthetized rats, they showed that the incidence and severity of ventricular arrhythmias following the release of short periods of coronary artery occlusion of the same duration (five minutes) was markedly attenuated if the recovery period between the repeated occlusions was 10 or 20 minutes. This antiarrhythmic effect was, however, greatly attenuated if the second occlusion–reperfusion insult was performed hours or even days after the first occlusion. This study was, in effect, similar to that of Barber [14] except that reperfusion arrhythmias were examined and that very much longer "rest" (reperfusion) periods were used. Neither of these studies provided evidence relevant to the important question of whether brief ischemic episodes also protect the heart against those ventricular arrhythmias that occur during a longer period of ischemia–reperfusion. Shiki and Hearse [5] also failed to observe the reappearance of protection against arrhythmias 24 hours after the initial preconditioning stimulus, i.e., the late, or delayed, second window of protection that has since aroused such considerable interest. Perhaps the explanation for this is that the preconditioning stimulus (a single five-minute coronary artery occlusion) was too weak.

THE ANTIARRHYTHMIC EFFECTS OF ISCHEMIC PRECONDITIONING

The first demonstration that short periods of sublethal ischemic stress are protective against those ventricular arrhythmias that occur during a subsequent longer period of ischemia–reperfusion was provided by a collaborative study, performed in parallel in Glasgow and in Szeged, using two different species [18,19]. Komori, working in the Glasgow department, examined in an anesthetized rat model whether brief periods of ischemia reduced the severity of arrhythmias during a subsequent, more prolonged period of coronary artery occlusion. He found that the number of ventricular premature beats and both the incidence and duration of ventricular tachycardia (VT) during a 30-minute occlusion were markedly suppressed if that prolonged occlusion had been preceded 10 minutes earlier by a single three-minute occlusion [7,19]. A single one-minute preconditioning occlusion was not effective, whereas a five-minute occlusion in this model [20] resulted in a high incidence of ventricular arrhythmias following reperfusion of the ischemic myocardium. It seemed from these results that in anesthetized rats, a single three-minute preconditioning occlusion is optimal for triggering protection against those ventricular arrhythmias that occur during a subsequent 30-minute period of ischemia, commencing shortly (10 minutes) after this preconditioning stimulus (table 1). This protection was, however, much less pronounced if the time interval between the single preconditioning

Table 1. The number of ventricular premature beats (VPBs), the incidence and duration of ventricular tachycardia (VT), and the incidence of ventricular fibrillation (VF) in rats following preconditioning

Duration of preconditioning occlusion (min)	*n*	Number of VPBs	Incidence of VT (%)	Duration of VT (s)	Incidence of VF (%)
0 (Control)	12	1236 ± 262	100	95.6 ± 28.7	42
1	8	1230 ± 370	100	109.8 ± 30.9	50
3	10	200 ± 60[a]	70	12.6 ± 3.8[a]	10
5	10	394 ± 152[b]	60[b]	55.1 ± 20.3	30

[a] $p < 0.01$ cp. nonpreconditioned animals.
[b] $p < 0.05$ cp. nonpreconditioned animals.
Note: Rats were subjected to a 30-minute occlusion of the left coronary artery. In the preconditioned rats, this was preceded 10 minutes earlier by a preconditioning occlusion of 1, 3, or 5 minutes. Preconditioning periods of 3 or 5 minutes significantly reduced the arrhythmias occurring during a longer occlusion. Values are mean (±SEM).

occlusion and the prolonged occlusion was increased from 10 minutes to 30 minutes and was entirely lost if the reperfusion period was further increased to one hour [7].

This marked antiarrhythmic effect of ischemic preconditioning was also confirmed in a large animal arrhythmia model, which had been used for many years in the Szeged department. In mongrel dogs, anesthetized with a mixture of chloralose and urethane, we showed that if a prolonged, 25-minute occlusion of the anterior descending branch of the left coronary artery (LAD) was preceded, 20 minutes earlier, by one or two brief (five-minute) occlusions of that same artery, the severity of the arrhythmias during the prolonged occlusion was markedly reduced [6,7]. This pronounced antiarrhythmic effect of preconditioning is illustrated in figure 1. Usually these early postocclusion ventricular arrhythmias (e.g., ventricular premature beats) occur in two phases: phase 1a and phase 1b (figure 1) [21]. However, when this prolonged occlusion was preceded 20 minutes earlier by two five-minute periods of occlusion, separated by a 20-minute reperfusion interval, the number of premature ectopic beats was markedly suppressed over the entire 25-minute occlusion period. This was real protection, i.e., there was an absolute reduction in the number of ectopic beats and there was no shift in the distribution of these to a later time. This was demonstrated in experiments in which the occlusion time was prolonged from 25 minutes to 60 minutes [7].

There is considerable evidence that the severity of ventricular arrhythmias during coronary artery occlusion depends, among other factors, upon the site of occlusion, the area at risk, the anesthetic used, the weight of the dogs (heart: body weight ratio), the extent of the preexisting coronary collateral circulation, the electrolyte balance, and the degree of cardiac sympathetic drive. The influence of these various factors has been discussed recently [22,23]. In the chloralose-urethane anesthetized canine model, occlusion of the LAD proximal to the first main diagonal branch produces severe myocardial ischemia and results in an area at risk of infarction of about 40%–43% of the mass of the left ventricle (including the septum), a great

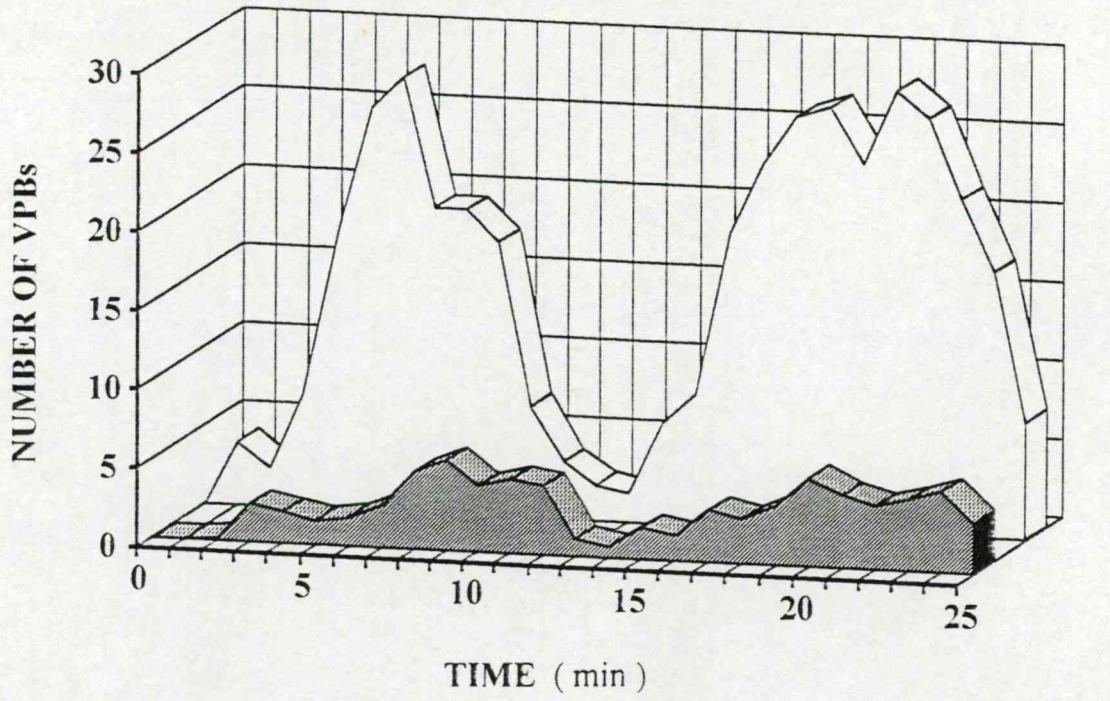

Figure 1. Distribution of ventricular premature beats (VPBs) at one-minute intervals over a 25-minute occlusion period of the left anterior descending (LAD) coronary artery, in control dogs (open histograms) and in dogs subjected to preconditioning by two five-minute occlusions of the LAD (hatched histograms). VPBs over a 25-minute occlusion of a coronary artery occur in two phases: phase Ia and phase Ib. When this prolonged occlusion is preceded by two brief (five-minute) occlusions of that same artery, there is a marked reduction in the number of VPBs throughout the entire occlusion period.

number of ventricular premature ectopic beats (VPBs; more than 400 during a 25-minute occlusion), and a high incidence (90%–100%) of ventricular tachycardia (VT), with many episodes of VT (more than nine per dog; see figure 2). In 40%–50% of these dogs, ventricular fibrillation (VF) results. Clearly, this is a very severe arrhythmia model. Further, the subsequent reperfusion of the ischemic myocardium results in ventricular fibrillation in all the dogs (figure 2). All these severe ventricular arrhythmias were reduced dramatically when the heart was previously subjected to short periods of ischemia. For example, the incidence and number of episodes of VT during occlusion were significantly less in these preconditioned dogs. However, the most striking feature of this protection was the absence of VF during occlusion and the increased survival from the combined ischemia–reperfusion insult (figure 2).

There is no doubt that ischemic preconditioning also reduces the severity of arrhythmias in rat hearts subjected to coronary artery occlusion both in vivo [7,18,19,24] and in vitro [25–27]. Some studies using a canine model of preconditioning have shown a similar reduction in arrhythmia severity [6,7,28], but some investigators [29,30] have been unable to confirm this marked antiarrhythmic effect. There is only one example of preconditioning inducing protection against

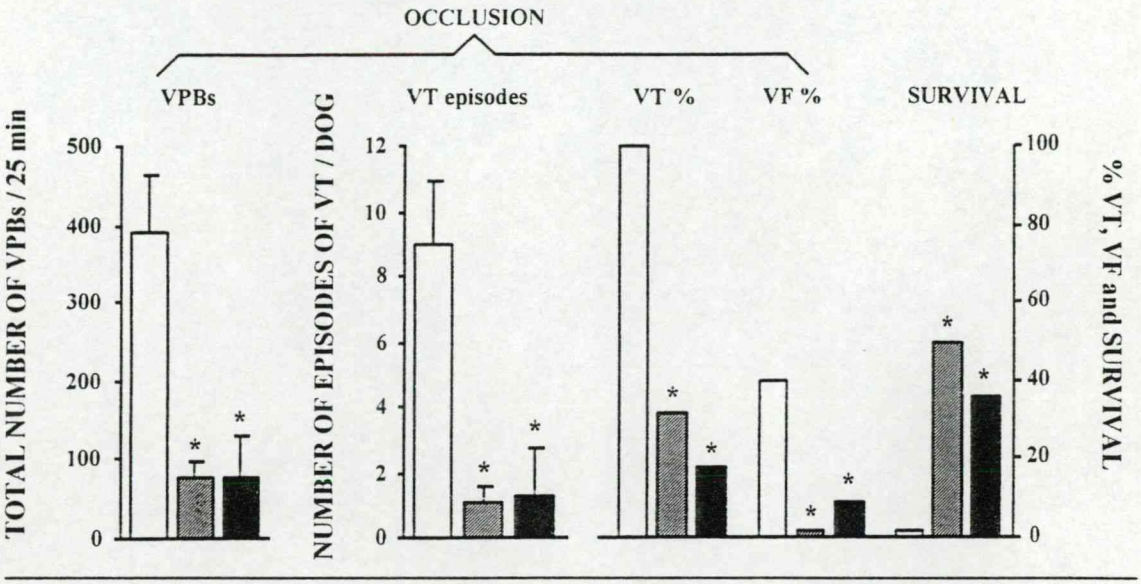

Figure 2. The incidence and severity of ventricular arrhythmias during a 25-minute occlusion of the anterior descending branch of the left coronary artery, and survival following reperfusion at the end of the occlusion period, in control dogs (open columns) and in dogs subjected to preconditioning, either by two (hatched columns) or four five-minute (solid columns) coronary artery occlusions. The severity of these ventricular arrhythmias during such a prolonged occlusion is markedly reduced when the dogs had been previously preconditioned, either by two or four brief periods of occlusion of that same artery. *, $p < 0.05$ cp. control dogs.

arrhythmias in anesthetized pigs [31]. Most of the laboratories using a pig model for assessing ischemic injury as a primary endpoint failed to demonstrate such an antiarrhythmic protection [32].

A number of possible explanations for these discrepancies have been previously discussed in detail [22,23], and this is not the place to repeat them. Apart from the variability in the protocols used by the different groups, one difference of some potential importance is the fact that multiple preconditioning occlusions (usually four five-minute episodes with short reperfusion intervals), or longer periods of ischemia (10 minutes), were used in those studies that aimed primarily to examine the effects of preconditioning on infarct size [4]. We surmised that these protocols might have detrimental effects on arrhythmias during a subsequent prolonged occlusion. Certainly, it would be difficult in our model to use a preconditioning occlusion of as long as 10 minutes because reperfusion after such a long preconditioning occlusion would certainly result in immediate ventricular fibrillation. Indeed, Li and his colleagues [28] showed, also in dogs, that whereas a single five-minute preconditioning occlusion reduced the incidence of ventricular fibrillation, a greater number of preconditioning occlusions (12 × 5 minutes) resulted in a high mortality during the subsequent prolonged occlusion.

It seemed of interest to determine in our model, which has consistently demonstrated an antiarrhythmic effect of preconditioning, whether multiple occlusions still protect the myocardium against ischemia-induced life-threatening ventricular

arrhythmias in a similar way to that achieved by one or two such occlusions. In a series of experiments, we preconditioned dogs with four five-minute periods of LAD occlusion interspersed with five-minute reperfusion periods, and five minutes later subjected the heart to a 25-minute coronary artery occlusion. Figure 2 shows that four five-minute periods of coronary artery occlusion resulted in a similar protection against ventricular arrhythmias to that which occurred in dogs preconditioned by only two such occlusions. Thus, in our hands, increasing the number of preconditioning occlusions (at least up to four) with short reperfusion intervals (five instead of 20 minutes) still maintained the antiarrhythmic effect during a prolonged occlusion. This finding might mean that other factors, such as the anesthesia used, the risk zone, sympathetic drive, etc., are particularly important in the generation of these early ventricular arrhythmias. Certainly, there seems little point in attempting to examine possible antiarrhythmic effects of preconditioning in an inappropriate model in which coronary artery occlusion results in only a few premature beats (a mean of less than 50 over a 60-minute occlusion period) [29] even under control conditions. This outcome probably reflects the depressant effects of barbiturate anesthesia on the myocardium.

