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The antiarrhythmic effects of ischaemic preconditioning in anaesthetized dogs are prevented by atropine; role of changes in baroreceptor reflex sensitivity

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1 Dogs, anaesthetized with chloralose and urethane, were subjected to a 25 min occlusion of the left anterior descending coronary artery. This resulted in ventricular ectopic activity, a reduction in baroreflex sensitivity (BRS, measured following the intravenous administration of phenylephrine), elevations in the epicardial ST-segment and increases in the degree of inhomogeneity of electrical activation, both measured from the ischaemic region of the left ventricular wall.

2 These changes were markedly reduced when the 25 min occlusion was preceded, 20 min earlier, by a 5 min (preconditioning) occlusion of the same coronary artery (e.g. VF during ischaemia reduced from 40% in the controls to 0%; $P<0.05$; BRS increased from 1.22 ± 0.23 pre-occlusion to 1.61 ± 0.25 mmHg ms^{-1} post-occlusion in preconditioned dogs; cf. 1.28 ± 0.29 to 0.45 ± 0.12 mmHg ms^{-1} respectively in the controls, $P<0.05$).

3 These beneficial effects of preconditioning were prevented by the administration, 10 min prior to the 25 min coronary artery occlusion, of atropine (1 mg kg^{-1} i.v. followed by a continuous infusion of 0.04 mg kg^{-1} h^{-1}). For example, VF during occlusion was increased from 0% in the preconditioned dogs to 40% ($P<0.05$) in the presence of atropine and BRS was again reduced during occlusion (from 1.75 ± 0.29 to 0.30 ± 0.08 mmHg ms^{-1}; $P<0.05$).

4 We conclude that preconditioning reduces arrhythmia severity during ischaemia by favourably modifying cardiac autonomic receptor mechanism through enhancing vagal influences.
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Keywords: Atropine; ischaemic preconditioning; baroreceptor reflex sensitivity; ventricular arrhythmias; anaesthetized dogs; reperfusion

Abbreviations: Atr, atropine; BRS, baroreceptor reflex sensitivity; DABP, diastolic arterial blood pressure; HR, heart rate; LVEDP, left ventricular end-diastolic pressure; LVSP, left ventricular systolic pressure; MABP, mean arterial blood pressure; PC, preconditioning; RP, resting period; SABP, systolic arterial blood pressure; VF, ventricular fibrillation; VPBs, ventricular premature beats; VT, ventricular tachycardia

Introduction

Ventricular fibrillation resulting from an acute ischaemic and/or reperfusion insult is the main factor responsible for sudden cardiac death in both humans and experimental animals. There is evidence that ischaemic preconditioning can provide protection against these ischaemia (Végh *et al*., 1992a) and reperfusion-induced (Shiki & Hearse, 1987) fatal ventricular arrhythmias. The evidence for this comes mainly from animal studies, performed primarily in anaesthetized dogs, in which preconditioning was induced by brief coronary artery occlusions (Végh *et al*., 1992a), rapid cardiac pacing (Végh *et al*., 1991a; Kaszala *et al*., 1996) or exercise (Végh *et al*., 2000). There is also recent evidence that preconditioning may protect the heart against life threatening ventricular arrhythmias in man (Airaksinen & Huikuri, 1997). Although the mechanisms of this remarkably protective phenomenon are still not precisely known, there is evidence that endogenous protective substances, such as bradykinin, prostacylin and nitric oxide are involved (reviewed in Végh & Parratt, 1996). These substances then trigger intracellular cascade mechanism(s) which ultimately lead to protection. One possible mechanism involved in this protection is elevation of cyclic GMP, through activation of soluble guanylate cyclase by nitric oxide (reviewed in Parratt, 1994; Végh & Parratt, 1996), leading to a reduction in calcium influx in cardiac myocytes (Tohse & Sperelakis, 1991) during ischaemia.

Myocardial cyclic AMP levels are increased during ischaemia as a result of increased sympathetic activation (Krause *et al*., 1978) and this is involved in the generation of ventricular arrhythmias that result when a coronary artery is occluded (recently reviewed by Du & Dart, 1999). In contrast, the parasympathetic nervous system, which can also be activated during myocardial ischaemia, is antifibrillatory (Corr *et al*., 1986; Verrier, 1986). The evidence for this comes from studies demonstrating that electrical or pharmacological stimulation of parasympathetic nerves and receptors reduces the severity of ischaemia-induced ventricular arrhythmias

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(Sneddon *et al*., 1993; Vanoli *et al*., 1991; Cerati & Schwartz, 1991), whereas, blockade of muscarinic receptors (or section of the vagus nerve) increases the severity of these arrhythmias (De Ferrari *et al*., 1992). Thus, it seems that the balance between the cardiac sympathetic and parasympathetic systems is an important determinant of the consequences of acute coronary artery occlusion (Toubes & Brody, 1970; Takeshita *et al*., 1980; Schwartz *et al*., 1988; De Ferrari *et al*., 1991) and, that in particular, a reduced baroreflex sensitivity (BRS) is a predictor of the increased risk of ventricular fibrillation that results from acute coronary artery occlusion (Farrell *et al*., 1992; Kim & Euler, 1997; Airaksinen *et al*., 1998).

