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Modification by bradykinin B_2 receptor blockade of protection by pacing against ischaemia-induced arrhythmias

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Abstract

In dogs, rapid cardiac pacing, by way of a pacing electrode in the right ventricle, protects against ventricular arrhythmias when a coronary artery is occluded immediately after cessation of the pacing period. This represents a form of ischaemic preconditioning. The role of bradykinin in mediating the protective effects of rapid cardiac pacing in this model was investigated using a selective antagonist of bradykinin at B_2 receptors (icatibant; HOE 140). In the presence of icatibant cardiac pacing (220 beats min^{-1}) resulted in more severe ischaemia (as assessed by ST-segment elevation from the pacing electrode at the end of the stimulus) and to a higher incidence of ventricular arrhythmias during the pacing protocol. When the coronary artery was occluded under such conditions the antiarrhythmic protection afforded by cardiac pacing was not seen although other indices of reduced ischaemia severity (epicardial ST-segment mapping; changes in the degree of inhomogeneity of electrical activation within the ischaemic area) were not affected by icatibant treatment. These results suggest that bradykinin is an important trigger mediator involved in the protective effects of cardiac pacing. Whether this is due to the generation of endothelium-derived protective substances (such as nitric oxide and prostacyclin) or whether it results from a direct effect on B_2 receptors in cardiac myocytes is unclear.

Keywords: Bradykinin; Icatibant; Cardiac pacing, rapid; Ischemic preconditioning; Ventricular arrhythmia

1. Introduction

Since the original observation that a reduction in myocardial ischaemic injury can be achieved by repeated short periods of coronary artery occlusion (Murry et al., 1986), there has been increasing evidence that preconditioning can be induced by means other than by complete coronary artery occlusion, for example by rapid cardiac pacing (Végh et al., 1991a; Kaszala et al., 1995, 1996; Gho et al., 1996). The most important consequences of preconditioning are marked reductions in the severity of occlusion and reperfusion-induced ventricular arrhythmias and in the extent of myocardial necrosis (reviewed by Wainwright and Parratt, 1996).

Despite extensive research, the precise mechanisms of this protection afforded by preconditioning are still unclear. One suggestion (Parratt and Végh, 1996) is that endothelium-derived vasoactive substances play an important role in mediating some of the effects of preconditioning when this is induced by brief periods of coronary artery occlusion. Bradykinin is thought to be especially important (Parratt et al., 1995). It has been known for several years that kinins are generated under conditions of myocardial ischaemia (Pitt et al., 1970; Kimura et al., 1973); this leads to a reduction in coronary venous kininogen levels (Marshall and Parratt, 1980). Further, intracoronary infusions of bradykinin markedly reduce the severity of ischaemia-induced ventricular arrhythmias (Végh et al., 1991b). Bradykinin also reduces myocardial lactate production, preserves myocardial tissue levels of glycogen and energy-rich phosphates (Linz et al., 1990) and increases glucose uptake and utilisation (Rösen et al., 1983) during periods of myocardial ischaemia. Bradykinin also contributes to the protective effects of brief periods of coronary artery occlusion (ischaemic preconditioning) in reducing myocardial necrosis (Wall et al., 1994) and arrhythmia severity (Végh et al., 1994) and of angiotensin converting enzyme inhibitors (Hartman et al., 1993) in limiting myocardial infarct size. All these beneficial effects

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of bradykinin are mediated through stimulation of B_2 receptors since they can be abolished by the antagonist icatibant (HOE 140; Martorana et al., 1990; Hartman et al., 1993; Végh et al., 1994; Wall et al., 1994) which, in the dose used in the present study (Linz and Schölkens, 1992; Végh et al., 1994) is particularly selective for these receptors (Wirth et al., 1991).

Recently we have demonstrated that, in dogs, cardiac pacing results in both short-term and delayed protection against ischaemia (and reperfusion)-induced ventricular arrhythmias (Kaszala et al., 1996). In the present study, using a similar anaesthetised, open-chest canine model, we have examined whether bradykinin is also involved in the protection afforded by cardiac pacing.

2. Materials and methods

2.1. Animals

Mongrel dogs of either sex with a mean body weight of 24.9 ± 1.6 kg were used in these experiments. They were anaesthetised with a mixture of chloralose and urethane (60 and 200 mg kg^{-1} i.v., respectively) and ventilated with room air using an Ugo Basile Respirator (Hugo Sachs, Germany) at a rate and volume sufficient to maintain arterial gases and pH within the normal limits (Végh et al., 1992a). Oesophageal temperature was monitored using a thermocouple and body temperature was maintained at 37 ± 0.5°C by means of a heating pad.