There was clear evidence from our studies that preconditioning reduces the severity of myocardial ischemia. This result was demonstrated by the measurement of epicardial ST-segment elevation and the degree of inhomogeneity of electrical activation, within the ischemic area [6,7]. Preconditioning by an increased number of occlusions also confirmed this antiischemic effect of preconditioning. Thus, when dogs were preconditioned by four, rather than only two, five-minute periods of coronary artery occlusion and, five minutes later, subjected to a 25-minute occlusion, there was marked epicardial ST-segment elevation, particularly during the first five-minute preconditioning occlusion. The following preconditioning occlusions and the prolonged occlusion resulted in significantly less pronounced epicardial ST-segment elevation (figure 3) and less marked changes in the degree of inhomogeneity (figure 4). These changes were significantly less marked than those seen in control dogs. This modification of ischemia severity occurred without a significant change in collateral blood flow, since the reduction in peripheral coronary perfusion pressure, measured in a small branch of the LAD distal to the occlusion site, was the same during each coronary artery occlusion (figure 5). This finding might indicate that the anti-ischemic, and perhaps also the antiarrhythmic, effects of ischemic preconditioning are not dependent upon changes in collateral blood flow. This question was particularly important to clarify in our experiments because there has been some debate as to whether species with a variable coronary collateral circulation, such as dogs, can be preconditioned in such a way as to reduce ventricular arrhythmias induced by ischemia. However, there is no evidence that coronary blood flow is higher when the artery is occluded a second time, i.e., that an increase in collateral (overlap) blood flow is responsible for the decreased injury seen at sites immediately below surface electrodes [14,28], or that there is any significant change in peripheral coronary pressure (an index of collateral perfusion) during successive coronary artery occlusions (figure 5). Even Murry and colleagues

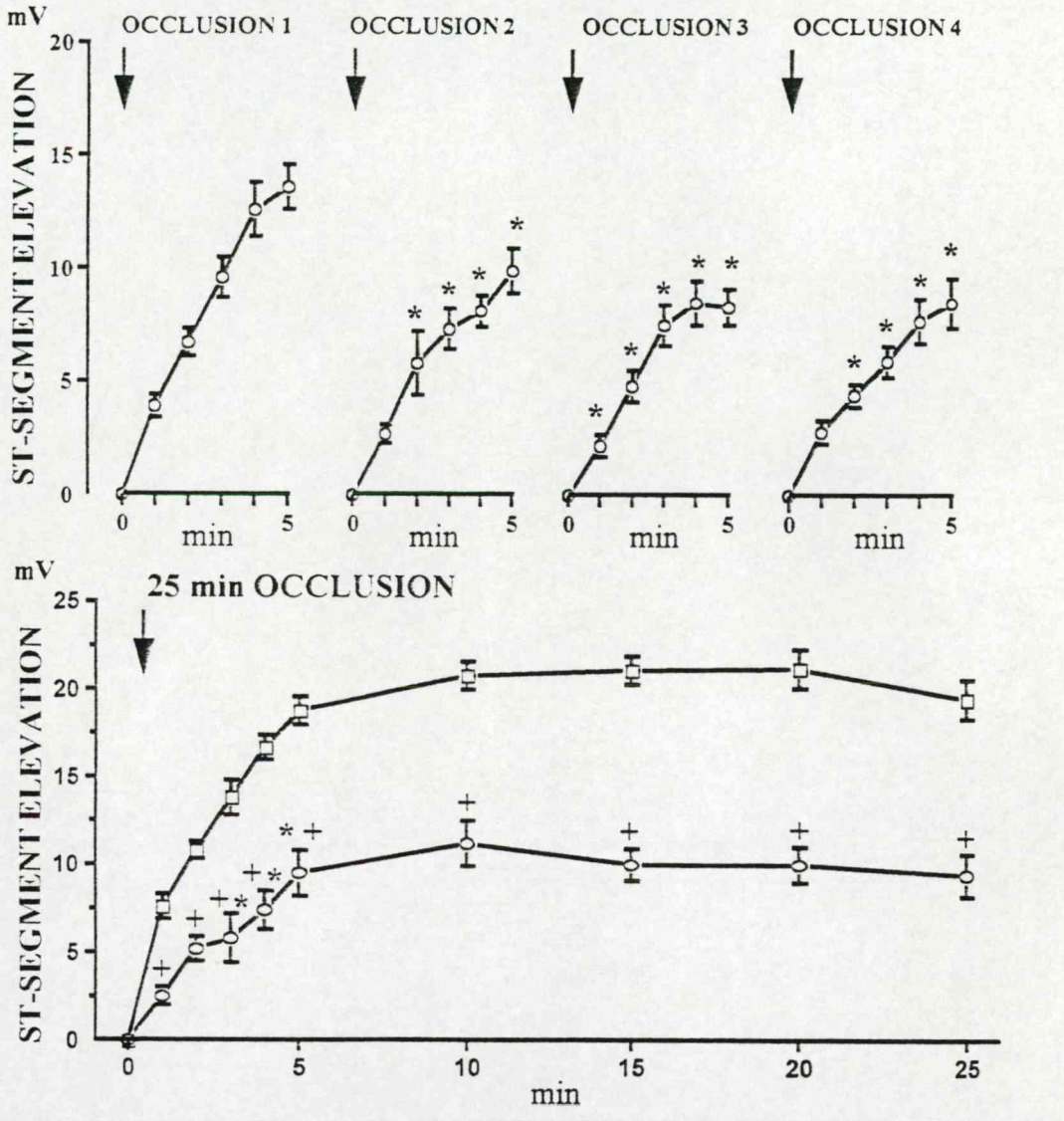

Figure 3. Changes in epicardial ST-segment elevation in anaesthetized control dogs (open squares), and in dogs preconditioned by four five-minute occlusions and then, five minutes later, subjected to a 25-minute occlusion of the left anterior descending coronary artery (open circles). This figure shows that the most marked epicardial ST-segment elevation occurs during the first five-minute preconditioning occlusion. The following preconditioning occlusions, and the prolonged occlusion, result in a significantly less pronounced ST-segment elevation. This increase in epicardial ST-segment during prolonged occlusion is significantly less marked than in control dogs during the same occlusion period. *, $p < 0.05$ cp. first preconditioning occlusion; +, $p < 0.05$ cp. control group.

in their initial study demonstrated that the reduction in infarct size was apparent at any level of collateral circulation [4]. Further, the protective effects of preconditioning, including arrhythmia suppression, are present in those species with very little coronary collateral development (e.g., rats).

The antiarrhythmic effect of ischemic preconditioning has thus been demonstrated in at least two species (rats and dogs). This protection is *marked* (reduction in

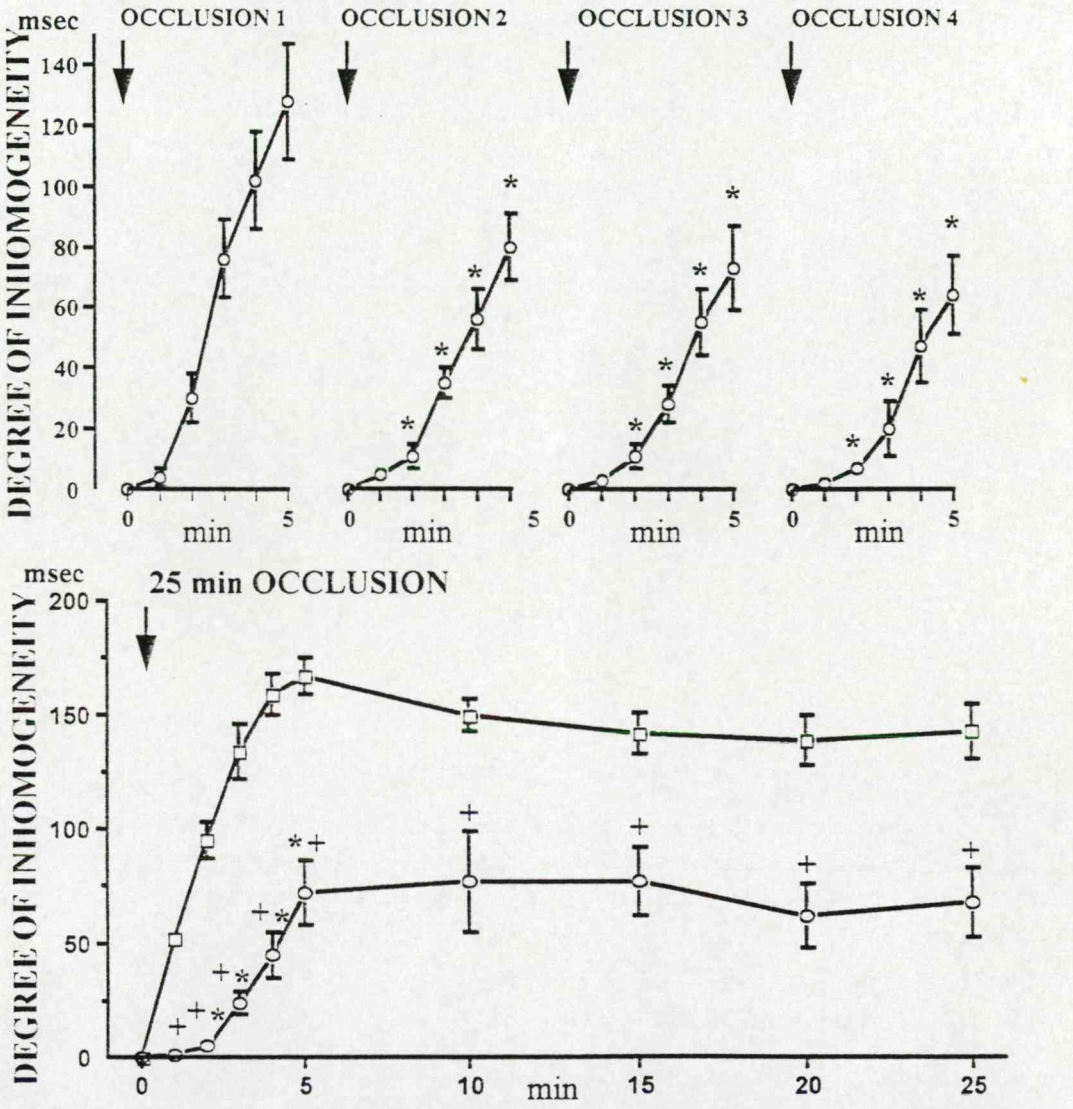

Figure 4. Changes in the degree of inhomogeneity of electrical activation in anaesthetized control dogs subjected to a 25-minute coronary artery occlusion (open squares), and in dogs preconditioned by four five-minute occlusions and then, five minutes later, subjected to a 25-minute occlusion of the left anterior descending coronary artery (open circles). The most pronounced increase in the degree of inhomogeneity is seen during the first five-minute preconditioning occlusion. The following preconditioning occlusions, and the prolonged occlusion itself, result in significantly less marked changes in the degree of inhomogeneity. The increase in the degree of inhomogeneity is also significantly less marked during the prolonged occlusion in the preconditioned dogs than in the control dogs. *, $p < 0.05$ cp. first preconditioning occlusion; +, $p < 0.05$ cp. control group.

the severity of arrhythmias) and *real* (absolute reduction in the number and incidence of ventricular arrhythmias without shifting to a later time period). However, this marked antiarrhythmic effect of preconditioning is transient; the protection wanes with time. For example, if the time interval between the preconditioning occlusions and the prolonged occlusion is increased from 20 minutes to 60 minutes, the antiarrhythmic effect is markedly attenuated (figure 6). The number of VPBs

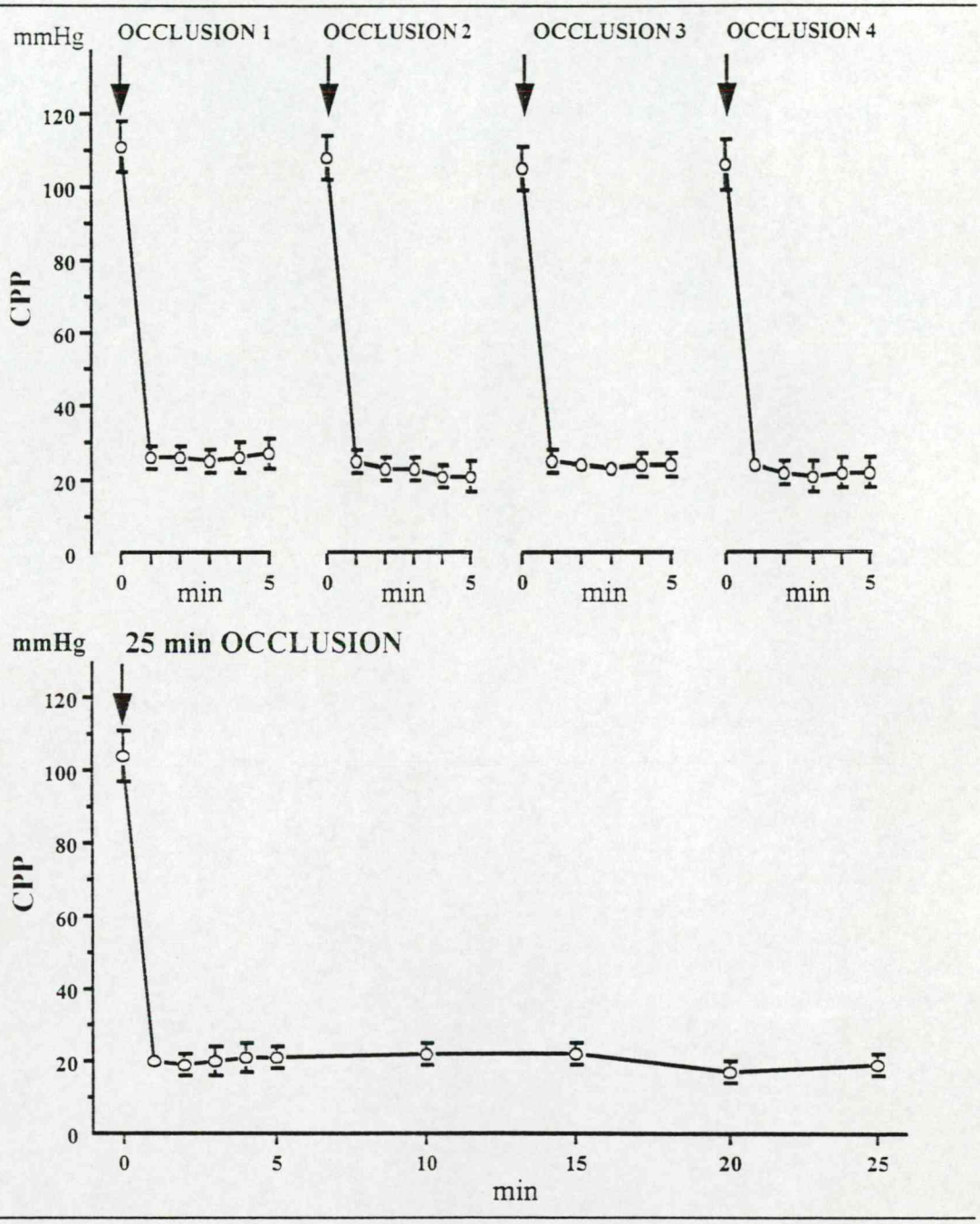

Figure 5. Changes in peripheral coronary perfusion pressure (CPP; mmHg) during brief (five-minute) and prolonged (25-minute) occlusions, measured in a small branch of the left anterior descending coronary artery distal to the occlusion site. The reduction in peripheral CPP is the same during repeated occlusions of the LAD, suggesting there is no collateral vessel recruitment.

and the incidence and number of episodes of VT during coronary artery occlusion are similar to those in control dogs and, although the incidence of VF during occlusion is still reduced, nearly all the dogs fibrillated on reperfusion. Survival, as in control dogs, is either not observed at all or is much reduced.