Although some attempts have been made to explore the role of the autonomic nervous system in some the protective effects of ischaemic preconditioning, e.g. on infarct size reduction, the results are conflicting. For example, there is evidence that both noradrenaline (Banerjee *et al*., 1993; Asimakis *et al*., 1994; Bankwala *et al*., 1994) and acetylcholine (Yao & Gross, 1993) are able to induce a protection which is similar to that resulting from preconditioning itself. However, no studies have attempted to examine the antiarrhythmic effects of ischaemic preconditioning in relation to effects on arterial baroreflex sensitivity. The aim of the present study were therefore to examine (i) how ischaemic preconditioning, induced by brief coronary artery occlusion, modifies baroreflex sensitivity during a subsequent, more prolonged period of ischaemia, and (ii) whether the antiarrhythmic effect of ischaemic preconditioning is mediated by acetylcholine.

Methods

Animals and surgical preparation

Adult mongrel dogs of both sexes, weighing between 18 and 26 kg (mean: 24.9 ± 0.6 kg), were used in this study. The origin and upkeep of these dogs were in accord with Hungarian law (XXVIII, chapter IV, paragraph 31) regarding large experimental animals which comply with those of the European Commission as described in the regulations dated December 16, 1991. The dogs were given intramuscular ketamine (Richter, 50 mg kg^{-1}) and then anaesthetized with intravenous α-chloralose and urethane (80 and 200 mg kg^{-1}, respectively; Sigma). If necessary, additional chloralose and urethane (10 and 25 mg kg^{-1} $hour^{-1}$, respectively) was infused to maintain anaesthesia. Dogs were ventilated with room air at a rate of 13 min^{-1} (Harvard respirator, U.S.A.). Blood gases (pO_2, pCO_2 and pH) were continuously monitored and maintained within the normal range (Végh *et al*., 1992a). Temperature was recorded from the midoesophagus and maintained at 37 ± 0.5°C by means of a heating pad.

Polyethylene catheters were inserted into the right femoral artery (for monitoring blood pressure) and into the right femoral vein (for drug and anaesthetic administration). Another catheter was introduced into the cavity of the left ventricle, through the left femoral artery, for the measurement of left ventricular systolic (LVSP) and end-diastolic (LVEDP) pressures, as well as changes in positive and negative dP/dt_{max}.

Thoracotomy was performed at the left fifth intercostal space and the pericardium opened. The descending branch of the left anterior coronary artery (LAD) was prepared for occlusion, proximal to the first main diagonal branch, and a silk thread loosely placed around it. A composite electrode was sutured onto the epicardial surface of the left ventricle within the proposed ischaemic area and changes in the epicardial ST-segment, and in the degree of inhomogeneity of electrical activation, were evaluated as described previously (Végh *et al*., 1992a). 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 since all sites are activated almost simultaneously. Following coronary occlusion, however, widening and fractionation of this summarized R-wave occurs because, as a result of the inhomogeneity of conduction within the ischaemic myocardium, fibres are not simultaneously activated. This inhomogeneity of conduction is expressed as 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) and data were analysed off-line by the Advanced CODAS Analysing System using the Windaq Waveform Browser playback and analysing software (DATAQ Instruments, U.S.A.).

Determination of baroreflex sensitivity

Baroreflex sensitivity (BRS) was assessed by the method of Smyth *et al*. (1969), which involves examining the haemodynamic responses following the rapid intravenous bolus injection of 10 μg kg^{-1} phenylephrine. The slope of the linear relationship between the duration of the RR interval (in ms) and the preceding systolic blood pressure (in mmHg) was calculated. As previously suggested (Smyth *et al*., 1969), those dogs in which the correlation between RR interval and the preceding systolic blood pressure, measured at baseline, was less than 0.85 were excluded from further experimentation.

Assessment of ventricular arrhythmias and area at risk

Ventricular arrhythmias during coronary artery occlusion and following reperfusion were assessed as previously described (Végh *et al*., 1992a). 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 evaluated. Following reperfusion, only the incidence of VF was assessed. Those dogs were pronounced survivors if they were still alive, and predominantly in sinus rhythm, 10 min after reperfusion. These animals were euthanized by an excess of anaesthetic.

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. It was expressed as a percentage of the left ventricular wall together with the septum (Kaszala *et al*., 1996).

Experimental protocol

This is illustrated in Figure 1. Six groups of dogs were used. The animals were randomly assigned to control or treated groups by providing equal distribution of males and females in

each group. In all groups, following a 30 min period of recovery from the surgical interventions, baroreflex sensitivity (BRS) was determined at baseline (0 min) which was followed by a 1 h resting period. Control dogs (groups 1 and 2) were subjected to a 25 min occlusion of the anterior branch of the left coronary artery (LAD) and, if the dogs had survived up to this time, the ischaemic myocardium was rapidly reperfused. In these control dogs, BRS was determined either once (group 1, $n=10$), 1 h prior to coronary artery occlusion or twice (group 2; $n=11$), 1 h prior to, and again 3 min after, the commencement of coronary artery occlusion. In another group of dogs (group 3, $n=8$), in which the BRS was also determined twice, atropine was administered intravenously, starting with a bolus injection of 1 mg kg^{-1} given 10 min prior to the occlusion and immediately followed by an infusion of 0.04 mg kg^{-1} h^{-1} which was maintained throughout the entire 25 min occlusion period. Further dogs (groups 4, 5 and 6) were subjected to preconditioning by occluding the LAD for 5 min. In some of these preconditioned dogs (group 4, $n=13$) BRS was determined twice (ie. 1 h prior to and 23 min after the preconditioning occlusion); these dogs were not subjected to prolonged ischaemia. Thirteen dogs (group 5) were subjected to preconditioning and then, 20 min later, to a prolonged (25 min) occlusion, whereas 10 dogs (group 6), subjected to this same protocol, were given atropine as detailed above. In these dogs BRS was also determined twice, 1 h prior to the preconditioning occlusion, and 3 min after the prolonged (25 min) coronary artery occlusion.