Catheters were inserted into the right femoral artery (for monitoring arterial blood pressure), into the left ventricle, via the left carotid artery, for measuring left ventricular systolic (LVSP) and end-diastolic (LVEDP) pressures and LV dP/dt, and into the right femoral vein for drug administration. A bipolar pacing electrode (Cordat F4) was introduced, via the right jugular vein, into the right ventricle for pacing and also for the assessment of endocardial ST-segment changes. The correct placing of the electrode was confirmed from the endocardial electrocardiogram. The animals were then thoracotomised at the fifth intercostal space and, after suspending the heart in a pericardial cradle, the anterior descending branch of the left coronary artery, proximal to the first main diagonal branch, was prepared for occlusion. Epicardial ST-segment elevation and the degree of inhomogeneity of electrical activation were measured within the area supplied by the anterior descending branch by means of the composite electrode described previously (Végh et al., 1987). Subendocardial ST-segment elevation was also measured by means of a unipolar needle electrode (Végh et al., 1987) inserted into the subendocardial region of the left ventricle.

These parameters, together with a Standard II limb lead electrocardiogram, were recorded on a Graphtec Thermal Arreyrecorder (Hugo Sachs, Germany).

2.2. Assessment of arrhythmias

Ventricular arrhythmias were assessed as described previously (Végh et al., 1992a). Thus, the total number of ventricular premature beats, the number of episodes and

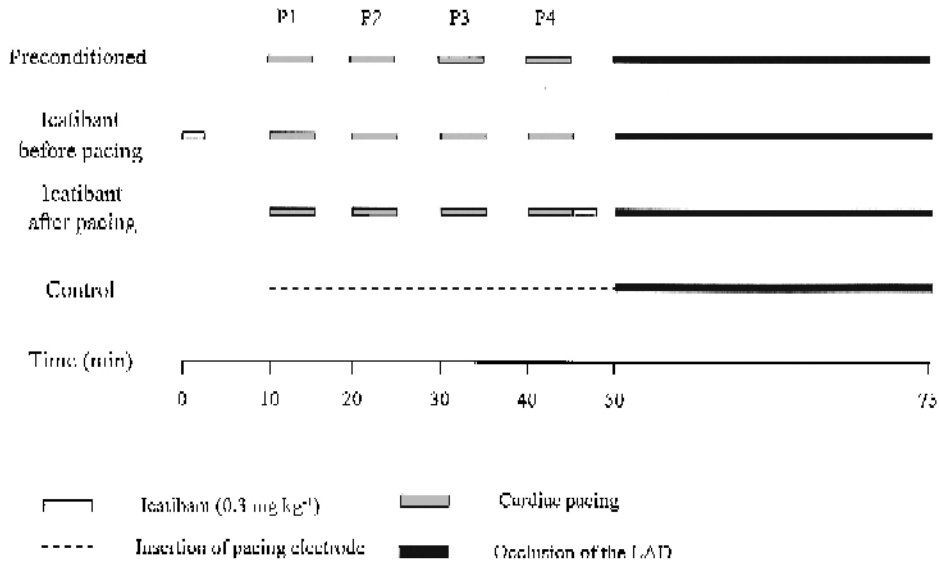

Fig. 1. The experimental protocol used to determine the effect of blockade of bradykinin B_2 receptors on the protective effects of cardiac pacing. The heart was paced (220 beats min^{-1}) for four 5 min periods (P1–P4) with 5 min rest periods between. Five minutes after the last pacing stimulus the left anterior descending coronary artery was occluded for 25 min and the ischaemic area was then reperfused. Icatibant was given either 10 min before the initial pacing stimulus or immediately after the last pacing stimulus.

the incidence of ventricular tachycardia (VT, defined as four or more consecutive ventricular premature beats at a rate faster than the normal sinus rate) and the incidence of ventricular fibrillation (VF) were determined.

Since the area at risk is one of the major determinants of the severity of ventricular arrhythmias after a coronary artery occlusion, this was measured at the end of each experiment by injecting patent blue V dye into the occluded (or re-occluded) coronary artery; the dyed area was then weighed and compared with the non-dyed area. This is the area at risk of necrosis as expressed as a percentage of the left ventricular wall together with the septum.

2.3. Statistics

All the data are expressed as means ± S.E.M. and differences between means were compared by one-way analysis of variance or two-way ANOVA. VPBs were compared by using the Kruskal-Wallis rank test, and the incidence of arrhythmias was compared using a chi-square test or Fisher's exact test. Differences between groups were considered significant when $P < 0.05$.