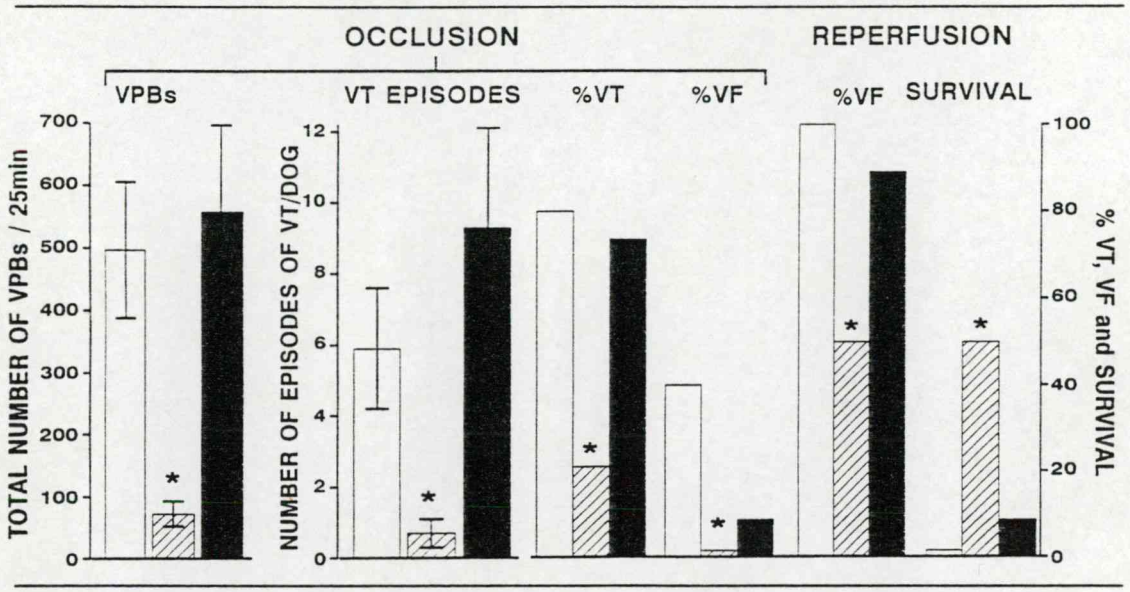

Figure 6. The transient nature of protection afforded by ischemic preconditioning (by brief coronary artery occlusions) against ventricular arrhythmias in anesthetized dogs. The protection is lost if the time between the preconditioning occlusions and the prolonged occlusion is increased from 20 minutes to 60 minutes. The figure shows the number of ventricular premature beats (VPBs), the number of episodes of ventricular tachycardia (VT), and the incidences of VT and ventricular fibrillation (VF) during a 25-minute occlusion of the left anterior descending coronary artery and during subsequent reperfusion. There is a reduction in the number of VPBs and VT episodes and in the incidences of both VT and VF (and an increased survival) in those dogs that were preconditioned by two five-minute occlusions (striped columns), provided that the reperfusion time was 20 minutes, but not if the reperfusion time was increased to one hour (solid columns). *, $p < 0.05$ cp. control nonpreconditioned dogs (open columns).

PRECONDITIONING BY RAPID CARDIAC PACING

In most studies concerned with suppression of ischemia- and reperfusion-induced ventricular arrhythmias, the preconditioning stimulus has been complete occlusion of a major branch of a coronary artery (or global ischemia in isolated hearts). The question as to whether it is possible to induce preconditioning of the myocardium by means other than short coronary artery occlusion was initially investigated by rapid cardiac pacing. In 1991 we reported that rapid (overdrive) ventricular pacing reduces the consequences of subsequent periods of regional ischemia in anesthetized dogs [33]. Thus, the epicardial ST-segment elevation, the changes in the degree of inhomogeneity of electrical activation within the ischemic area, and ventricular arrhythmias that normally occur during a 25-minute LAD occlusion were markedly reduced when the dogs were subjected to two brief (two-minute) periods of rapid right ventricular pacing (300 beats/min) two minutes prior to the occlusion. In conscious rabbits, overdrive pacing also reduced changes in left ventricular end-diastolic pressure and endocardial ST-segment elevation induced by subsequent periods of overdrive pacing [34]. Perhaps these were the first attempts to induce preconditioning of the myocardium by means other than by short coronary artery occlusions. Since then it has become well established that preconditioning can be

induced by stimuli other than complete occlusion of a coronary artery. Thus, similar infarct size limitation can be achieved by partial coronary artery occlusion [35,36], by hypoxia [37], by increasing stretch in the left ventricular wall via acute volume overload [38], by transient ischemia in adjacent myocardial regions [39], and even following ischemia in organs other than the heart (preconditioning at a distance; [40,41]). However, none of these studies was designed to investigate, as a primary endpoint, the effects of these various preconditioning stimuli on ventricular arrhythmias.

We do not understand precisely why cardiac pacing protects the heart against the effects of a subsequent coronary artery occlusion. One possibility is that these short periods of cardiac overdrive pacing result in transient ischemia, especially in the endocardial regions of the left ventricular wall, because subendocardial perfusion pressure would be drastically reduced due to the combination of reduced coronary artery diastolic perfusion pressure and a markedly elevated left ventricular end-diastolic pressure [42]. Evidence for subendocardial ischemia was obtained in experiments in which we recorded the electrocardiogram from the endocardium of the *left* ventricle during *right* ventricular pacing [43]. Immediately following cessation of pacing, there were transient electrographic changes (4–5 mV ST-segment elevation) indicative of myocardial ischemia. This ischemia was limited to the endocardium, since there was no evidence for ST-segment changes in the epicardial surface of the left ventricle at this time [43].

A similar marked protection was seen against occlusion and reperfusion arrhythmias if the heart was paced at a lower frequency (220 beats/min) but for a longer period (four times for five minutes) [43,44]. In these experiments, dogs were paced four times for five minutes at a rate of 220 beats/min, using a bipolar pacing electrode introduced into the right ventricle. This procedure was followed, at various time intervals after cessation of pacing, by a 25-minute coronary artery occlusion and then by reperfusion. Such cardiac pacing markedly reduced the severity of ventricular arrhythmias during coronary artery occlusion when this was induced five minutes later. These results are illustrated in figure 7. In contrast to the control group, in which 43% of the dogs fibrillated during occlusion and all the dogs died following reperfusion, no dog in the paced group died during occlusion and 55% of the dogs survived reperfusion. However, as with the antiarhythmic effects of classical preconditioning, the protection was lost if the time between the pacing stimulus and the commencement of the prolonged coronary artery occlusion was increased. When dogs were preconditioned by cardiac pacing, the duration of the protection was much shorter than when preconditioning was induced by brief coronary artery occlusions, suggesting that the duration of the protection may depend on the *intensity* of the proceeding ischemic stimulus. Thus, when the artery was occluded five minutes after the end of the pacing stimulus, there was marked antiarrhythmic protection (figure 7). However, this protection was attenuated if the interval between the pacing period and the occlusion was increased to 15 minutes, and protection was almost lost when this interval was increased to one or six hours (figure 8).

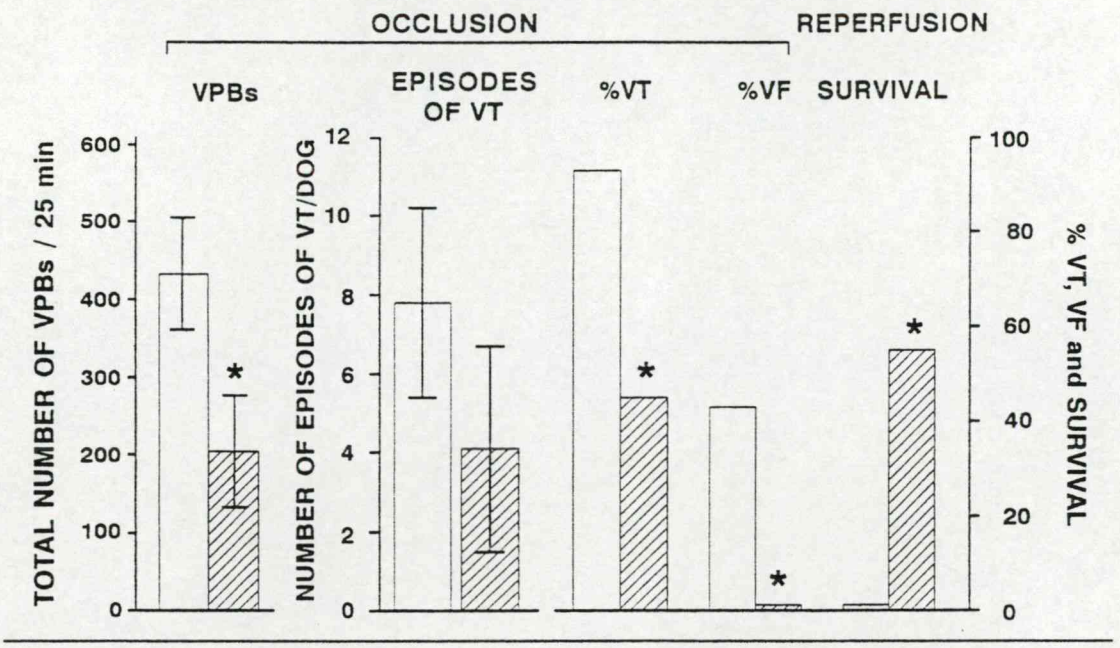

Figure 7. The severity of ventricular arrhythmias during a 25-minute coronary artery occlusion in control dogs (open columns) and in dogs subjected to right ventricular pacing five minutes previously (striped columns). Cardiac pacing (four times for five minutes at a rate of 220 beats/min) five minutes before occlusion of the left anterior descending coronary artery markedly reduces the number of ventricular premature beats (VPBs), the number of episodes of ventricular tachycardia (VT), and the incidences of VT and ventricular fibrillation (VF) during occlusion, and also increases survival from the combined ischemia–reperfusion insult. *, $p < 0.05$ cp. control, nonpaced dogs.

On the basis of these results, we conclude that preconditioning induced either by short coronary artery occlusions or by cardiac pacing results in a marked antiarrhythmic effect against those ventricular arrhythmias that occur during a subsequent, more prolonged ischemia–reperfusion insult.

DELAYED ANTIARRHYTHMIC EFFECTS OF CARDIAC PACING

As outlined above, one of the disappointments of classical ischemic preconditioning is that the protection is relatively short-lived. In most experiments, the protection is lost as the time interval between the preconditioning stimulus and the prolonged ischemic insult is increased to one or two hours; the protection induced by cardiac pacing disappears in an even shorter time. A very important step in preconditioning research was the discovery that the protection afforded by short coronary artery occlusions returned several hours later [45,46]. Two groups, in Japan and in the United Kingdom, showed that brief periods of coronary artery occlusion resulted in *both early protection* against myocardial necrosis, disappearing within two hours, *and delayed protection*, with a time course of many hours. This phenomenon is now known as late or delayed myocardial protection or the *second window* [47].

A similar delayed protection against ischemia-induced ventricular arrhythmias, resulting from cardiac pacing, has been observed in our canine mode ([43,48,49]; see

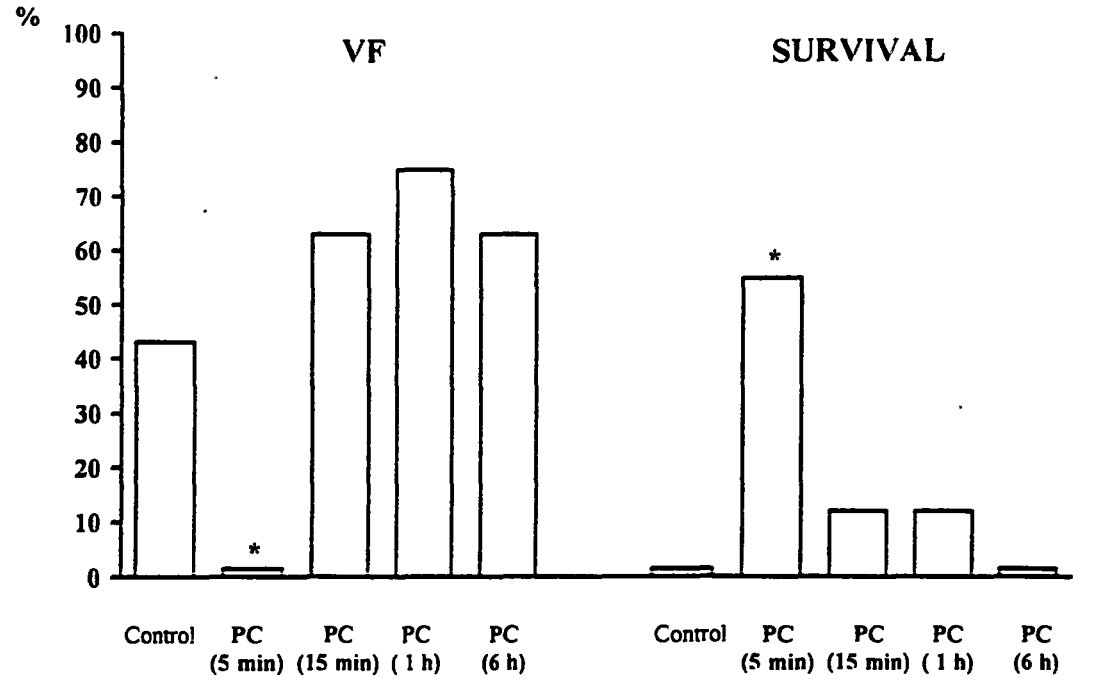

Figure 8. The incidence of ventricular fibrillation during a 25-minute occlusion of the left anterior descending coronary artery, and the overall survival from the combined ischemia–reperfusion insult, in control dogs and in dogs subjected to preconditioning by cardiac pacing at different times prior to the coronary occlusion. The incidence of ventricular fibrillation during occlusion is markedly reduced five minutes after cessation of pacing. This protection is attenuated, or abolished, if the time between the last pacing period and the occlusion is increased to 15 minutes, one hour, or six hours. Survival from the ischemia–reperfusion episode is markedly increased five minutes, but not 15 minutes, one hour, or six hours after pacing. *, $p < 0.05$ cp. control dogs.

figure 9). The total number of VPBs, the incidence and number of episodes of VT, and the incidence of ventricular fibrillation during coronary artery occlusion were all reduced in dogs paced 24 hours previously. Further, 50% of these dogs survived the combined ischemia–reperfusion insult; in contrast, there were few survivors in the sham-operated control dogs. This delayed protection against ventricular fibrillation was not observed when the time between the pacing stimulus and the occlusion was extended to 48 or 72 hours (figure 10), although these was still evidence of protection against VPBs and VT.