A preliminary account of these studies was given to the Joint Meeting of the British and Hungarian Physiological Societies in Budapest in May 2000 (Babai *et al*., 2000).

Statistical evaluation

Data are expressed as mean ± 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 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 measurements before, and after, a 25 min occlusion of the LAD

Table 1 illustrates that there were no significant differences between groups in arterial blood pressure, LVSP, LVEDP and in positive and negative $LVdP/dt_{max}$, measured at baseline (0 min) and just prior to the 25 min occlusion of the LAD, except that heart rate was significantly increased in dogs given atropine (AtrC and PC + Atr) 10 min prior to prolonged ischaemia.

Atropine itself, administered to control (AtrC) and preconditioned (PC + Atr) dogs 10 min prior to prolonged ischaemia, significantly ($P<0.05$) reduced mean arterial blood pressure (by 8 ± 2 and 9 ± 4 mmHg, respectively) and increased heart rate (by 17 ± 7 and 21 ± 6 beats min^{-1}, respectively; Figure 2).

Occlusion of the LAD resulted in marked haemodynamic changes in all experimental groups (Table 1). There were reductions in arterial blood pressure and in positive and negative $LVdP/dt_{max}$, and increases in LVEDP and heart rate when these parameters were measured 2 min after coronary artery occlusion and just prior to the assessment of BRS (see below). Coronary artery occlusion resulted in less significant haemodynamic changes in the preconditioned dogs than in the controls.

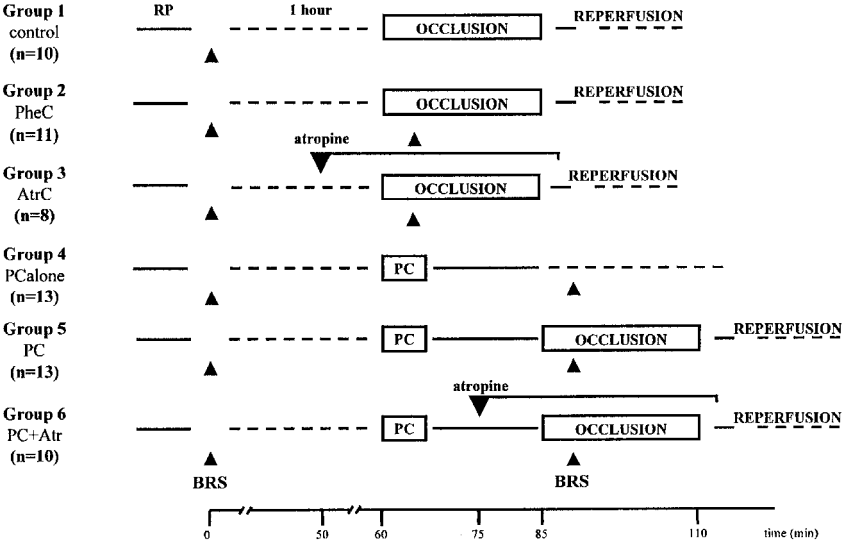

Figure 1 Experimental protocol. Baroreceptor reflex sensitivity (BRS; at the arrows) was measured following the injection of phenylephrine (Phe) 1 h before (except in the control group 1) and 3 min after coronary artery occlusion in control (group 2) dogs and in dogs preconditioned (PC) by a 5 min coronary artery occlusion 20 min prior to a 25 min occlusion of the same (LAD) coronary artery (groups 4–6). Some dogs (groups 3 and 6) were given atropine (1 mg kg^{-1} 10 min prior to the occlusion followed by a continuous infusion of 0.04 mg kg^{-1} h^{-1}).

Table 1 Haemodynamic changes before, and after, a 25 min coronary artery occlusion in control, preconditioned (PC) dogs and in dogs given atropine (Atr)