2.4. Experimental protocol

Preconditioning was induced by four 5 min periods of right ventricular pacing at a rate of 220 beats min^{-1}, with 5 min reperfusion periods between the pacing stimuli (Kaszala et al., 1996). A 25 min occlusion of the anterior descending branch of the left coronary artery was commenced 5 min after the last pacing stimuli; the ischaemic myocardium was reperfused at the end of this period and the incidence of VF during reperfusion assessed. Icatibant, an antagonist of bradykinin at B_2 receptors (Wirth et al., 1991); a gift from Professor B. Schölkens, was administered intravenously in a dose of 0.3 mg kg^{-1} either 10 min before the first pacing period (i.e., prior to preconditioning by pacing; $n = 8$) or immediately after the last ($n = 9$) pacing preconditioning period. Eleven dogs were paced without treatment with icatibant. The controls ($n = 14$) were those dogs which were subjected to a 25 min coronary artery occlusion-reperfusion insult but without prior pacing. This experimental protocol is illustrated in Fig. 1.

Table 2
Changes in the ST segment in mV recorded from the pacing electrode in the right ventricle (RV; ST endo) and from a recording electrode in the subendocardial region of the left ventricle (LV; ST subendo) immediately after cessation of four periods (P1–P4) of cardiac pacing at a rate of 220 beats min^{-1} – these elevations in endocardial (and subendocardial) electrocardiograms may indicate transient ischaemia

	RV; ST endo	Change from baseline	LV; ST subendo	Change from baseline
Baseline	1.4 ± 0.44		1.1 ± 0.28	
P1		+ 3.7 ± 0.73 [a]		+ 0.7 ± 0.29 [a]
P2		+ 2.3 ± 0.32 [a]		+ 0.6 ± 0.16 [a]
P3		+ 2.4 ± 0.24 [a]		+ 0.7 ± 0.16 [a]
P4		+ 2.9 ± 0.30 [a]		+ 0.7 ± 0.17 [a]

[a] $P < 0.05$ compared to the change over a similar time period in which pacing was not induced (baseline).

3. Results

3.1. Haemodynamic effects of cardiac pacing

The haemodynamic effects of right ventricular pacing at a rate of 220 beats min^{-1}, i.e., sufficient to increase heart rate by about 30%, are summarised in Table 1 and were similar in each of the four pacing periods. There were prompt decreases in (mean) arterial blood pressure (from 100 ± 5 to 60 ± 4 mmHg, $P < 0.05$) and significant reductions in both positive and negative LV dP/dt_{max}. Left ventricular end-diastolic pressure (LVEDP) was markedly elevated during pacing (from 5.5 ± 0.7 to 11.3 ± 2.0 mmHg; $P < 0.05$). However, during the intervals between the pacing periods, heart rate, blood pressure, LVEDP and LV positive and negative dP/dt_{max} rapidly returned to pre-pacing levels (Table 1). Further, when pacing was stopped a marked, but transient (i.e., for less than 1 min) ST-segment elevation occurred as assessed from both the

Table 1
The haemodynamic effects of cardiac pacing at a rate of 220 beats min^{-1} in anaesthetised dogs

	Pre-pacing	During pacing (at 1 min)	After pacing (at 1 min)
Systolic arterial blood pressure (mmHg)	129 ± 6	71 ± 4 [a]	131 ± 10
Diastolic arterial blood pressure (mmHg)	86 ± 5	52 ± 5 [a]	87 ± 7
Mean arterial blood pressure (mmHg)	100 ± 5	60 ± 4 [a]	103 ± 8
LVSP (mmHg)	127 ± 5	71 ± 8 [a]	137 ± 8
LVEDP (mmHg)	5.5 ± 0.7	11.3 ± 2.0 [a]	7.0 ± 0.8
LV dP/dt_{max} positive (mmHg s^{-1})	4114 ± 590	1587 ± 128 [a]	4998 ± 634
LV dP/dt_{max} negative (mmHg s^{-1})	3788 ± 502	3254 ± 4.5	4683 ± 647
Heart rate (beats min^{-1})	154 ± 5	222 ± 5 [a]	159 ± 6

[a] $P < 0.05$ compared to the immediate pre-pacing values.