Recently we have tried to widen this time window of protection by repeating the preconditioning pacing stimulus at a time when the antiarrhythmic effect of the previous pacing had already waned [50]. In these experiments, we paced dogs, under light pentobarbitone anesthesia, on day 1 but instead of occluding the coronary artery on day 3, when we had determined that the antiarrhythmic protection would be virtually lost, we repeated the pacing stimulus at this time. Forty-eight hours after this second pacing stimulus (on day 5), we reanesthetized the dogs and occluded the left anterior descending coronary artery for 25 minutes. Repeated pacing during the period when the protection had faded resulted in a more prolonged protection

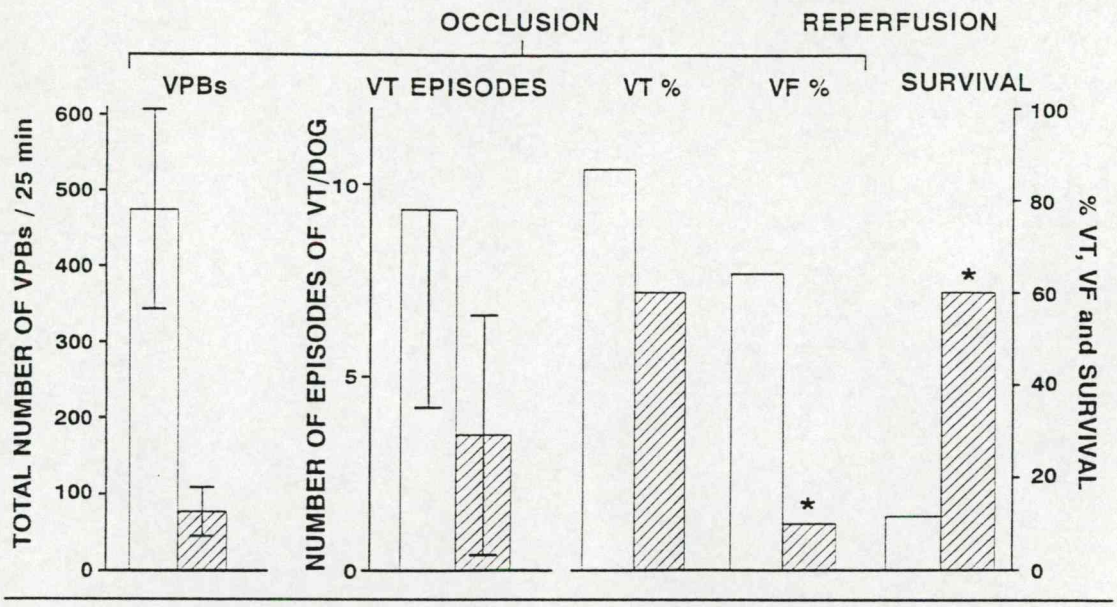

Figure 9. The incidence and severity of ventricular arrhythmias during a 25-minute coronary artery (LAD) occlusion in control dogs (open columns) and in dogs subjected to right ventricular pacing 20–24 hours previously (striped columns). Cardiac pacing markedly reduced the number of ventricular premature beats (VPBs), the number of episodes of ventricular tachycardia (VT), and the incidences of VT and ventricular fibrillation (VF) in these dogs when they were subjected to a 25-minute occlusion 20–24 hours later. Sixty percent of these dogs survived the combined ischemia–reperfusion insult; in contrast, there were few survivors in the control group. *, $p < 0.05$ cp. control, non-paced dogs.

against severe ventricular arrhythmias (VT and VF) than when dogs were paced only once, i.e., 48 hours after the first pacing [50]. Thus, at this time no dog in the repeatedly paced group died during occlusion, in contrast to a high mortality (from VF) in both the sham-operated controls and in those dogs paced only once. Further, 50% of the dogs subjected to repeated pacing survived the combined ischemia–reperfusion insult (figure 11). *It seems from these results that repeated pacing widens the time window of protection against these life-threatening ventricular arrhythmias.*

AN HYPOTHESIS FOR THE MECHANISM OF THE ANTIARRHYTHMIC EFFECT OF ISCHEMIC PRECONDITIONING

It is well accepted that cardiac adapatation induced by preconditioning is a general phenomenon; i.e., the hearts of all species so far studied can be preconditioned. Whereas the phenomenon is well described, little is known at present concerning the mechanisms involved in this protection. A number of possibilities that have been suggested to explain this marked cardioprotection have been reviewed elsewhere [51]. The most likely of these involve alterations in the generation and release of endogenous substances from either ischemic cardiac myocytes, endothelial cells, or both. These mediators may either be protective or potentially injurious [10,11,52]. It is very likely that several mediators, both protective and injurious, are released during the early phase of ischemia, that is, in response to the transient ischemic

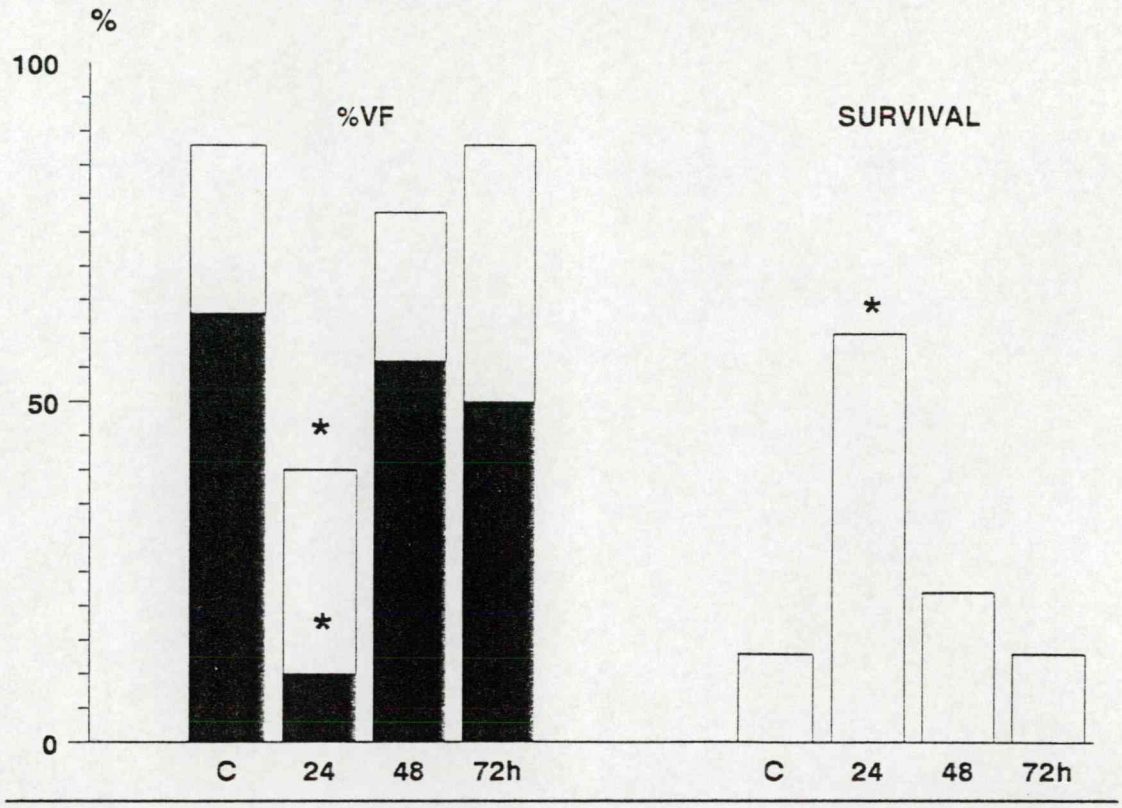

Figure 10. Delayed antiarrhythmic protection induced by cardiac pacing. This figure illustrates the incidence of ventricular fibrillation during a 25-minute occlusion of the left anterior descending coronary artery (solid columns) and following reperfusion (open columns), as well as the overall survival from the combined ischemia–reperfusion insult, in control dogs and in dogs subjected to preconditioning by cardiac pacing at different times prior to the coronary occlusion. The most marked suppression of occlusion and reperfusion ventricular fibrillation occurred 24 hours after cardiac pacing. This delayed protection was not observed if the time between the pacing stimulus and the occlusion was increased to 48 or 72 hours. *, $p < 0.05$ cp. sham-operated controls.

injury such as would occur during a preconditioning stimulus. A strong case can be made for the hypothesis that, after preconditioning, there is an increased liberation of protective substances such as adenosine, nitric oxide, and bradykinin, and a reduced release of potentially injurious substances such as endothelin and noradrenaline. It is also very likely that more than one protective mediator is released to compensate for the harmful consequences of ischemic stress. These mediators, acting at different receptors, might induce protection in different parallel ways, or there might he a final, common pathway. Some believe that this pathway involves the translocation of protein kinase C (PKC) from the cytosol to the sarcolemmal and nuclear membranes. This subject has been recently reviewed in depth [53].

The role of adenosine, as originally proposed by Downey and colleagues [54], with the subsequent involvement of PKC activation, in mediating the infarct size limitation associated with ischemic preconditioning seems clear in most species except rats (reviewed recently by Miura [55]). However, there is no evidence that adenosine plays any role in the antiarrhythmic effect of ischemic preconditioning

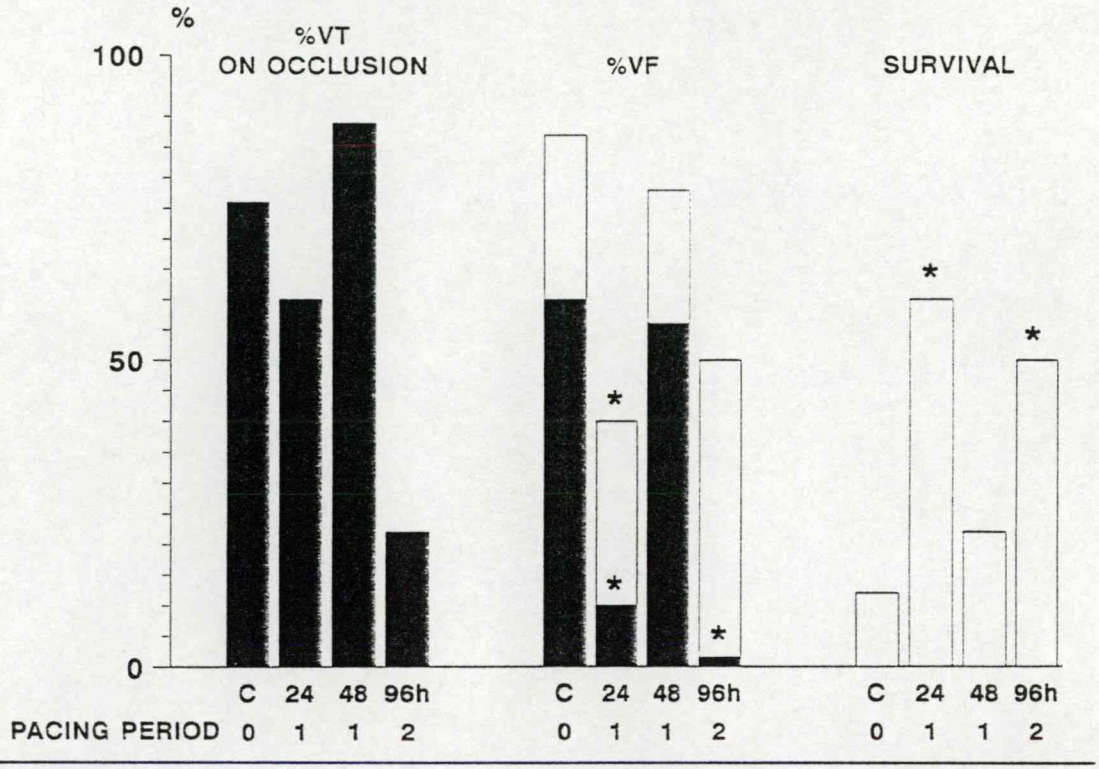

Figure 11. Delayed antiarrhythmic protection induced by repeated pacing. The incidences of ventricular tachycardia (VT) and ventricular fibrillation (VF) during occlusion (solid columns) and following reperfusion (open columns) are shown, as well as survival from the combined ischemia–reperfusion insult, in control dogs, in dogs paced 24 or 48 hours before coronary artery occlusion, and in dogs subjected to repeated pacing both 48 hours before coronary artery occlusion and again 48 hours after this first pacing stimulus. Repeated pacing at a time when the protection from the initial pacing stimulus had faded resulted in more prolonged protection against VT and VF and an increased survival (at 96h) compared to when dogs were paced only once 48 hours before the occlusion. *, $p < 0.05$ cp. sham-operated controls.

either in rats [56] or dogs [57], despite the fact that in both these species adenosine can act as an endogenous antiarrhythmic substance [58].

The hypothesis that other endogenous protective substances, particularly those derived from the coronary vascular and endocardial endothelium, are involved in the antiarrhythmic effects of ischemic preconditioning comes mainly from studies performed in the canine model described above. This hypothesis [59] is outlined in figure 12. It assumes that the target organ for preconditioning is the coronary vascular endothelium and that mediators, derived from such endothelial cells, modify arrhythmogenesis by direct communication, via diffusable mediators, with cardiac myocytes. In brief, the evidence, which has been recently summarized in some depth [60], is as follows:

1. Evidence from coronary endothelial denudation using a detergent. Although this approach is not possible in the *in vivo* canine heart, studies using rat isolated perfused hearts in which the coronary vascular endothelium had been removed

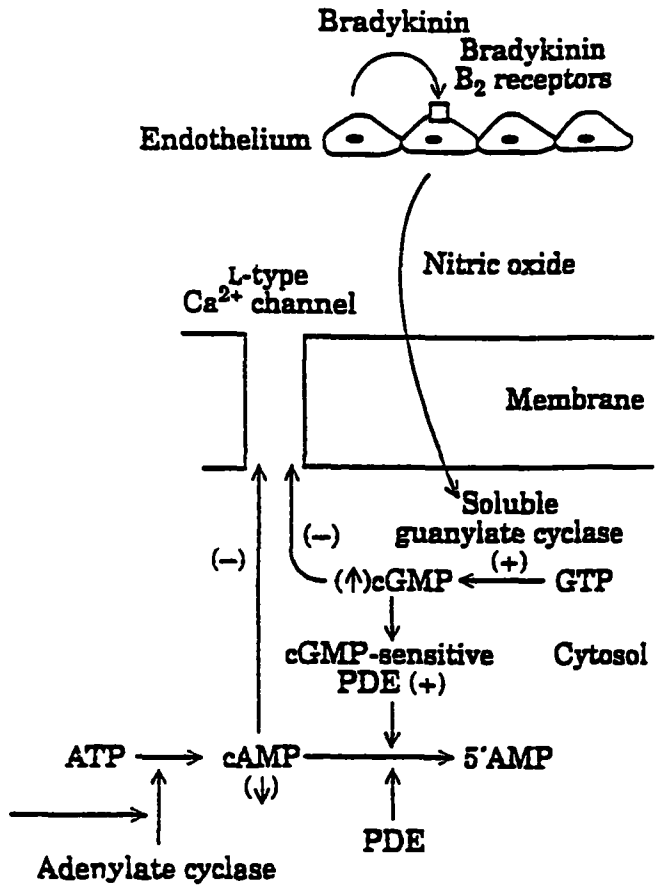

Figure 12. Role of endothelium-derived endogenous protective mediators in ischemic preconditioning—an hypothesis. Bradykinin is released, probably from endothelial cells (which have the mechanisms for generating and releasing kinins), and then acts on B2 receptors on the endothelial surface to increase the calcium transient within these cells and to activate the L-arginine nitric oxide pathway. Nitric oxide then "talks" to the cardiac myocyte, stimulates soluble guanylyl cyclase, and elevates cyclic GMP. This stimulates cGMP-sensitive phosphodiesterase (thus reducing cAMP levels), inhibits calcium entry through L-type calcium channels, and depresses myocardial contractility. From [59], with permission.

revealed that coronary artery occlusion resulted in a higher incidence, and greater severity, of arrhythmias than in control, endothelium-intact hearts [61]. For example, in Sprague–Dawley rats there was a mean of 2724 ± 434 ventricular premature beats over the 20-minute occlusion period in endothelium-denuded hearts compared with only 255 ± 39 beats in hearts in which the coronary vascular endothelium was intact. The incidence and duration of ventricular tachycardia during the occlusion period were also much greater in endothelium-denuded hearts (100% and 711 ± 186 seconds compared to 75% and 14.5 ± 5.4 seconds) [61]. These results suggest that, following coronary occlusion, protective substances are released from the coronary vascular endothelium that modify arrhythmia severity.