	SABP (mmHg)	*DABP* (mmHg)	*MABP* (mmHg)	*LVSP* (mmHg)	*LVEDP* (mmHg)	$+dP/dt$ (mmHg s^{-1})	$-dP/dt$ (mmHg s^{-1})	*HR* (beats min^{-1})
Baseline (0.min)								
C ($n=10$)	146±8	98±9	114±8	132±7	5.2±0.6	2692±185	3000±229	157±4
PheC ($n=11$)	146±7	93±3	111±4	136±5	4.5±0.8	2570±155	2765±235	163±8
AtrC ($n=8$)	162±10	106±8	125±8	144±7	5.5±1.4	3181±245	3275±202	158±6
PCalone ($n=13$)	143±3	92±3	113±3	129±3	5.0±0.8	2574±149	3571±238	140±10
PC ($n=13$)	136±6	91±4	105±4	128±4	4.7±0.8	2925±131	3424±206	149±7
PC+Atr ($n=10$)	157±6	105±5	122±5	144±4	4.1±0.7	3007±219	3163±376	149±6
Pre-occlusion								
C ($n=10$)	139±8	94±6	109±6	133±12	5.1±0.4	2616±206	2785±202	151±8
PheC ($n=11$)	147±8	91±4	110±5	127±6	5.2±1.5	2635±174	2863±255	155±9
AtrC ($n=8$)	155±9	99±6	118±6	139±6	5.0±1.8	3211±316	3232±350	172±6*
PC ($n=13$)	137±9	86±5	103±7	126±8	5.0±0.9	2822±140	3145±237	144±8
PC+Atr ($n=10$)	146±8	98±7	117±8	130±7	3.9±0.7	2798±297	3384±236	173±7*
2. Min of occlusion								
C ($n=10$)	130±8	86±6$^{\#}$	101±7$^{\#}$	119±10$^{\#}$	12.0±1.1$^{\#}$	2083±223$^{\#}$	2360±244$^{\#}$	154±8$^{\#}$
PheC ($n=11$)	139±9	88±5	105±6	121±7	10.9±2.0$^{\#}$	2220±202$^{\#}$	2555±282$^{\#}$	158±8$^{\#}$
AtrC ($n=8$)	151±10	103±8	119±8	137±9	13.3±1.6$^{\#}$	3009±274	3224±453	175±7†
PC ($n=13$)	133±9$^{\#}$	85±7	99±7	125±9	8.8±1.3$^{\#}$	2763±184	3067±269	148±9$^{\#}$
PC+Atr ($n=10$)	141±11	95±8	113±10	131±9	7.9±1.8$^{\#}$	2854±302	3219±227	176±7$^{\#\dagger}$

*$P<0.05$ vs. baseline; $^{\#}$ $P<0.05$ vs pre-occlusion; † $P<0.05$ vs PC. SABP = systolic arterial blood pressure; DABP = diastolic arterial blood pressure; MABP = mean arterial blood pressure; LVSP = left ventricular systolic pressure; LVEDP = left ventricular end-diastolic pressure; HR = heart rate; C = control; PC = preconditioning; Phe = phenylephrine; Atr = atropine.

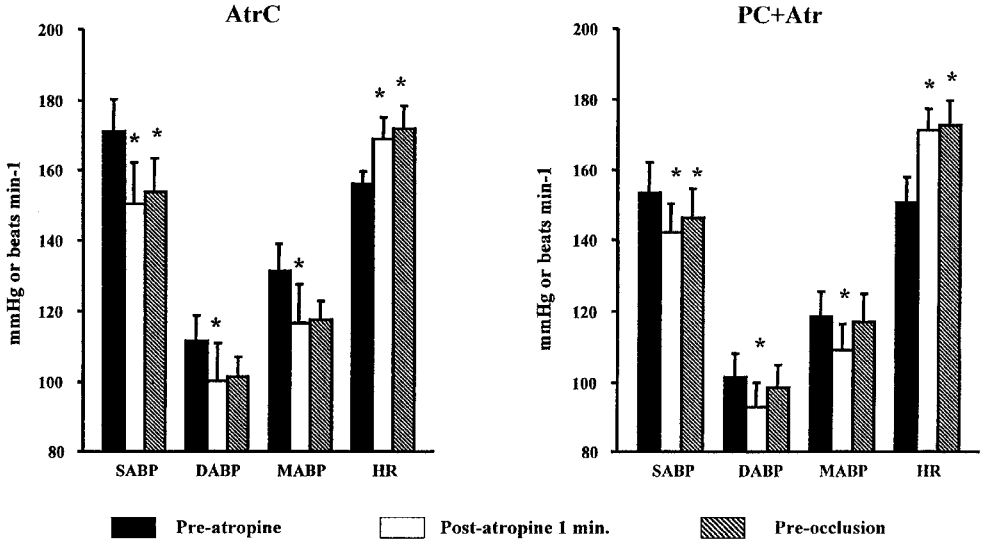

Figure 2 Blood pressure and heart rate effects of atropine prior to (pre-atropine) 1 min after atropine (post-atropine) and immediately prior to (pre-occlusion) coronary artery occlusion in control (AtrC) and in preconditioned dogs (PC+Atr). SABP = systolic arterial blood pressure, DABP = diastolic arterial blood pressure, MABP = mean arterial blood pressure, HR = heart rate. Values are means±s.e.m. *$P<0.05$ compared to the pre-atropine value.