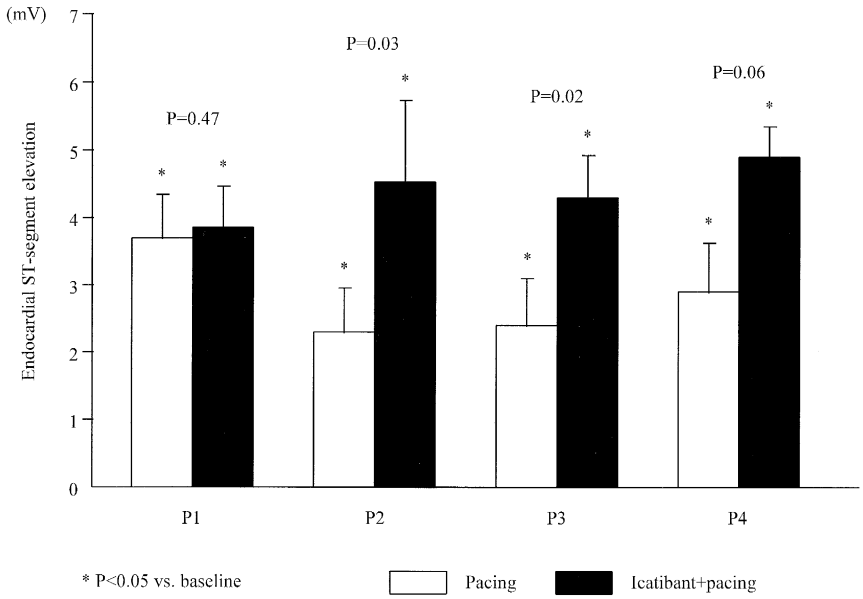

Fig. 2. ST-segment elevation recorded from the pacing electrode in the right ventricle immediately after the cessation of four periods (P1–P4) of pacing at a rate of 220 beats min^{-1}. In contrast to dogs paced without the administration of icatibant (open histograms), following administration of this bradykinin B_2 receptor blocking drug (filled histograms) the ST-segment elevation was significantly ($P < 0.05$) higher immediately after cessation of pacing periods 2 and 3, and there was no evidence of preconditioning.

left ventricular subendocardial electrode and from the right ventricular endocardial pacing electrode in the right ventricle (Table 2). The ST-segment elevation recorded from the pacing electrode was less pronounced following pacing periods 2–4 than it was following the first pacing period, perhaps indicating a form of ischaemic preconditioning. A more pronounced elevation of endocardial ST-segment occurred following cessation of pacing in the presence of icatibant (Fig. 2) and this was particularly marked (and significant, $P < 0.05$) following pacing periods 2 and 3.

3.2. Haemodynamic effects of icatibant

Administration of icatibant had no significant effect on any haemodynamic parameter (Table 3).

Table 3
The haemodynamic effects of icatibant (0.3 mg kg^{-1} i.v., 5 min after administration) in ($n = 8$) anaesthetised dogs

	Pre-drug	After icatibant
Systolic arterial blood pressure (mmHg)	134 ± 6	132 ± 9
Diastolic arterial blood pressure (mmHg)	90 ± 7	89 ± 8
Mean arterial blood pressure (mmHg)	105 ± 6	103 ± 8
LVSP (mmHg)	116 ± 7	115 ± 9
LVEDP (mmHg)	5.6 ± 1.3	6.2 ± 2.3
LV dP/dt_{max} positive (mmHg s^{-1})	2191 ± 273	2036 ± 227
LV dP/dt_{max} negative (mmHg s^{-1})	2285 ± 133	2127 ± 71
Heart rate (beats min^{-1})	142 ± 7	138 ± 8

3.3. Ventricular arrhythmias arising during the pacing procedure

Only a few ectopic beats (18 ± 1) occurred in untreated dogs when the pacing stimulus was terminated. None of these dogs fibrillated either during or after pacing. However, when pacing was performed in the presence of icatibant, the total number of ventricular premature beats was increased (to 47 ± 2, $P < 0.05$); further, 3/8 of these animals fibrillated either during the first (two dogs) or the second (one dog) pacing period.

3.4. Effects of a 25 min occlusion of the left anterior descending coronary artery on ventricular arrhythmias, on the degree of inhomogeneity of electrical activation and on epicardial ST-segment changes; effects of the prior administration of icatibant

The changes that occurred in the various haemodynamic parameters during a 25 min occlusion of the anterior descending branch of the left coronary artery are also summarised in Table 4.

Under the conditions of myocardial ischaemia resulting from coronary artery occlusion, there was a marked inhomogeneity of electrical activation (Fig. 3) and elevations in the epicardial ST segment (Fig. 4), as measured over the ischaemic area. These indices of myocardial ischaemia were significantly less in those dogs in which the coronary artery occlusion was preceded by cardiac pacing. Administration of icatibant either before or after pacing did not modify the protection afforded by cardiac pacing on these indices of ischaemia (Figs. 3 and 4).