2. Substances normally derived from vascular endothelial cells (for example, bradykinin and prostacyclin), when infused locally into a side branch of the coronary

artery to be occluded or directly into the artery itself, are profoundly antiarrhythmic [62,63]. In the case of bradykinin, this protection is mediated through nitric oxide generation, since it is markedly attenuated in the presence of an inhibitor of the L-arginine nitric oxide pathway [64].

There is also some evidence that the amount of prostacyclin released under conditions of coronary artery occlusion is related to the number of ventricular premature beats that occurs up to that sampling time [65].

3. Potentiation of mediator release also suppresses early ischemia-induced ventricular arrhythmias. For example, nafazatrom, which inhibits prostacyclin breakdown, is antiarrhythmic in the canine model [66]. We have yet to examine whether treatment with angiotensin-converting enzyme (ACE) inhibitors modifies early ischemia-induced arrhythmias in the canine model that we have used to demonstrate the antiarrhythmic effects of ischemic preconditioning. Whether this is true in other models, and in humans, remains uncertain but has been recently reviewed [67].

4. Evidence for the release of protective mediators during preconditioning comes from studies in which their generation by endothelial cells, or their effects at receptor level, have been inhibited. Thus, blockade of bradykinin B_2 receptors with icatibant increases the severity of arrhythmias following coronary artery occlusion [68,69] (figure 13), as well as markedly attenuating the protective effects of ischemic preconditioning. This finding suggests that *bradykinin* is a key mediator in protection against ischemia-induced arrhythmias.

The situation regarding *prostacyclin* is more complicated because it is not possible to selectively inhibit its generation without influencing the release of a variety of cyclooxygenase products that influence coronary vascular dynamics and arrhythmogenesis. There is some evidence in the canine model that the nonspecific inhibition of all cyclooxygenase products markedly attenuates the antiarrhythmic effects of preconditioning [6] and that the dual inhibition of both the cyclooxygenase *and* L-arginine nitric oxide pathways completely prevents this protection [70]. The effects of prostacyclin on arrhythmogenesis during coronary artery occlusion have been extensively reviewed [71,72].

The role of *nitric oxide* in modulating arrhythmia severity during ischemia and preconditioning has been analyzed in two ways. First, the antiarrhythmic effects of preconditioning are attenuated following inhibition of the L-arginine nitric oxide pathway [73]. Second, the local intracoronary administration of methylene blue (which inhibits the effect of nitric oxide on soluble guanylyl cyclase) completely abolishes the antiarrhythmic effect of preconditioning [74]. This outcome is illustrated in figure 14.

The above evidence suggests that endothelial-derived substances protect against the consequences of ischemia by suppressing life-threatening ventricular arrhythmias and that preconditioning in some way stimulates this release. This conclusion would provide a partial explanation why, when there is coronary vascular endothelial dysfunction (as in hypertension, atherosclerosis, ventricular hypertrophy and

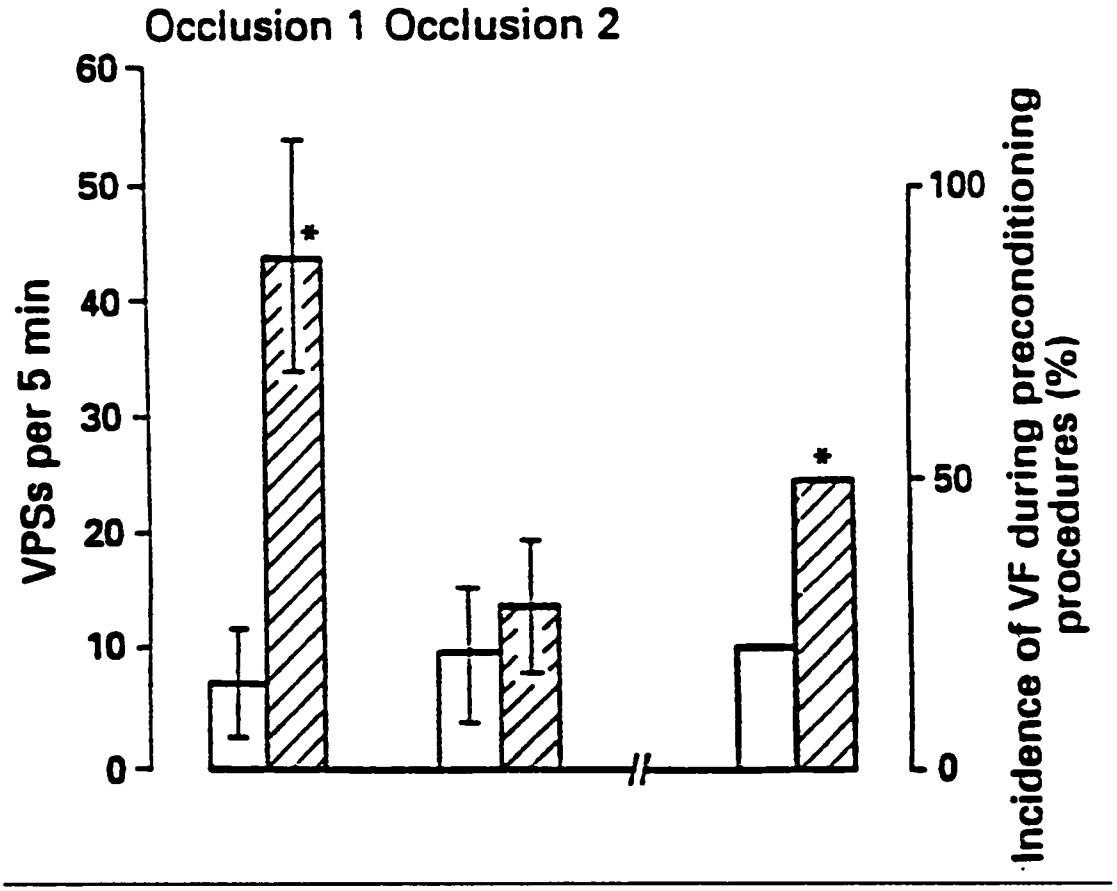

Figure 13. Ventricular premature beats during two five-minute preconditioning coronary artery occlusion periods in dogs in the absence (open columns) and presence (hatched columns) of the bradykinin B2 antagonist icatibant. On the right is shown the incidence of ventricular fibrillation that occurred during the preconditioning procedure in the two groups of dogs. Arrhythmia severity is increased during the preconditioning stimulus in the presence of icatibant. From [68], with permission.

ischemic heart disease), arrhythmia severity might be increased, although the precise relationship between endothelial dysfunction and arrhythmia severity in patients is almost impossible to document. Certainly, in spontaneously hypertensive rats, where there is evidence of endothelial dysfunction, there is an increased arrhythmia severity following coronary artery occlusion [75].

POSSIBLE MECHANISMS OF DELAYED MYOCARDIAL PROTECTION AFFORDED BY RAPID CARDIAC PACING

We know much less about the mechanisms involved in the delayed protection against ischemia-induced arrhythmias described above than we do regarding the protective effects of "classical" ischemic preconditiong. The possible mechanisms of this second window of protection in reducing myocardial necrosis have been recently reviewed [76]. They include the induction of heat-shock (stress) proteins, an increase in antioxidant activity, the induction of nitric oxide synthase (iNOS),

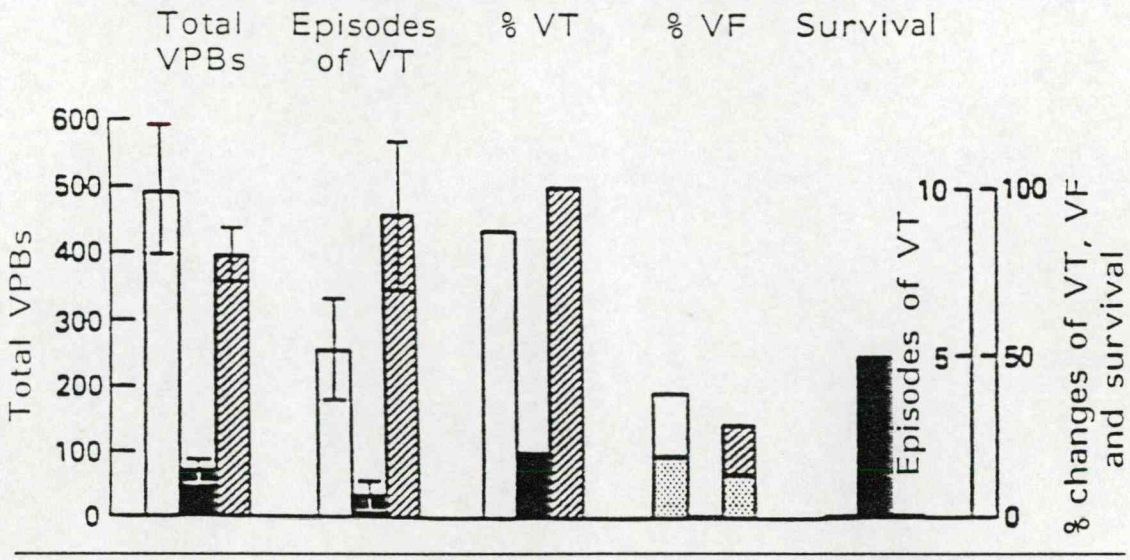

Figure 14. The effect of infusing methylene blue (by intracoronary infusion and given during both preconditioning and the prolonged occlusion in a total dose of 325 mg; shaded columns) on the protective effects of ischemic preconditioning (solid columns) in anesthetized mongrel dogs. The control data are seen in the initial open columns. Shown are the total number of ventricular premature beats (VPBs) during the 25-minute occlusion period, the number of episodes and incidence of ventricular tachycardia (VT), the incidence of ventricular fibrillation (VF), and survival from the combined ischemia–reperfusion insult. The incidence of VF is given both as the total incidence throughout the 25-minute occlusion period and during the first five minutes (stippled column). Reproduced from [74], with permission.

and the role of adenosine-mediated protein kinase C translocation and the influence this has on nuclear transcription events through activation of other kinase signal cascades.

There are only two series of experiments that shed light on the mechanisms of the delayed antiarrhythmic effect of cardiac pacing outlined above. These show that protection is abolished by prior treatment with dexamethasone [49] or by prior administration of icatibant [77]. These results again suggest that mediators normally derived from endothelial cells are also involved in this delayed protection. One possible explanation for the reversal of the protection by dexamethasone is prevention of the induction of nitric oxide synthase and cyclooxygenase-2, although, of course, dexamethasone has many other actions. The induction of these enzymes has also been suggested as a possible mechanism for the delayed antiarrhythmic effects of bacterial endotoxin (and the nontoxic monophosphoryl derivative of the lipid A component of the endotoxin molecule) that have been recently described [78–80]. These protective effects of endotoxin are cytokine mediated [81].

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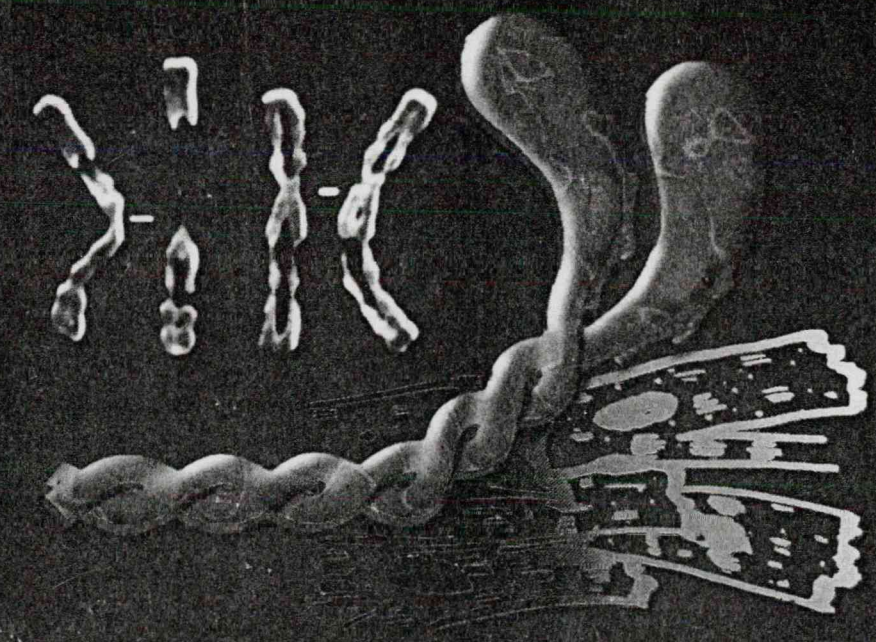

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DUAL BLOCKADE OF THE CYCLO-OXYGENASE AND L-ARGININE-NITRIC OXIDE PATHWAYS PREVENTS THE ANTIARRHYTHMIC EFFECT OF PRECONDITIONING Tu136

Adrienn Kis, Agnes Vegh, Julius Gy Papp, *James R Parratt.
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The mechanism of the antiarrhythmic effect of preconditioning (PC) involves the generation of nitric oxide (NO) and prostacyclin (PGI_2), since separate blockade of the cyclo-oxygenase and L-arginine-NO pathways attenuates this protection. We have now examined whether blockade of both pathways is able to abolish completely this marked cardioprotection. In anaesthetized dogs PC was induced by two 5 min occlusions of the left anterior descending (LAD) coronary artery, followed, 20 min later, by a 25 min occlusion of that same artery. Meclofenamate (M; 2 $mgkg^{-1}$, iv) and L-NAME (5 $mgkg^{-1}$,ic) were given either before the PC procedure or after PC but before the long occlusion. PC markedly reduced the severity of arrhythmias (VPBs: 72±21 v 429±68 in controls: $P<0.05$, %VT; 21% v 95%; VT episodes: 0.7±0.4 v 7.6±2.1; $P<0.05$; %VF 0% v 44%; $P<0.05$), and increased survival (47% v 0%). However, it was difficult to precondition dogs in the presence of M and L-NAME: 77% of the dogs died during the PC procedure and the remaining dogs fibrillated during prolonged occlusion. When M and L-NAME were given before the long occlusion, the protection was attenuated but still present (VPBs: 118±74; VT episodes; 2.6±2.2, %VT: 50%, %VF: 17%). The results show that NO and PGI_2 generated during the PC procedure contribute to the antiarrhythmic effect of PC. Furthermore, dual blockade of the cyclo-oxygenase and L-arginine-NO pathways prevents the protection more effectively than inhibition of either pathway alone. Supported by OTKA (T-5725), British Council, OMFB and the European Commission (ERB CT 924009).