Changes in baroreflex sensitivity in control dogs, in preconditioned dogs and in dogs treated with atropine

These are illustrated in Figure 3. There were no differences between groups in BRS measured at baseline. However, when the coronary artery was occluded, there was a marked reduction in BRS in control dogs (PheC). In contrast, BRS was largely maintained in the preconditioned dogs and in many of the dogs was even increased (Figure 3). This was despite the fact that preconditioning itself did not modify BRS (PC alone: 1.20±0.27 vs 1.13±0.29; not shown in the figure). Atropine given to control (AtrC) dogs did not modify the reduction of BRS that resulted from coronary artery occlusion. Thus, as with the controls, BRS was markedly reduced in dogs given atropine and then subjected to coronary artery occlusion (Figure 3). However, atropine administered to preconditioned dogs (PC+Atr) reversed the BRS preservation that resulted from preconditioning; as with

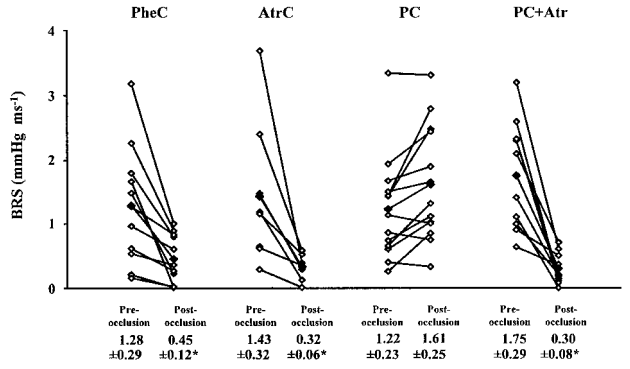

Figure 3 Changes in baroreceptor reflex sensitivity (BRS, mmHg ms^{-1}) in individual dogs (open rhombus) before (left hand symbols) and after (right hand symbols) coronary artery occlusion in controls (PheC), controls given atropine (AtrC), in preconditioned (PC) dogs and in preconditioned dogs given atropine (PC + Atr). Ischaemia results in a significant reduction in BRS which is not observed in PC dogs; this maintenance of BRS by preconditioning is abolished by atropine. The mean results for each group are shown by the solid (rhombus) symbols. Values are means ± s.e.m. $^*P<0.05$ compared to pre-occlusion (Student's paired *t*-test).

the controls, BRS in these atropine treated preconditioned dogs was markedly reduced during coronary artery occlusion (Figure 3).

The severity of ventricular arrhythmias during coronary artery occlusion

The severity of ventricular arrhythmias is illustrated in Figures 4 and 5. In control dogs (C and PheC), there was marked ventricular ectopic activity (409 ± 89 and 480 ± 175 VPBs respectively during the 25 min occlusion period) and high incidences (100 and 80%), and many episodes, of ventricular tachycardia during coronary artery occlusion (Figure 4). In contrast, these arrhythmias were significantly less in dogs subjected to preconditioning. Thus, there were only 115 ± 38 VPBs, and 0.9 ± 0.6 episodes of VT ($P<0.05$), during the prolonged occlusion. Further, VT occurred in only two out of the 13 preconditioned dogs. Atropine did not modify the severity of these arrhythmias but significantly attenuated the protective effect of preconditioning. Thus, in preconditioned dogs in the presence of atropine, the number of VPBs (275 ± 58) and the number of episodes of VT (4.3 ± 1.6) were again increased and 80% of the preconditioned dogs exhibited VT at some time during the prolonged occlusion (Figure 4).

The incidences of ventricular fibrillation were also modified by preconditioning. Following coronary artery occlusion ventricular fibrillation occurred in 40% (C) and 36% (PheC) of the control dogs and only one dog in each of these groups survived the combined ischaemia-reperfusion insult (Figure 5). In contrast, no preconditioned dog died during occlusion and there was a 38% survival from the occlusion/reperfusion insult. This marked antiarrhythmic effect resulting from preconditioning was prevented by atropine; in the presence of atropine 40% of the preconditioned dogs fibrillated during occlusion and all the remaining dogs died following reperfusion. Atropine itself did not significantly modify the incidence of ventricular fibrillation resulting from either occlusion or reperfusion (Figure 5).

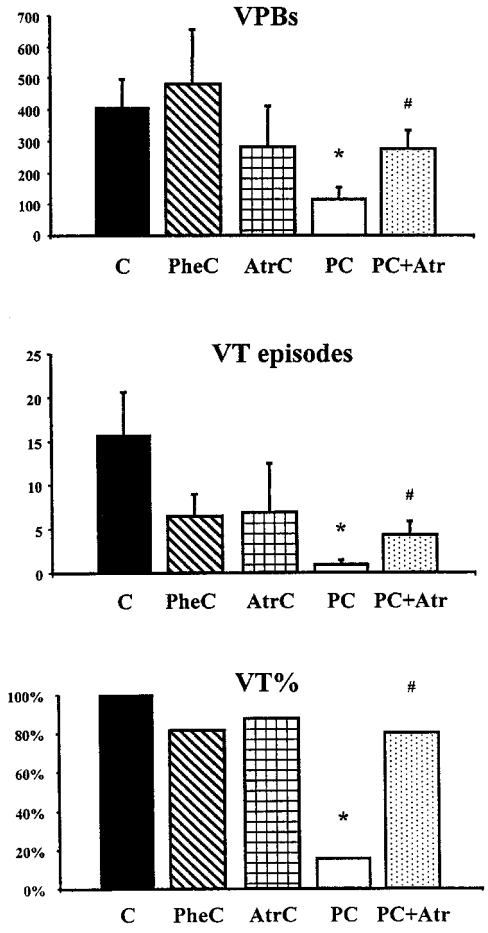

Figure 4 Ventricular premature beats (VPBs) and the incidence (%) and number of episodes of ventricular tachycardia (VT) in control dogs with (PheC) and without (C) phenylephrine, in preconditioned dogs (PC) and in control (AtrC) and preconditioned (PC + Atr) dogs given atropine. The antiarrhythmic effect of PC is prevented by atropine. Values are means ± s.e.m. $^*P<0.05$ compared to controls; $^{\#}P<0.05$ compared to preconditioned dogs.