Table 4
Haemodynamic changes prior to and immediately after the commencement of a 25 min occlusion of the left anterior descending coronary artery in anaesthetised dogs without pacing (controls) and following pacing with and without the administration of icatibant (see Fig. 1)

	Controls		Paced dogs		Icatibant (before pacing)		Icatibant (after pacing)	
	Pre-occlusion	Post-occlusion	Pre-occlusion	Post-occlusion	Pre-occlusion	Post-occlusion	Pre-occlusion	Post-occlusion
Systolic arterial blood pressure (mmHg)	126±5	−17±2 [a]	106±8	−13±3 [a]	132±7	−8±4	130±7	−8±4
Diastolic arterial blood pressure (mmHg)	85±4	−15±1 a	71±7	−9±3[a]	86±9	−7.7±4	88±4	−10±5
Mean arterial blood pressure (mmHg)	99±5	−15±1 [a]	83±7	−10±2 [a]	101±8	−7±2	100±5	−8±15
LVSP (mmHg)	138±7	−22±2 a	110±6	−19±3[a]	104±10	−8±2	117±4	−11±17
LVEDP (mmHg)	5.6±0.4	+16.7±2.0 a	6.0±5	+11.3±1.0[a]	7.0±1.4	+4.0±0.4	6.0±1.9	+3.9±1
LV dP/dt_{max} positive (mmHg s^{-1})	3027±355	−1019±159 [a]	2545±102	−503±83 [a]	2043±168	−341±58	4103±428	−530±495
LV$dP/dt_{max}$ negative (mmHg s^{-1})	2269±271	−570±149 [a]	1949±120	−368±72	1982±110	−189±26	3824±275	−428±460
Heart rate (beats min^{-1})	146±5	+1±2	147±7	−2±3	152±8	+7±1	168±5	−4±5.2

[a] $P < 0.05$ compared to immediate pre-occlusion values. There were no significant differences between any of the groups in the pre-occlusion values.

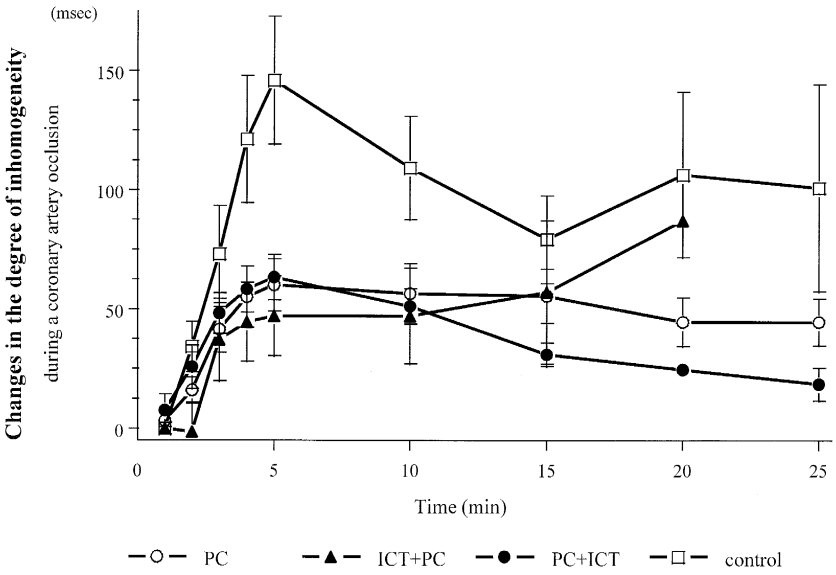

Fig. 3. Changes in the degree of inhomogeneity of electrical activation in ms (as recorded from a composite electrode) during a 25 min occlusion of the left anterior descending coronary artery. Pacing (open circles) reduced the degree of inhomogeneity during occlusion in comparison with the controls (open squares) and this was not sufficiently changed by the administration of icatibant either before (closed triangles) or after (closed circles) the preconditioning pacing stimulus. The changes in the controls were significantly different (at a level of $P < 0.05$) at each time point (from 4 min of ischaemia) from the three other groups.