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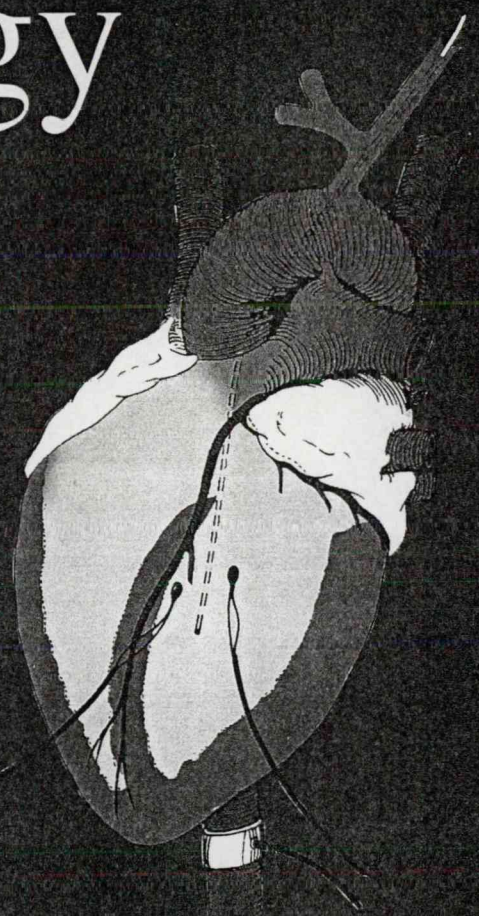

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ABSTRACTS

Bologna, June 18-21, 1996

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ISHR International Society for Heart Research

Published for the International Society for Heart Research

Kis Adrienn, Végh Ágnes, Papp Gyula, *Parratt James
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A PREKONDICIONÁLÁS ANTIARITMIÁS HATÁSÁNAK BEFOLYÁSOLÁSA A CIKLOOXIGENÁZ ÉS L-ARGININ-NITROGÉN MONOXID SZINTÉZIS UTAK GÁTLÁSÁVAL

miokardiális infarktus, prekondicionálás, aritmia

A miokardiális iszkémia során fellépő kamrai aritmiák súlyossága jelentős mértékben csökkenthető, ha a koszorúéren előzetesen rövid ideig tartó okklúziókat végzünk. A jelenség, amely prekondicionálásként (PC) ismert, olyan endogén anyagok keletkezésének tulajdonítható mint a prosztaciklin vagy a nitrogén monoxid (NO), mivel mind a cikloogigenáz enzim gátlása meclofenamattal, mind az L-arginin-NO szintézis út blokkolása L-NAME-el mérsékeli a PC antiaritmiás hatását. Jelen kisérleteinkben azt vizsgáltuk, hogy vajon a védelem teljesen megszüntethető-e, ha a két szintézis utat együttesen gátoljuk. Kisérleteinket altatott kutyák három csoportjában végeztük. A kontroll csoportban a bal koronária arteria descendens anterior ágát (LAD) 25 percre elzártuk, melyet reperfúzió követett. A második, PC csoportban, kétszer 5 perces LAD lefogást végeztünk, majd 20 perc elteltével a koszorúeret 25 percre ismételten elzártuk. A harmadik csoportban meclofenamátot (2 mg kg^{-1}, iv) és L-NAME-t (5 mg kg^{-1}, ic) az első PC okklúzió előtt 30 illetve 20 perccel adtuk. Ezután az állatokat hasonló PC protokollnak vetettük alá mint a második csoportban. A kezeletlen, PC csoportban jelentősen csökkent a kamrai aritmiák súlyossága a hosszú koronária okklúzió alatt. Így a kamrai extraszisztolék száma (ES: 72 ± 21 v 429 ± 68; P < 0.05), a kamrai tachikardia gyakorisága (VT: 21 % v 94 %; P < 0.05), a VT epizódok száma (0.7 ± 0.4 v 7.6 ± 2.1; P < 0.05) és a kamrafibrilláció gyakorisága (VF: 0 % v 44 %) szignifikánsan kisebb volt mint a kontroll csoportban. Ugyanakkor azokban a kutyákban, amelyekben a prekondicionálást meclofenamat és L-NAME jelenlétében végeztük, az állatok 50 %-a a rövid okklúziók alatt, a fennmaradó 50 % pedig a hosszú okklúzió alatt kamrafibrillációban elpusztult. Így túlélés nem volt, szemben a PC csoporttal, amelyben az állatok 47 %-a túlélte az okklúziós-reperfúziós inzultust. Eredményeink arra utalnak, hogy a PC során keletkező prosztaciklin és NO jelentős szerepet játszik a prekondicionálás antiaritmiás hatásában. A ciklooxigenáz és az L-arginin-NO szintézis utak együttes gátlása hatékonyabban csökkenti a PC nyújtotta védelmet, mint az utak külön-külön történő blokkolása. Készült az OTKA (T-5725) támogatásával.

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ANTIARRHYTHMIC EFFECTS OF PRECONDITIONING ARE PREVENTED BY A DUAL BLOCKADE OF CYCLO-OXYGENASE AND L-ARGININE-NITRIC OXIDE PATHWAYS

myocardial infarction, preconditioning, arrhythmias

Short periods of myocardial ischemia induced by coronary artery occlusion reduce the severity of ventricular arrtyhtmias the occur during a subsequent more prolonged period of ischaemia. This phenomenon, which is known as preconditioning (PC), might be due, in part, to the generation of nitric oxide (NO), or prostacyclin, since either the inhibition of the cyclooxygenase pathway by meclofenamate or the blockade of the L-arginine-NO pathway by L-NAME attenuated the antiarrhythmic effect of PC. We have now examined whether this protection would be completely prevented by a dual blockade of these pathways. Three groups of anaesthetized, artificially ventilated dogs were used. In the control group the animals were subjected to a 25 min occlusion of the left anterior descending coronary artery (LAD), followed by reperfusion. In the second, PC group, dogs underwent two 5 min occlusions of the LAD followed, 20 min later, by a prolonged (25 min) occlusion. The myocardium was then reperfused. In the third group meclofenamate (2 mg/kg, iv) and L-NAME (5 mg/kg ic) were administered 30 and 20 min prior to the first PC occlusion, respectively. Then the animals were subjected to the usual preconditioning protocol. Preconditioning in the absence of meclofenamate and L-NAME markedly reduced the severity of ventricular arrhythmias during the long occlusion (ventricular premature beats (VPB): 72 ± 21 v 429 ± 68 in controls; P < 0.05, incidence of VT 21% v 94%: number of episodes of VT per dog 0.7 ± 7.6 ± 2.1; P < 0.05; incidence of VF 0% v 44%.). However, it was difficult to precondition dogs in the presence of meclofenamate and L-NAME. Most of the dogs died during the PC procedure (50%) and the remaining dogs fibrillated during long coronary occlusion. Furthermore, whereas in the PC group 47% of the dogs survived the combined ischaemia-reperfusion insult in the treated group neither of the dogs survived. The result show that the generation of NO and prostacyclin during short periods of ischaemia contributes to the protective effect of preconditioning. It seems also that the dual blockade of the cyclooxygenase and L-arginine-NO pathways more effectively prevents the protection than inhibition of these pathways alone. Supported by OTKA 8T-5725).

VII.

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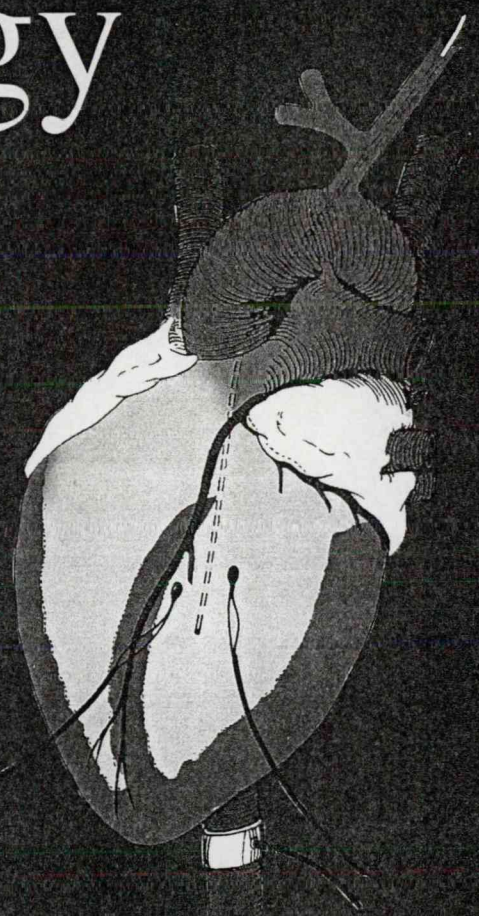

International Society for Heart Research
XVII European Congress

ABSTRACTS

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REPEATED PACING WIDENS THE TIME WINDOW OF DELAYED PROTECTION AGAINST VENTRICULAR ARRHYTHMIAS IN DOGS

Adrienn Kis, Ágnes Végh, Julius Gy. Papp, *James R. Parratt. Departments of Pharmacology, Albert Szent-Györgyi Medical University, Szeged, *University of Strathclyde, Glasgow, UK

We have shown earlier that right ventricular pacing (4x5 min, 220 beats min^{-1}) markedly reduces the severity of ventricular arrhythmias which occur during a subsequent, 25 min occlusion-reperfusion, 24h later. This protection is, however, largely lost when the time between pacing and the occlusion is increased to 48 or 72h. In the present study we examined, whether this protection can be regained or extended if the dogs are subjected to repeated pacing at that time (ie. 48h after the first pacing), when the antiarrhythmic protection has already wained. Thus, under light pentobarbitone anaesthesia dogs ($n=10$) were paced two times, ie. 96 and 48h before a 25 min occlusion of the left anterior descending (LAD) coronary artery. Repeated pacing resulted in a lower incidence of VT (40 % v 89 %; $P<0.05$) and VF (10 % v 56 %; $P<0.05$) during occlusion and an increased survival (40 % v 22 %; $P<0.05$) from the combined ischaemia reperfusion insult compared to dogs paced only once 48h before the occlusion. Since more dogs in the repeat pacing group survived the 25 min occlusion period, both the number of VBPs (139 $\pm$ 62 v 79 $\pm$ 34) and the episodes of VT (2.3 $\pm$ 1.7 v 1.4 $\pm$ 0.6) was somewhat higher than in dogs paced only once before the occlusion. These results indicate that the time window of delayed protection against arrhythmias can be extended by repeated moderate pacing stimuli, applied at a time when the protection from the previous pacing had already disappeared.

Supported by the Hungarian Scientific Research Foundation (OTKA), the joint grant of the OMFB and The British Council and by the Hungarian Health Science Council (T-06521/93).

VIII.

Cardiologia Hungarica

SCIENTIFIC JOURNAL OF THE HUNGARIAN SOCIETY OF CARDIOLOGY

Főszerkesztő: Prof. DR. NASZLADY ATTILA • Szerkesztőségi titkár: DR. TOMCSÁNYI JÁNOS

A MAGYAR KARDIOLÓGUSOK TÁRSASÁGA TUDOMÁNYOS KONGRESSZUSA 1996. MÁJUS 8–11.

Kongresszusi előadás-kivonatok

(ABSTRACTS)

TARTALOM

Kis Adrienn, Kaszala Károly, Végh Ágnes, Papp Gyula, *Parratt James
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ISMÉTELT SZÍVINGERLÉS ERŐSÍTI A PREKONDICIONÁLÁS KÉSŐI ANTIARRHYTHMIÁS HATÁSÁT

prekondicionálás, arrhythmia, szívingerlés

Korábbi kísérleteink alapján ismert, hogy egy koszorúér elzárásával kialakított miokardiális iszkémia során fellépő arrhythmiák száma és súlyossága jelentősen csökken, ha 24 órával korábban a szívet mérsékelt frekvenciával ingereljük. Ez az ún. késői védelem (Second Window of Protection, SWOP) csökken illetve megszűnik, ha a prekondicionáló szívingerlés és az okklúzió között 48 illetve 72 óra telik el. Jelen kísérleteinkben arra kerestük a választ, vajon erősíthető-e a védelem, ha a szívet ismételten ingereljük egy olyan időpontban, amikor a védelem már gyengül. Pentobarbitallal felületesen narkotizált kutyákban, a jobb kamrába vezetett bipoláris elektród segítségével, egy (1 csoport, n = 9) illetve két alkalommal (2 csoport, n = 9), 48 órás időközökkel, 4-szer 5 percen keresztül, 220 ütés/perc frekvenciával ingereltük a szívet. Az utolsó ingerlést követően 48 óra múlva az állatokat kloralóz-uretán keverékével ismételten elaltattuk, a mellkast megnyitottuk és a bal koronária artéria descendens anterior ágát 25 percre elzártuk. Ezt követően az iszkémiás miokardiumot reperfundáltuk. Ellentétben azokkal a kutyákkal, amelyeket csak egy alkalommal prekondicionáltunk, a szív kétszeri ingerlése után csökkent az okklúzió alatti kamrai tachicardia gyakorisága (89 % vs 37 %), a kamrafibrilláció előfordulása (56 % vs 11 %) és jelentősen fokozódott az iszkémiás-reperfúziós inzultus utáni túlélés (22 % vs 45 %). Eredményeink arra utalnak, hogy a szív 48 órás időközökben történő ismételt ingerlése jelentősen nyújthatja a prekondicionálás arrhythmiákkal szemben megnyilvánuló késői védőhatását. Elképzelhető, hogy a szívet bizonyos időközökben mérsékelten ingerelve, a miokardium folyamatos védelme alakítható ki. Ennek kiderítése azonban további vizsgálatokat igényel.

Készült az OTKA (T 16602), az OMFB és The British Council közös pályázatának (NP-747/94 GB) valamint az Egészségügyi Tudományos Tanács (T-06521/93) támogatásával.

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Department of Pharmacology, Albert Szent-Györgyi Medical University, Szeged, Hungary and *Department of Physiology & Pharmacology, University of Strathclyde, Glasgow, UK.