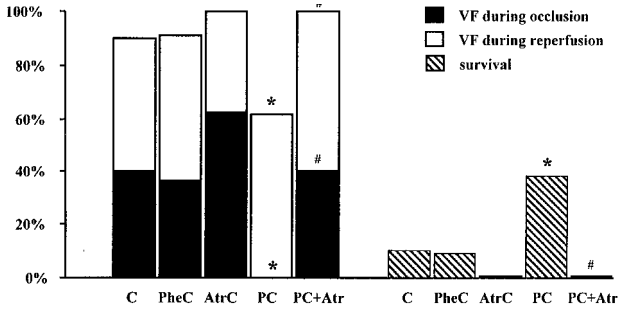

Figure 5 Ventricular fibrillation (VF; %) during a 25 min coronary artery occlusion, following reperfusion and survival following the combined ischaemia-reperfusion insult in control dogs with (PheC) and without (C) phenylephrine, in preconditioned dogs (PC) and in control (AtrC) and preconditioned (PC + Atr) dogs given atropine. The reduction in VF and the increase in survival following ischaemic preconditioning is prevented by atropine administration. $^*P<0.05$ compared to controls; $^{\#}P<0.05$ compared to preconditioned dogs.

The severity of myocardial ischaemia following a 25 min occlusion of the LAD

This was assessed in two ways; by changes in the ST-segment recorded from epicardial electrodes and in the degree of inhomogeneity of electrical activation, both measured from the ischaemic area. Compared to controls, preconditioning markedly reduced both epicardial ST-segment elevation (Figure 6) and the degree of inhomogeneity (Figure 7). Although atropine did not itself modify the severity of myocardial ischaemia as assessed in these ways, it reversed the effects of preconditioning. In the presence of atropine both epicardial ST-segment elevation and the degree of inhomogeneity were markedly increased in preconditioned dogs; indeed, the ST-segment changes were even somewhat more pronounced than in the controls (Figure 6).

There were no significant differences in the area at risk between controls (37±2%), controls given phenylephrine (33±2%), preconditioned dogs (34±1%), preconditioned dogs given atropine (32±2%) and atropine controls (33±2%).

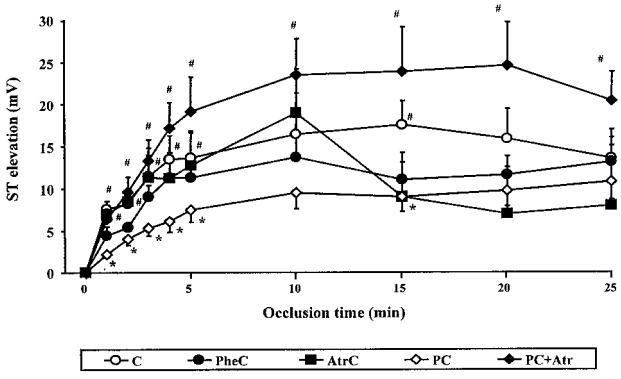

Figure 6 Changes in epicardial ST-segment elevation (mV) during a 25 min coronary artery occlusion in control (C) dogs with and without phenylephrine, in control dogs given atropine (AtrC) and in preconditioned dogs with (PC + Atr) and without (PC) atropine. The preconditioning-induced reductions in this index of ischaemia severity are prevented by atropine. Values are means ± s.e.m. $^{*}P<0.05$ compared to PheC; $^{\#}P<0.05$ compared to PC dogs.

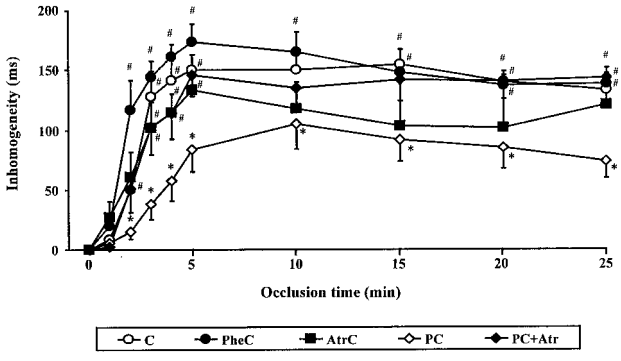

Figure 7 Changes in the degree of inhomogeneity of electrical activation (ms) within the ischaemic area during a 25 min coronary artery occlusion in control dogs (C) with and without phenylephrine, in control dogs given atropine (AtrC) and in preconditioned dogs with (PC + Atr) and without (PC) atropine. The increase in inhomogeneity during occlusion is reduced by preconditioning, and this is reversed by atropine. Values are means ± s.e.m. $^{*}P<0.05$ compared to PheC; $^{\#}P<0.05$ compared to PC dogs.