When the coronary artery was occluded 5 min after the last of the four pacing periods, there was a marked suppression of ventricular arrhythmic activity. Thus, the number of ventricular premature beats over the 25 min occlusion period (202 ± 19), the number of episodes of ventricular tachycardia (a mean of 4.1 ± 2.6 episodes per dog) and the incidence of ventricular tachycardia (45%) were all reduced ($P < 0.05$) in comparison to the controls (ventricular premature beats: 439 ± 72, VT episodes: 7.8 ± 2.4, VT%: 93%). Further, all the animals in the pacing group

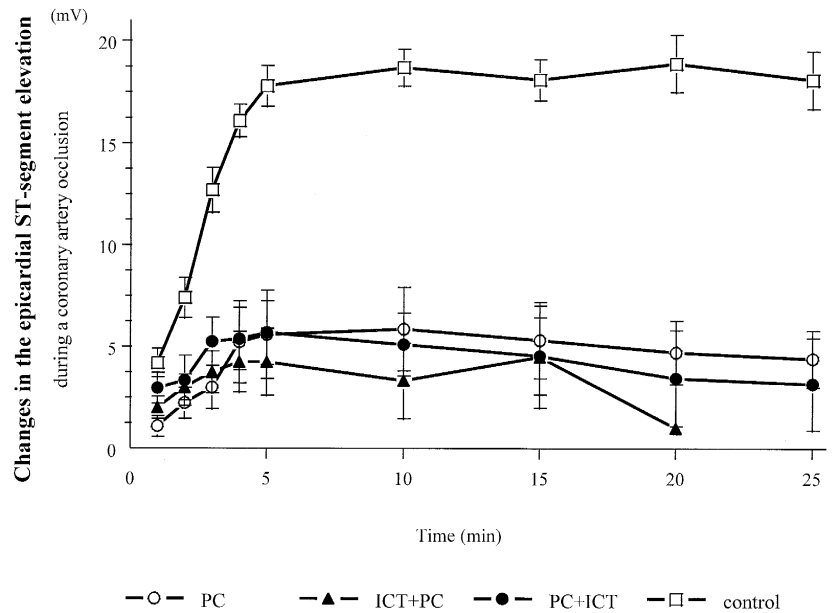

Fig. 4. Changes in epicardial ST-segment elevation (mV) during a 25 min coronary artery occlusion in control dogs (open squares) and in dogs subjected to cardiac pacing (open circles) and following the administration of icatibant, given either before the pacing stimulus (closed triangles) or after the preconditioning pacing stimulus but before occlusion (closed circles). The values for control dogs were significantly higher from 2 min of ischaemia onwards than those of the other three groups (at a level of $P < 0.05$) but there was no significant difference between those of the preconditioned groups, with or without icatibant.

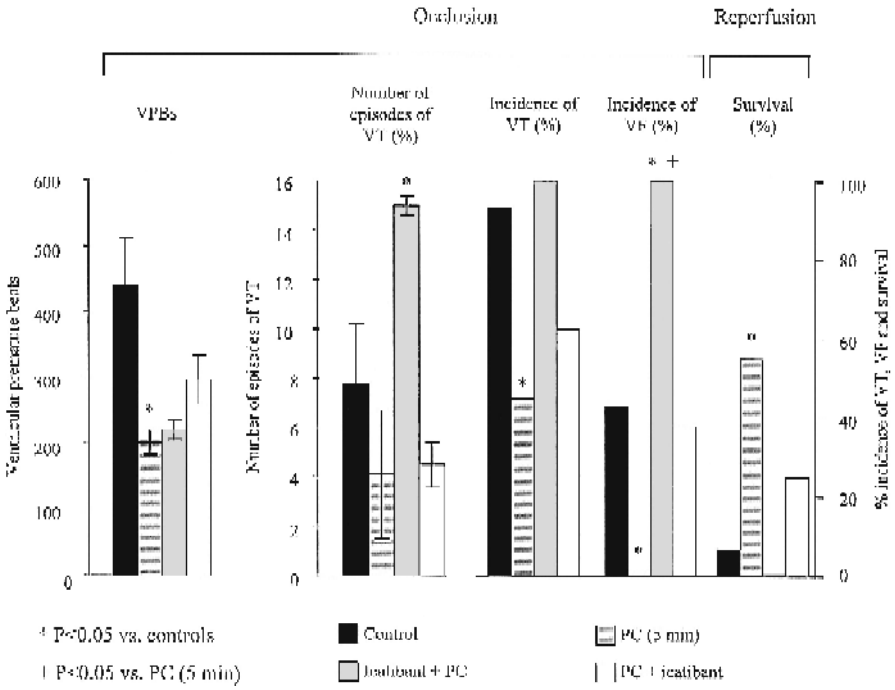

Fig. 5. The total number of ventricular premature beats (VPBs), the number of episodes of VT per dog, the incidences of VT and VF during coronary artery occlusion and the survival from the combined ischaemia-reperfusion insult in control dogs (closed histograms), in dogs preconditioned by cardiac pacing (hatched columns), in dogs given icatibant prior to the pacing stimulus (stippled histograms) and in dogs given icatibant immediately after the preconditioning stimulus (open histograms). Preconditioning by pacing suppressed all indices of arrhythmia severity and, apart from the incidence of VF, this was not markedly altered by the administration of icatibant when given after the preconditioning stimulus. However, when icatibant was given prior to the preconditioning stimulus the more serious arrhythmias (VT, VF) were as severe (or more severe) than in control, unpaced dogs (see text for discussion).