PROLONGATION OF THE SECOND WINDOW OF PROTECTION BY REPEATED CARDIAC PACING IN DOGS

preconditioning, arrhythmias, cardiac pacing

We have shown in our earlier experiments that the severity of ventricular arrhythmias occurring during a 25 min occlusion of a coronary artery is markedly reduced if the dogs are subjected to moderate cardiac pacing 24 h previously. This delayed protection (or Second Window of Protection, SWOP) is attenuated or abolished 48 or 72 h after cardiac pacing. The objective of the present study was to determine whether this protection can be extended if the dogs are subjected to a second series of cardiac pacing at that time when the antiarrhythmic effect of preconditioning resulting from the previous pacing has largely disappeared. Under light pentobarbitone anaesthesia dogs were paced (4 x 5 min, 220 beats/min) once (group 1, n = 9) or two times (group 2, n = 9) with an interval of 48 h, by means of a bipolar pacing electrode inserted into the right ventricle. 48 h later the dogs were re-anaesthetised with a mixture of chloralose and urethane, thoracotomized and the left anterior descending (LAD) coronary artery was occluded for 25 min, and then reperfused. In dogs subjected to repeated pacing the incidence of ventricular tachycardia (89 % vs 37 %) and ventricular fibrillation (56 % vs 11 %) during occlusion was markedly reduced, and there was an increased survival from the combined ischaemia/reperfusion insult (22 % vs 45 %) compared to those dogs which were preconditioned by pacing only once (ie. 48 h before coronary occlusion). It seems from our results that preconditioning by repeated cardiac pacing extends the time window of the delayed protection against ventricular arrhythmias occurring during a subsequent ischaemic episode. We suppose that a regular, frequent but moderate stimulation of the heart might provide a prolonged myocardial protection. To clarify this possibility further studies are required.

This work was supported by the Hungarian Scientific Research Foundation (OTKA, T 16602), the joint grant of the Hungarian Technological Development (OMFB) and The British Council (NP-747/94 GB) and by the Hungarian Health Science Council (T-06521/93).

IX.

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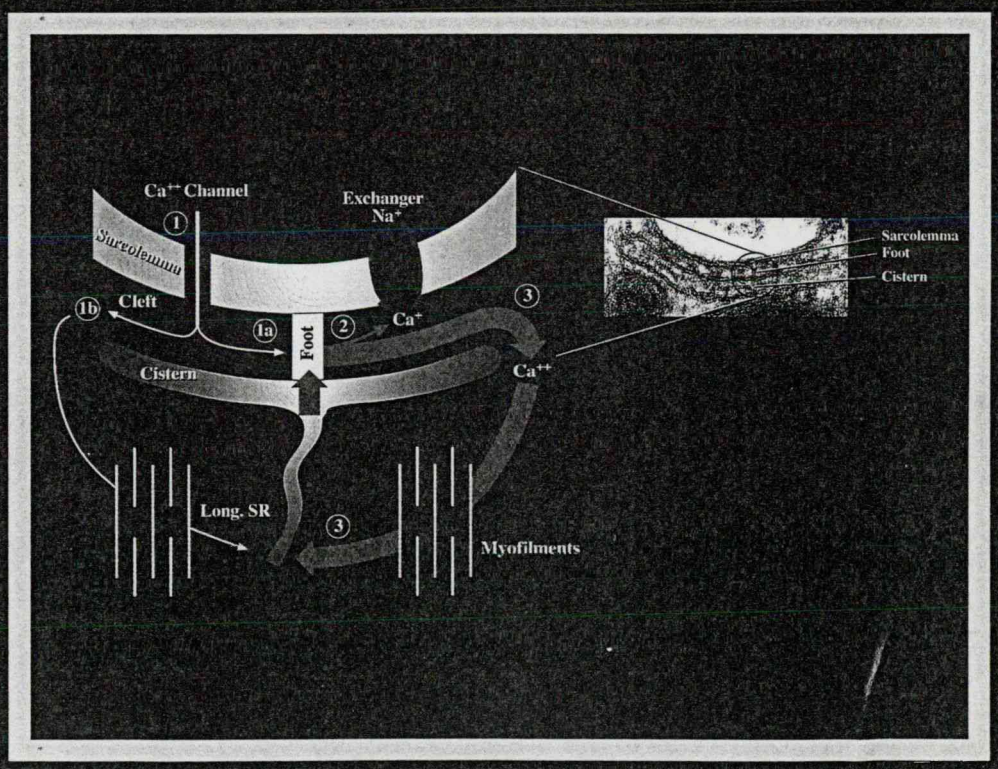

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REPEATED PACING MARKEDLY PROLONGS THE DELAYED ANTIARRHYTHMIC PROTECTION IN ANAESTHETISED DOGS

Sa62

Adrienn Kis, Ágnes Végh, Julius Gy. Papp, *James R. Parratt. Depts of Pharmacology, A. Szent-Györgyi Med. Univ. Szeged, Hungary and +Strathclyde Univ. Glasgow, UK.

We have demonstrated that repeated cardiac pacing prolongs the protection against those ventricular arrhythmias which occur during a 25 min occlusion/reperfusion of the left anterior descending (LAD) coronary artery, 48 h later (*Kis et al., J Mol Cell Cardiol., 28: 229, 1996*). In this study we examined whether this protection is still present if the time between the second pacing stimulus and the occlusion is increased to 72 h. Therefore we paced dogs, under light pentobarbitone anaesthesia, on day one and day three, four times for 5 min at a rate of 220 beats/min, and subjected to coronary artery occlusion 72 h later. Sham operated dogs served as controls. Repeated pacing resulted in a more prolonged protection than when dogs were paced only once. Compared to the sham-operated controls, occlusion of the LAD, 72 h after the second pacing stimulus, resulted in a reduced number of VPBs (78 ± 44 vs 219 ± 49, $P<0.05$) and episodes of VT (1.2 ± 1.2 vs 6.1 ± 2.9, $P<0.05$) and a lower incidences of VT (11% vs 75%, $P<0.05$) and VF (0% vs 50%,P<).)5). Survival from the combined ischaemia-reperfusion insult was also increased (78% vs 8%, $P<0.05$) in dogs subjected to repeated pacing, 72 h previously. It seems from these results that following repeated pacing a marked antiarrhythmic protection is still present even 72 h after the pacing stimulus.

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X.

JOURNAL FÜR KARDIOLOGIE

4. Jahrgang
Sonderheft 2/1997
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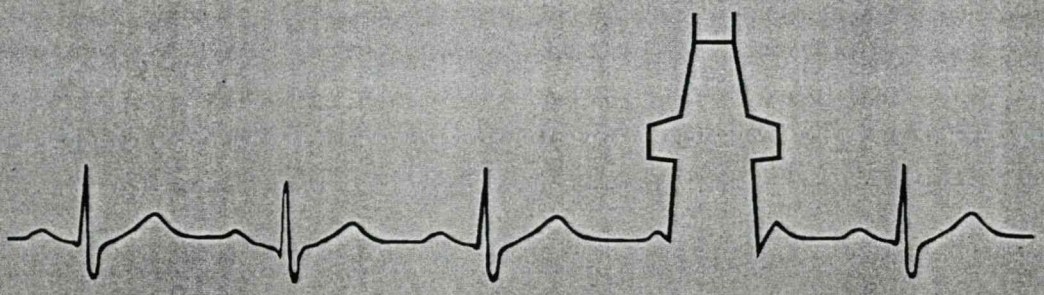
ÖSTERREICHISCHE ZEITSCHRIFT FÜR HERZ-KREISLAUFERKRANKUNGEN



5th Alpe-Adria Cardiology Meeting in Austria at the University of Graz / Vorklinische Institute Harrachgasse 21, Graz 8.–10. May 1997

Abstracts + Posters

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faded, widens the time window of protection against life-threatening ventricular arrhythmias.

REPEATED PACING WIDENS THE TIME WINDOW OF DELAYED ANTIARRHYTHMIC PROTECTION IN CANINE

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We have shown earlier that in anaesthetised dogs four 5 min periods of cardiac pacing via the right ventricle at a rate of 220 beats min^{-1} protect the myocardium against those ventricular arrhythmias which occur during a 25 min occlusion of the left anterior descendens coronary artery (LAD) 24 h later. This delayed protection is, however, markedly attenuated or abolished if the time between the pacing stimulus and occlusion is increased to 48 or 72 h. The present study examined whether this protection could be prolonged if the animals are subjected to an additional pacing stimulus at a time when the antiarrythmic effect of the previous pacing had already vanished. Thus, we paced dogs on day one under light pentobarbitone anaesthesia but instead of occluding the coronary artery on day three when we know that the antiarrhytmic protection is virtually lost, we repeated the pacing stimulus. Repeated pacing resulted in a more prolonged protection than when dogs were paced only once. Thus, at this time in dogs subjected to repeated pacing there were lower incidences of VT (25% v 89%) and VF (0% v 56%) during occlusion, and an increased survival from the combined ischaemia-reperfusion insult (50% v 22%) compared to those dogs which were paced only once 48 h before the ischaemia. It seems from these results that repeated pacing during the period when the protection has

XI.

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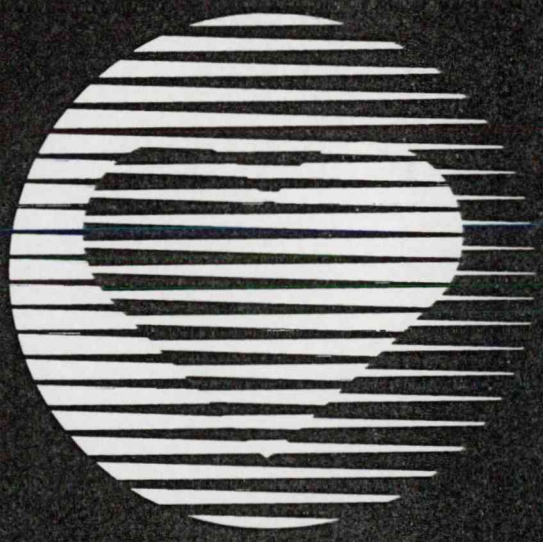
Journal of Molecular and Cellular Cardiology



XVI World Congress of the International Society for Heart Research, Cardiovascular Biology and Medicine into the 21st Century

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PACING-INDUCED DELAYED ANTIARRHYTHMIC PROTECTION IS ATTENUATED BY AMINOGUANIDINE IN DOGS

280

Adrienn Kis, Ágnes Végh, Julius Gy. Papp, *James R. Parratt. Depts. of Pharmacology, A. Szent-Györgyi Med. Univ. Szeged, Hungary, *Univ. Strathclyde, Glasgow, UK.

We have shown earlier that delayed antiarrhythmic protection, resulting from cardiac pacing 24 h before an ischaemic insult, was markedly reduced by the prior administration of dexamethasone. These results suggested that either induction of nitric oxide synthase (NOS) or a cyclooxygenase enzyme might be involved in this delayed antiarrhythmic protection. The aim of the present study was to examine whether aminoguanidine (AG), a more selective inhibitor of iNOS, reduces pacing-induced delayed antiarrhythmic protection. Preconditioning was induced by right ventricular pacing (4 x 5 min, 220 beats min^{-1}), 24 h before a 25 min occlusion of the left anterior descending coronary artery (LAD; n = 10). In nine dogs AG was administered intravenously (50 mg kg^{-1}) 30 min prior to the pacing procedure, ie. 24 h before coronary artery occlusion. Sham-operated controls (n = 8) were subjected to occlusion without pacing. Compared to the controls, cardiac pacing resulted in a marked reduction in the number of VPBs (77 ± 32 vs 474 ± 139, $P < 0.05$) and in the incidence of VF (10 % vs 63 %, $P < 0.05$) during occlusion. These were increased again in dogs given AG (VPBs: 244 ± 116, VF: 45 %). Survival from the combined ischaemia-reperfusion insult was increased in paced dogs (60 % vs 0 % in controls, $P < 0.05$), but this protection was reduced in AG treated dogs (22 %, $P < 0.05$ vs paced dogs). These results confirm that generation of NO is involved in the mechanism of delayed antiarrhythmic protection, perhaps through the inducion of iNOS.

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XII.

Cardiologia Hungarica

SCIENTIFIC JOURNAL OF THE HUNGARIAN SOCIETY OF CARDIOLOGY

A MAGYAR KARDIOLÓGUSOK TÁRSASÁGA TUDOMÁNYOS KONGRESSZUSA

SUPPL. 98/1

1997. MÁJUS 13–16.

Főszerkesztő: Prof. DR. NASZLADY ATTILA • Szerkesztőségi titkár: DR. TOMCSÁNYI JÁNOS

Kongresszusi előadás-kivonatok

(ABSTRACTS)

TARTALOM

Kis Adrienn, Végh Ágnes, Papp Gyula, James R. Parratt
Pharmacologiai Intézet, Szent-Györgyi Albert Orvostudományi Egyetem, Szeged, Élettani és Pharmacologiai Intézet, Strathclyde Egyetem, Glasgow, UK.

A NITROGÉN OXID SZEREPE A MAGAS FREKVENCIÁJÚ SZÍVINGERLÉSSEL VÉGZETT PREKONDICIONÁLÁS KÉSŐI ANTIARITMIÁS HATÁSÁBAN ALTATOTT KUTYÁKBAN

arrhythmias, ischaemia, myocardial protection

Korábbi kísérleteinkben kimutattuk, hogy a szív magas frekvenciájú ingerlése jelentős védelmet biztosít a 24 óra múlva bekövetkező akut miokardiális iszkémia során fellépő súlyos kamrai aritmiákkal szemben. Jelen kísérleteinkben a nitrogén oxid (NO) képződés szerepét vizsgáltuk a késői antiaritmiás védőhatásban az indukálható nitrogén oxid szintáz (iNOS) enzimet szelektíven gátló aminoguanidin alkalmazásával. Prekondicionálást a szív 4x5 percig tartó 220 ütés/perc frekvenciájú ingerlésével idéztünk elő felületes pentobarbitál narkózisban a jobb kamrába vezetett bipoláris ingerlő elektód segítségével. 24 óra múlva a kutyákat kloralóz-uretán keverékével ismételten elaltattuk, a mellkast megnyitottuk és a bal elülső leszálló koronária artériát 25 percre elzártuk (PC, n=10). A prekondicionált kutyák másik csoportjában a szívingerlés előtt 30 perccel intravénásan aminoguanidint adtunk 50 mg/kg dózisban (AG, n=9). A kontroll csoportban (K, n=8) a műtéti beavatkozásokat, ill. 24 óra múlva a koszorúér elzárását elvégeztük, de a szívet nem ingereltük. A szív magas frekvenciájú ingerlése jelentősen csökkentette a kamrai aritmiák súlyosságát. A kontroll csoporthoz képest a szívingerelt állatokban a kamrai extraszisztolék száma 333±120-ról 77±30-ra (P<0.05), a tachikardia gyakorisága 88%-ról 50%-ra, a kamrafibrilláció gyakorisága 63%-ról 10%-ra (P<0.05) csökkent. Míg a szívingerelt állatok 60%-a túlélte a reperfúziót, túlélés nem volt a kontroll csoportban. A szívingerlés előtt alkalmazott AG jelentősen csökkentette a a 24 órával korábban végzett prekondicionálás késői antiaritmiás hatását. Az aminoguanidinnel kezelt állatokban az extraszisztolék száma 244±116, a tachikardia gyakorisága 78%, a kamrafibrilláció gyakorisága 45% volt koszorúér elzárása alatt. 24 óra múlva a szívingerlés hatására a kombinált iszkémia-reperfúzió utáni túlélés igen magas volt (60%), de lényegesen csökkent (22%) az előzetes aminoguanidin kezelés hatására. Eredményeinkből arra következtetünk, hogy mivel az AG, az iNOS szelektív inhibitora, jelentősen csökkentette a szívingerlés antiaritmiás hatását, a NO-nak szerepe lehet a késői antiaritmiás védelemben.