Discussion

In the present study we confirmed our previous findings that, in anaesthetized dogs, preconditioning induced by a brief (5 min) coronary artery occlusion, protects the myocardium against the severe ventricular arrhythmias that occur during a subsequent, more prolonged period of ischaemia (Végh *et al*., 1992a). We now demonstrate that this reduction in arrhythmia severity during ischaemia is accompanied by a preserved baroreflex sensitivity (BRS); in contrast, arrhythmias are severe, and BRS is markedly reduced following coronary artery occlusion, in control, non-preconditioned, dogs. Further, both the antiarrhythmic and the BRS maintaining effects of preconditioning were abolished by atropine. We conclude therefore that preconditioning modifies autonomic control, perhaps by enhancing the vagal influence on the heart and that this contributes to the suppression of those life-threatening ventricular arrhythmias that result from an ischaemia and reperfusion insult.

It is established that the autonomic nervous system plays an important role in the genesis of severe ventricular arrhythmias, particularly under conditions of myocardial ischaemia (Webb *et al*., 1972; Schwartz & Stone, 1982; Lombardi *et al*., 1983; Schwartz *et al*., 1984; Collins & Billman, 1989; Armour, 1999) and it is well documented that autonomic nervous system activity is abruptly changed after the onset of ischaemia. It is this that largely determines the outcome of an acute ischaemic event (Theroux *et al*., 1974; Longhurst, 1984). Thus, sympathetic hyperactivity results in an increase in electrical instability favouring the genesis of life-threatening ventricular arrhythmias (Euler *et al*., 1985; Puddu *et al*., 1988), whereas vagal activation exerts an opposite, antifibrillatory effect (Corr *et al*., 1986; Verrier, 1986). For example, Collins & Billman (1989) showed that coronary artery occlusion elicits a significantly greater increase in sympathetic activity, coupled with greater reduction in parasympathetic activity, in animals subsequently shown to be susceptible to VF. Furthermore, more recent studies have suggested that the loss of protective vagal reflexes is associated with an increased incidence of arrhythmias and sudden cardiac death (Cerati & Schwartz, 1991). Indeed, numerous experimental (Kerzner *et al*., 1973; Myers *et al*., 1974; Cerati & Schwartz, 1991; Vanoli *et al*., 1991) and clinical (Airaksinen *et al*., 1999) studies have demonstrated that stimulation of the vagus nerve during ischaemia reduces the severity of ventricular arrhythmias and is particularly protective against ischaemia-induced ventricular fibrillation.

Arterial baroreflex sensitivity is generally accepted as a marker of vagal reflex activity and has become a useful tool in exploring reflex autonomic control of the heart (Farrell *et al*., 1992; Kim & Euler, 1997). Several studies, in both humans (Imaizumi *et al*., 1984; Airaksinen *et al*., 1998) and experimental animals (Toubes & Brody, 1970; Holmberg *et al*., 1983; Hageman & Gantenberg, 1993), have shown that arterial baroreflex control of heart rate and ventricular resistance is impaired during acute myocardial ischaemia and that the reduced BRS is associated with an increased

incidence of arrhythmic events (Farrell *et al*., 1992). It is also well documented that a low BRS, measured in hearts prior to ischaemia, has a predictive value for the increased risk of fatal ventricular fibrillation during a subsequent coronary artery occlusion (Billman *et al*., 1982; Schwartz *et al*., 1984; La Rovere *et al*., 1988; De Ferrari *et al*., 1991).

Normally, afferent nerve transmission from the arterial baroreceptors to the higher centres results in inhibition of sympathetic outflow and a subsequent increase in efferent parasympathetic activity. Myocardial ischaemia may interfere with this mechanism in several ways. Occlusion of a coronary artery abruptly increases the firing of nonmyelinated vagal afferent fibers (Thoren, 1976; Cerati & Schwartz, 1991) and that this exerts a profound inhibitory influence on the carotid baroreflex (Victor & Mark, 1985). Vagal activity can also decrease after myocardial infarction because of an increase, secondary to abnormal stretch of the cardiac mechanoreceptors, in cardiac sympathetic afferent activity (Malliani *et al*., 1972; Schwartz *et al*., 1973) which exerts a tonic restraint on vagal outflow (Cerati & Schwartz, 1991). Barber *et al*. (1985) have reported that soon after the occlusion of a coronary artery the efferent and afferent nerve endings in the non-infarcted myocardium are denervated; this might be due to attenuation of neurotransmission in the autonomic nerves transversing the ischaemic myocardium. This heterogeneous loss of autonomic innervation results in areas of myocardium becoming supersensitive to catecholamines whilst the destruction of afferent neural fibres (and receptors) leads to the impairment of protective cardiac reflexes resulting in electrical instability (Inoue & Zipes, 1987).

There are several possible mechanisms for the anti-arrhythmic effect of vagal activation. Although such activity has direct electrophysiological effects on the ischaemic myocardium, what is perhaps more important is that increased vagal activity antagonizes sympathetic influences on the myocardium (Prystowsky *et al*., 1981). Gilmore & Zipes (1984) suggested that presynaptic muscarinic receptor inhibition of noradrenaline release might represent a cellular mechanism for the observed sympathetic-parasympathetic antagonism. More recently Kawada *et al*. (2000) have suggested that, during the very early phase of myocardial ischaemia, the different modes of acetylcholine release (Ca^{2+}-dependent local mechanisms versus increased vagal efferent nerve activity) between the ischaemic and non-ischaemic regions, together with an inhomogenous release of noradrenaline, result in heterogeneity of noradrenaline and acetylcholine levels within the ventricle leading to the generation of fatal ventricular arrhythmias.