survived the occlusion period (VF during occlusion: 0%), and only 45% fibrillated on reperfusion. Thus, the overall survival from the combined occlusion-reperfusion insult was 55%. In contrast, 43% of the control group fibrillated during occlusion and none of these animals survived reperfusion (Fig. 5).

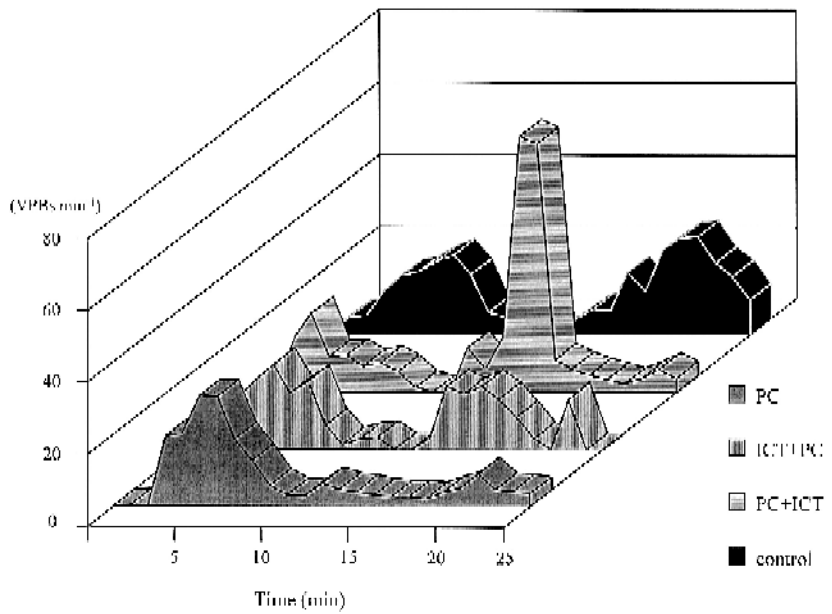

Fig. 6. The distribution of ventricular premature beats over a 25 min coronary artery occlusion period in control dogs and in paced dogs given icatibant prior to, or after, the preconditioning stimulus. Pacing completely suppressed phase Ib arrhythmias (those occurring between 12 and 25 min) but this protection was not seen in the presence of icatibant.

In those dogs in which icatibant was given 10 min before pacing the protection against the more serious ventricular arrhythmias (VT, VF) was completely lost (Fig. 5); all the dogs had a considerable number of episodes of VT (15.0 ± 0.4; $P < 0.05$ compared to those dogs preconditioned by pacing) and all fibrillated during occlusion. In those dogs in which icatibant was administered immediately after the last pacing period, but 5 min prior to occlusion, the pacing-induced protection against arrhythmias was partially lost (Fig. 5). The reduction in the number of premature beats, in the number of episodes of VT, and in the incidence of VT was hardly influenced by icatibant but there was a higher incidence of VF during occlusion (38% vs. 0%; $P = 0.057$; Fig. 5) and the overall survival rate from the combined ischaemia-reperfusion insult was also less than in those dogs that were paced but not given icatibant prior to occlusion (25% vs. 55%; $P = 0.352$). There was an interesting change in the distribution of ventricular premature beats in those dogs subjected to pacing (Fig. 6). The marked suppression in phase Ib arrhythmias seen in the paced dogs was lost in the presence of icatibant given either prior to pacing, or after pacing but prior to occlusion.

3.5. Area at risk

There was no significant difference in the area at risk between any of the groups (controls: $38.5 \pm 2\%$, preconditioned: $38.5 \pm 1.9\%$, icatibant before pacing: $32.4 \pm 1.7\%$, icatibant after pacing: $37.5 \pm 6.4\%$).