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ROLE OF NITRIC OXID IN THE DELAYED ANTIARRHYTHMIC PROTECTION INDUCED BY RAPID CARDIAC PACING IN ANAESTHETISED DOGS

arrhythmias, ischaemia, myocardial protection

We have shown earlier that rapid cardiac pacing protects the heart against those ventricular arrhythmias which occur during myocardial ischaemia, resulting from coronary artery occlusion, 24 hours later. We have now examined the role of nitric oxid (NO) in the delayed antiarrhythmic protection by administration of aminoguanidine, a selective inhibitor of the inducible nitric oxide synthase (iNOS). Under light pentobarbitone anaesthesia dogs were preconditioned by right ventricular pacing 4 times for 5 minutes at a rate of 220 beats min^{-1} by means of a bipolar pacing electrode introduced into the right ventricle. 24 hours later the dogs were re-anaesthetised with a mixture of chloralose and urethane, thoracotomised, and the left anterior descending coronary artery (LAD) was occluded for 25 minutes, followed by reperfusion (PC, n=10). In another group of preconditioned dogs aminoguanidine (AG, n=9) was given intravenously 30 minutes prior to right ventricular pacing. Control dogs (C, n=8) were subjected to the same surgical procedure and occlusion of the LAD 24 hours later, but the dogs were not paced. Right ventricular pacing significantly reduced the severity of ventricular arrhythmias. Compared to the controls, in the paced dogs the number of ventricular premature beats (VPBs, 333±120 vs. 77±30, P<0.05), the incidence of ventricular tachycardia (VT 88% vs. 50%) and the incidence of ventricular fibrillation (VF, 63% vs. 10%, P<0.05) decreased. Further, 60% of the paced dogs survived reperfusion. In contrast, no dog in the control group survived. Administration of AG markedly attenuated the antiarrhythmic protection resulted from right ventricular pacing 24 hours previously. Thus, the number of VPBs was 244±116, and the incidences of VT and VF during occlusion were 78% and 45%, respectively. Increase in survival from combined ischaemia-reperfusion insult following cardiac pacing (60%) was reduced to 22% in the AG treated group. We concluded from these results that AG, a selective inhibitor of iNOS, attenuated the delayed antiarrhythmic effect of cardiac pacing. This might indicate that NO plays an important role in this delayed phase of protection.

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1726 Repeated Cardiac Pacing Prolongs Delayed Protection Against Ventricular Arrhythmias in Canine

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We have shown earlier that brief periods of cardiac pacing from the right ventricle (4 × 5 min at a rate of 220 beats min^{-1}) markedly reduce the severity of ventricular arrhythmias which occur during a subsequent 25 min occlusion of the Left Anterior Descending (LAD) coronary artery, 24 h later. This delayed protection is attenuated or even abolished 48 or 72 h after the pacing stimulus. The objective of the present study was to examine, whether repeating the pacing stimulus at a time, when the antiarrhythmic effect of the previous stimulus has already faded (ie. 48 h) prolongs the protection against life-threatening ventricular arrhythmias. We paced dogs (4 times for 5 min at a rate of 220 beats min^{-1}) on day one and day three, under light pentobarbitone anaesthesia, and then, at various times afterwards (48, 72 and 96 h) subjected to a 25 min occlusion of the LAD. Sham-operated dogs served as controls. Repeated pacing resulted in a more prolonged protection than when dogs were paced only once. Thus, 48 and 72 h after the second pacing stimulus the number of VPBs during occlusion was still suppressed (87 ± 32 and 78 ± 44 cp 219 ± 49 in controls, $P < 0.05$) and the number of episodes of VT was markedly reduced (0.6 ± 0.4 and 1.2 ± 1.2 cp 6.1 ± 2.9 in controls, $P < 0.05$). No dog in the repeatedly paced group fibrillated during occlusion, in contrast 65% of the sham operated controls and 55% of the dogs that were paced only once (ie. 48 h before occlusion) fibrillated during occlusion. Survival from the combined ischaemia-reperfusion insult following repeated pacing was also increased (50% and 78% cp. 8% in controls, $P < 0.05$). This prolonged protection was not apparent if the time interval between the second pacing stimulus and the occlusion had been increased to 96 h. It seems from these results, that repeating the preconditioning pacing stimulus, a marked and prolonged protection could be achieved against life-threatening ventricular arrhythmias which result from coronary artery occlusion and reperfusion in canine.

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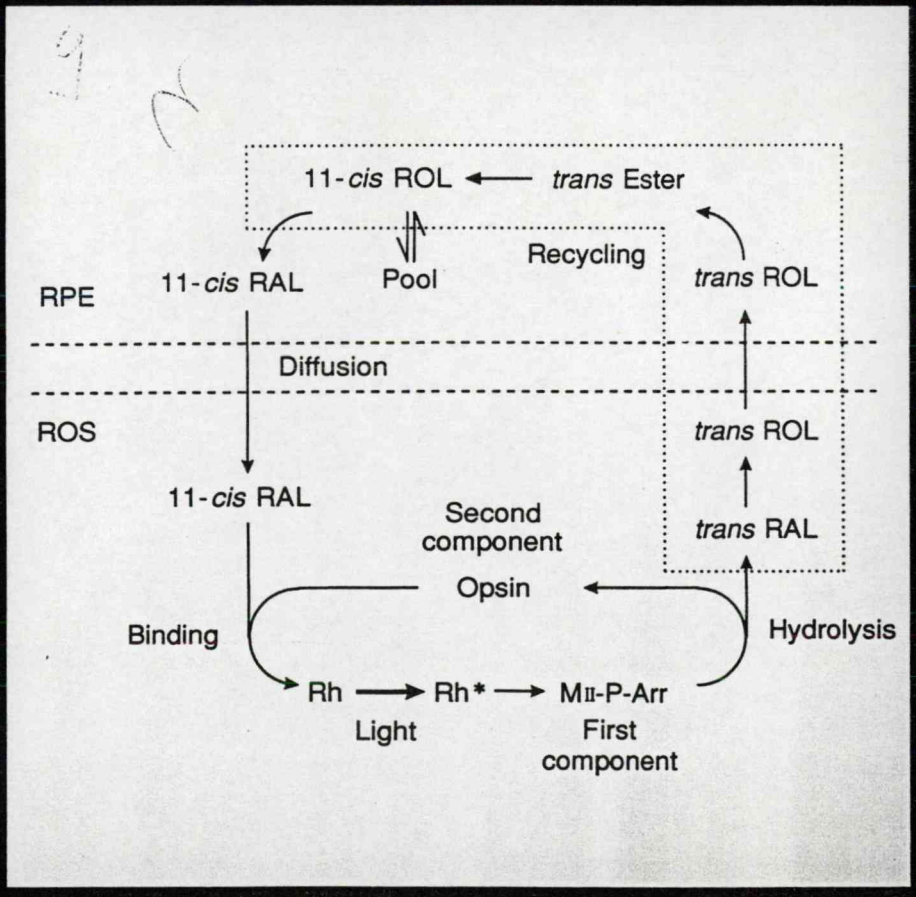

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Repeated cardiac pacing prolongs protection against ischaemia-induced ventricular arrhythmias in anaesthetized dogs

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We have previously shown that right ventricular pacing in dogs results in a delayed protection of the heart against the arrhythmic consequences of coronary artery occlusion (Vegh *et al.* 1994; Kaszala *et al.* 1996). This is a form of ischaemic preconditioning (Parratt & Szekeres, 1995). The protection is maximal 24 h after the pacing stimulus but then fades and is lost at 48 h. We have now examined whether this protection can be renewed, and even prolonged, if the pacing stimulus is repeated at a time when protection from the initial stimulus has already faded. Mongrel dogs were lightly anaesthetized with sodium pentobarbitone and a pacing catheter inserted into the right ventricle via the right external jugular vein; endocardial electrograms were recorded, following pacing, from the same electrode. The dogs were paced at a rate of 220 beats min^{-1} for four periods of 5 min, with 5 min rest periods between (Kaszala *et al.* 1996). Control dogs were subjected to the same procedure but were not paced. The same pacing stimulus was repeated 48 h later, i.e. when protection from a single pacing stimulus was no longer apparent. At different times after this second pacing stimulus (48, 72 and 96 h), the dogs were re-anaesthetized (chloralose and urethane, 60 and 200 mg kg^{-1}, respectively), thoracotomized and subjected to occlusion of the left anterior decending coronary artery (Vegh *et al.* 1992).

The incidence of ventricular fibrillation during occlusion was greatly reduced in the repeat paced dogs, e.g. 8% at 48 h ($n = 10$) compared with 56% at 48 h after a single pacing stimulus and 46% in the controls ($n = 19$; $P < 0.05$ using a χ^2 test). At 72 h ($n = 9$) no dog fibrillated during occlusion compared with 50% after one pacing stimulus ($P < 0.05$). Survival from a combined 25 min period of ischaemia followed by reperfusion was also markedly increased by the repeat pacing stimulus (42% at 48 h; 78% at 72 h compared with 8% in the sham-operated controls; $P < 0.05$). The protection was lost 96 h after the repeat pacing stimulus ($n = 9$).

Two indices of ischaemia severity (epicardial ST-segment elevation and changes in the inhomogeneity of electrical activation within the ischaemic area) were also significantly reduced 48 and 72 h following the repeat pacing stimulus. These results suggest that it may be possible to keep the heart protected against an ischaemic attack in the long term by repeating a pacing (or exercise?) stimulus.

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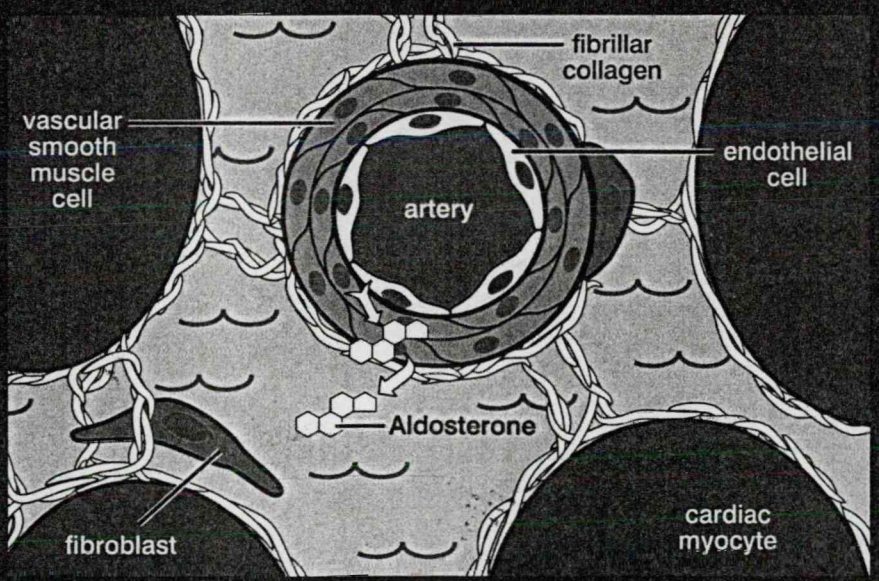

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DELAYED ANTIARRHYTHMIC PROTECTION INDUCED BY REPEATED PACING IS ABOLISHED BY S-(2-AMINOETHYL)-METHYL-ISOTIOUREA, A SELECTIVE INHIBITOR OF iNOS IN DOGS

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We have shown earlier that repeated cardiac pacing provides the delayed antiarrhythmic protection over a 72 hour period in anaesthetised dogs (*Kis et al, J. Mol. Cell. Cardiol. 1997, 29:A122*). Here we have examined whether nitric oxide (NO) plays a role in this marked protection by the administration of a selective inhibitor of the inducible nitric oxide synthase (iNOS), S-(2-aminoethyl)-methyl-isotiourea (AEST). Dogs were paced from the right ventricle under light pentobarbitone anaesthesia at a rate of 220 beat min^{-1} four times for 5 minutes, and this pacing protocol was repeated 48 h later. 72 hours after repeated pacing dogs were subjected to a 25 min occlusion of the left anterior descending coronary artery (LAD) (n=11). Sham-operated, non-paced dogs served as controls. 120 hours later these dogs were subjected to myocardial ischaemia (SC, n=10). In 8 repeatedly paced dogs AEST (n=8) was given in a dose of 2 mg kg^{-1} intravenously 90 minutes prior to the occlusion of LAD. Repeated pacing markedly suppressed the number of ventricular premature beats (VPBs) from 228±94 to 101±49, and the incidence of ventricular tachycardia from 50% to 18%, and significatnly increased the incidence of survival from 30% to 73% compared to controls. In those repaced dogs in which AEST was given the number of VPBs was (236±98) and the incidence of VT (63%) were increased during occlusion, and only 1 dog (12%) survived the combined occlusion-reperfusion insult. These results indicate that NO generated by iNOS is involved in the delayed antiarrhythmic protection induced by repeated cardiac pacing.

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DELAYED CARDIOPROTECTION INDUCED BY RAPID CARDIAC PACING IS ATTENUATED BY AMINOGUANIDINE

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We have shown earlier that nitric oxide (NO) plays a role in the delayed antiarrhythmic protection induced by cardiac pacing 24 hours previously since dexamethasone, an inhibitor of inducible nitric oxide synthase (iNOS) and cyclooxigenase-2 (COX2) prevented this protection. In this study we have examined the role of iNOS by the administration of aminoguanidine (AG), a more selective inhibitor of this enzyme. Under light pentobarbitone anaesthesia dogs were paced at a rate of 220 beats min^{-1} four times for 5 minutes from the right ventricle. 24 hours later these dogs were reanaesthetised with a mixture of chloralose and urethane, thoracotomised and the left anterior descending coronary artery (LAD) was occluded for 25 minutes followed by reperfusion (n=13). In 9 paced dogs AG was given intravenously in a dose of 50 mg kg^{-1} 30 minutes prior to the occlusion of the LAD. Sham operated (not paced) dogs served as controls. 24 hours later these dogs were subjected to myocardial ischaemia (n=12). Right ventricular pacing induced a marked delayed protection against ischaemia- and reperfusion-induced ventricular arrhythmias. Thus, compared to the controls the number of ventricular premature beats (VPBs) (330±110 vs. 70±28, P<0.05) and the episodes of ventricular tachycardia (VT) (6.4±3.6 vs. 2.1±1.9, P<0.05), and the incidence of ventricular fibrillation (VF) (58% vs. 15%, P<0.05) were significantly reduced. In the presence of AG the severity of arrhythmias during occlusion was markedly increased (VPBs: 284±120, VTepisodes: 5.2±3.2, VF:33%). Compared to controls the incidence of survival from the combined occlusion-reperfusion insult resulting from cardiac pacing was significantly increased (17% vs. 62%, P<0.05). This was 11% following AG treatment It seems from these results that induction of iNOS and the resultant formation of NO may play a role in the delayed antiarrhythmic protection induced by rapid cardiac pacing 24 hours previously.

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