As yet we do not know precisely how preconditioning modifies these autonomic reactions, but it seems likely that the preconditioning stimulus alters, during a subsequent ischaemic episode, autonomic responses (by increasing vagal activity and/or by reducing sympathetic activity). This leads to the suppression of fatal arrhythmias, a preserved BRS and an attenuation of the ischaemic changes. The fact that, in the present study, administration of atropine completely abolished the protective effects of preconditioning on arrhythmias, BRS and the two measured indices of ischaemic changes, suggests a role for a vagal component in this protection. Miyazaki & Zipes (1989) demonstrated that preconditioning, induced, as here, by brief coronary artery occlusions, preserves both efferent sympathetic and vagal responses by reducing the heterogenous development of sympathetic and parasympathetic denervation during the early phase of ischaemia. This helps to suppress the occurrence of fatal ventricular arrhythmias. Similarly, Airaksinen & Huikuri (1997) showed in clinical studies, that repeated occlusion and reperfusion cycles during PTCA increases the electrical stability of the myocardium; although they thought that the beneficial effect of this form of preconditioning was possibly due to the modulation of higher centres, rather than to a modification of local autonomic control at effector level (Airaksinen *et al*., 1995). More recently Takasaki *et al*. (1998) have shown, in a rat model, that preconditioning reduced the activity of sympathetic nervous system in all regions of the heart; this effect was attributed to inhibition of noradrenaline release (Seyfarth *et al*., 1996).

The more direct involvement of parasympathetic stimulation in the cardioprotective effects of preconditioning is suggested by studies showing that muscarinic receptor activation results in a similar protection against ischaemic damage to that induced by ischaemic preconditioning (Yao & Gross, 1993), and that increased vagal afferent activity reduces ischaemia-induced myocardial noradrenaline release (Kawada *et al*., 2001). Further, Niroomand *et al*. (1995) have reported that the ischaemia-induced decreased responsiveness of Gi proteins was completely reversed by preconditioning. They proposed that this maintained, or increased, receptor sensitization to muscarinic stimulants; the subsequent inhibition of adenylate cyclase during prolonged ischaemia was implicated in the protective effects of preconditioning.

We have previously suggested (Parratt, 1994; Végh & Parratt, 1996) that the antiarrhythmic effect of preconditioning involves the generation and release of 'endogenous myocardial protective substances'. These include bradykinin (Végh *et al*., 1991b; 1994), prostanoids (Végh *et al*., 1990) and nitric oxide (Végh *et al*., 1992b). Each of these substances can modulate neuronal noradrenaline release and thus interfere with sympathetic activation during ischaemia (Junstad & Wennmalm, 1973; Chahine *et al*., 1993; Addicks *et al*., 1994; Schwartz *et al*., 1995; Chowdhary & Townend, 1999). Nitric oxide seems to be of especial importance as a mediator of preconditioning (Végh *et al*., 1992b; Végh & Parratt, 1996) and there is recent evidence that NO enhances vagal activity by a presynaptic cyclic GMP dependent mechanism (Addicks *et al*., 1994; Schwartz *et al*., 1995; Sears *et al*., 1999). An attractive, unifying hypothesis to explain many of the protective effects of ischaemic preconditioning (suppression of arrhythmias, infarct size limitation, favourable metabolic alterations) would be inhibition of cardiac noradrenaline release (Parratt & Piacentini, 1993). Recent evidence, in the same canine model as that used to demonstrate the antiarrhythmic effects of preconditioning, revealed that inhibition of noradrenaline release with the dopamine agonist Z-1046 is also profoundly antiarrhythmic (Végh *et al*., 1998). Furthermore, we have proposed that exogenously administered noradrenaline, which can itself mimic ischaemic preconditioning in a variety of animal models (Banerjee *et al*., 1993; Bankwala *et al*., 1994) and also reduces the severity of ventricular arrhythmias when locally infused (Végh *et al*., 1995), might also act by suppressing endogenous neuronal noradrenaline release by stimulating presynaptic α-adrenoceptors.

We conclude that preconditioning reduces arrhythmia severity during ischaemia by favourably modifying autonomic regulatory mechanisms in the heart. This beneficial antiarrhythmic effect of preconditioning was associated with a preserved arterial baroreflex sensitivity and by less pronounced changes in epicardial ST-segment elevation and in the degree of inhomogeneity. Since these protective effects of preconditioning were completely abolished by the administration of atropine, we suggest that the transient preconditioning ischaemic episode enhances vagal influences on the myocardium during a subsequent, more prolonged ischaemia. This results in a better autonomic balance leading to the myocardium becoming less susceptible to the generation of arrhythmias. Although it is still not clear, how precisely preconditioning restores this autonomic imbalance, one possibility is that the release of endogenous substances, and especially of nitric oxide, attenuate the sympathetic influence on the ischaemic myocardium by inhibiting noradrenaline release. This results in a relative increase in the vagal component. The increased responsiveness to vagal influences, probably by cyclic GMP-mediated intracellular pathways, and the attenuated sympathetic outflow resulting from presynaptic inhibition of noradrenaline release, both seem to be involved in this antiarrhythmic protection. This may also explain the preserved baroreflex function during prolonged ischaemia in hearts that have been preconditioned.

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