4. Discussion

We have shown previously that four 5 min episodes of pacing at a rate of 220 beats min^{-1} markedly reduces the incidence and severity of ventricular arrhythmias that result from coronary artery occlusion (Kaszala et al., 1995, 1996) and that this protection against arrhythmias is similar to that seen in classical preconditioning (reviewed by Parratt et al., 1996). In the present study we show that this antiarrhythmic protection during coronary artery occlusion afforded by cardiac pacing is lost when pacing is performed in the presence of icatibant, an antagonist of bradykinin at B_2 receptors. Further, protection is only somewhat attenuated when bradykinin B_2 receptors are blocked before the occlusion period but after pacing. These results are in accord with our previous findings that intracoronary infusions of bradykinin exert a nitric oxide-dependent antiarrhythmic effect in this species (Végh et al., 1991b, 1993) and that preconditioning by short coronary artery occlusions results in higher coronary venous bradykinin levels and increased bradykinin generation during ischaemia (Parratt et al., 1997). As in our previous study using short periods of coronary artery occlusion to induce preconditioning (Végh et al., 1994), it was difficult to protect the heart by cardiac pacing in the presence of icatibant even though this procedure is much safer than using brief periods of coronary artery occlusion to precondition the heart. It seems then that a release of bradykinin is especially important as the triggering mechanism of preconditioning whether this is induced by short periods of ischaemia (by complete coronary artery occlusion) or by cardiac pacing.

What is at present unclear is whether these effects are mediated directly by bradykinin receptors on cardiac myocytes or whether bradykinin acts as a trigger for the release of other cardioprotective mediators, for example from coronary vascular endothelial cells. Bradykinin B_2 receptors are linked, by the G-protein-coupled stimulation of phospholipase C, to the formation of inositol phosphates and diacylglycerol. Inositol trisphosphate is known to elevate intracellular Ca^{2+} concentrations and this, in turn, releases arachidonic acid (and eicosanoids) via stimulation of phospholipase A_2 (Scherf et al., 1986). Diacylglycerol directly activates protein kinase (PK) C (Murray et al., 1991; Calixto and Madeiros, 1992) which is a key event in the signalling transduction mechanisms involved in preconditioning (reviewed by Cohen et al., 1996). Further, elevation of intracellular Ca^{2+}, and the resultant prostacyclin production, releases nitric oxide from endothelial cells (Hecker et al., 1994). Both prostacyclin (Végh et al., 1990; Parratt, 1993) and nitric oxide (Végh et al., 1992b), released from endothelial cells, have been suggested as possible protective mediators of the antiarrhythmic effect of ischaemic preconditioning in dogs. This would represent an example of 'cross-talk' between these vascular cells and cardiac myocytes (Parratt and Végh, 1996) with resultant protection against a reduction in coronary blood flow.

Noradrenaline is also released during acute myocardial ischaemia and may be particularly important in initiating ventricular ectopic activity (Sharma and Corr, 1983), particularly during phase Ib, and it is of interest that it was the pacing-induced suppression of ventricular ectopic activity during this particular period of occlusion that was abolished by icatibant. Bradykinin can inhibit noradrenaline overflow under conditions of ischaemia and protect against ventricular arrhythmias, an effect that appears to be mediated mainly through bradykinin B_1 receptors, although B_2 receptors may also be involved (Chahine et al., 1993).

An alternative explanation for the detrimental effects of icatibant in the present studies is the possibility that bradykinin may directly protect cardiac myocytes. As with the effects of bradykinin on endothelial cells outlined above this protection may involve G-protein coupling, inositol trisphosphate, diacylglycerol, activation of nitric oxide synthase and PKC and, perhaps, the opening of K_{ATP} channels. This concept of bradykinin as a paracrine in cardiac myocytes has been recently suggested by the experiments of Wall et al. (1996) showing that sarcolemmal damage resulting from hypoxia/reoxygenation injury

in cultured rat neonatal cardiac myocytes is significantly reduced by bradykinin, an effect mediated through bradykinin B_2 receptors.

In summary, our results indicate that pacing markedly reduces arrhythmias, particularly phase Ib arrhythmias, that occur soon after the onset of myocardial ischaemia and that this protection is attenuated by a selective antagonist of bradykinin at B_2 receptors. The mechanism of this protection by pacing thus seems to involve the early generation of bradykinin and indeed, there is direct evidence for this. In these dogs coronary sinus bradykinin levels are significantly higher after this pacing stimulus than before pacing (Parratt et al., 1997). These results add further support to the concept that the protection of the myocardium by ischaemic preconditioning involves the generation of endogenous myocardial protective substances (Parratt, 1993) of which bradykinin appears to be of particular importance.

Acknowledgements

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