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Preconditioning Modulates Susceptibility to Ischemia-Induced Arrhythmias in the Rat Heart: The Role of α-Adrenergic Stimulation and K(ATP) Channels

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Summary

A new concept of cardioprotection based on the exploitation of endogenous mechanisms is known as ischemic preconditioning (IPC). It has been hypothesized that substances released during brief ischemic stress (e.g. catecholamines) stimulate the receptors and trigger multiple cell signaling cascades. Opening of ATP-sensitive K^+ channels [K(ATP)] has been suggested as a possible final step in the mechanisms of protection. In this study, the role of adrenergic activation was tested in Langendorff-perfused rat hearts subjected to test ischemia (TI; 30 min occlusion of LAD coronary artery) by: 1) mimicking IPC (5 min ischemia, 10 min reperfusion) with short-term (5 min) administration of norepinephrine (NE, 1 μM), 15 min prior to TI; 2) blockade with β- or α_1-receptor antagonists, propranolol (10 μM) and prazosin (2 μM), respectively, applied 15 min prior to TI during IPC. The role of K(ATP) opening was examined by perfusion with a K(ATP) blocker glibenclamide (10 μM) during IPC. Both IPC and NE-induced PC effectively reduced the incidence of ventricular tachycardia (VT) to 33 % and 37 %, respectively, vs 100 % in the non-PC controls, whereby ventricular fibrillation (VF) was totally abolished by IPC and markedly suppressed by PC with NE (0 % and 10 %, respectively, vs 70 % in the non-PC hearts; $P<0.05$). The severity of arrhythmias (arrhythmia score, AS) was also markedly attenuated by both interventions (IPC: AS 1.7±0.4; NE-PC: AS 1.8±0.3 vs AS 4.1±0.2 in the controls; $P<0.05$). Protection was not suppressed by propranolol (VT 28 %; VF 14 %; AS 2.2±0.6), whereas prazosin reversed the protective effect of PC (VT 83 %; VF 67 %; AS 4.0±0.8). Antiarrhythmic protection afforded by NE-PC was abolished by pretreatment of rats with pertussis toxin (25 μg/kg, i.p.) given 48 h prior to the experiments. Glibenclamide did not suppress the IPC-induced protection. In conclusion, the sensitivity of the rat heart to ischemic arrhythmias can be modulated by IPC. Protection is mediated *via* stimulation of α_1-adrenergic receptors coupled with Gi-proteins but glibenclamide-sensitive K(ATP) channels do not appear to be involved in the mechanisms of antiarrhythmic protection in this model.

Key words
Ischemic preconditioning • Arrhythmias • Adrenergic stimulation • K(ATP) channels

Introduction

The last decade has witnessed the development of a novel approach to myocardial protection against ischemia that exploits the heart's own endogenous protective mechanisms. The concept of ischemic preconditioning (IPC) has offered new powerful tools to combat deleterious effects of long-lasting ischemia by way of

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adaptation of the heart during preceding short episodes of the same ischemic stress (Murry *et al.* 1986). Protection can be manifested by a reduced size of infarction (Thornton *et al.* 1993), improved postischemic contractile recovery (Cave 1995), as well as by suppression of malignant ischemia-induced arrhythmias (Vegh *et al.* 1992). This short-term adaptive phenomenon is believed to be mediated by mechanisms of cell signaling, which opens possibilities for pharmacological modulation at different levels of signal transduction (receptors, mediators, effectors). A number of substances, both protective and deleterious (e.g. adenosine, bradykinin, prostanoids, catecholamines), are known to be released locally in the myocardium during early ischemia and to modulate the severity of ischemic injury (Curtis *et al.* 1993, Parratt 1993). Receptor activation by endogenously released substances (e.g., by adenosine) is considered as a first step in the preconditioning mechanisms that trigger multiple signaling cascades leading to a protective response (Downey and Cohen 1995).

An alternative approach to eliciting IPC-like protection is the pharmacological stimulation of receptors by potentially deleterious substances, such as catecholamines, to induce short-term stress, but without harmful consequences of the ischemic injury. In general, under conditions of myocardial ischemia, catecholamines are believed to aggravate cell injury and exacerbate arrhythmias by facilitating calcium influx into the cells enhancing automaticity and triggered activity (Penny 1984). However, no clear correlation between increased concentration of plasma catecholamines and the incidence of arrhythmias has been demonstrated uNder clinical conditions (Bertel *et al.* 1982) and experimental studies have not revealed an essential role of increased sympathetic activity or circulating catecholamines for the occurrence of arrhythmias (Curtis *et al.* 1998).

On the contrary, under certain conditions (e.g. in the partially depolarized myocardium) adrenergic/sympathetic interventions can exert a protective effect and lead to attenuation of arrhythmogenesis (Li *et al.* 1993). Short administration of catecholamines before the onset of long-lasting ischemia has been found to precondition the heart against postischemic myocardial stunning in rats (Banerjee *et al.* 1993, Asimakis *et al.* 1994), to reduce infarct size in rabbits (Bankwala *et al.* 1994) and to suppress ischemia-induced arrhythmias in dogs (Vegh *et al.* 1994) and rats (Ravingerova *et al.* 1997). However, the role of catecholamines in the mechanisms of cardioprotection has not so far been sufficiently elucidated.

A number of receptor ligands interacting with G-proteins are known to induce activation of protein kinase C (Mitchell *et al.* 1995). The activation of protein kinase C (PKC), through phospholipase C or phospholipase D-mediated pathway, as well as participation of other kinases (Maulik *et al.* 1996), is considered as the mainstream process in signal transduction mechanisms triggered by classical IPC linked to the phosphorylation of some hypothetical end-effector proteins mediating the final protection (reviewed by Cohen *et al.* 2000). Opening of ATP-sensitive K^+ channels [K(ATP)] has been suggested as a most likely final step in preconditioning cascade since their blockade with the K(ATP) blocker glibenclamide abolished IPC in dogs (Gross and Auchampach 1992). The activation of these channels has been demonstrated in different forms of cardioprotection, and in many animal species, strengthened by the finding that K(ATP) openers could mimick IPC-induced protection (Grover *et al.* 1994, Gross and Fryer 1999). Although the reduction of infarct size is considered as the gold standard in the definition of cardioprotection conferred by IPC, sudden death due to ventricular fibrillation represents a major therapeutic challenge as well, due to the complexity of pathophysiological mechanisms initiating arrhythmias in ischemic heart disease, the absence of safe and effective preventive measures and due to proarrhythmic properties of many antiarrhythmic drugs. Hence, the development of a new approach to management of arrhythmias is urgently needed. Nevertheless, the role of K(ATP) channels activation in antiarrhythmic protection afforded by IPC has not been sufficiently elucidated so far. Moreover, K(ATP) modulations may exert both anti- and proarrhythmic effects on arrhythmias depending on experimental conditions, animal species and the mechanism of arrhythmias (Tosaki *et al.* 1992, Baczko *et al.* 1997, Wirth *et al.* 1999). The suppression of preconditioning by glibenclamide may be of major concern in humans (Tomai *et al.* 1994), since K(ATP) inhibition may be one of the causes of higher mortality in diabetic patients as a consequence of hypoglycemic therapy with sulphonylurea drugs (Brady *et al.* 1998).

The present study was designed to elucidate the role of adrenergic receptor stimulation and some postreceptor pathways. Our further goal was to test the effect of K(ATP) blockade by one of sulphonylurea drugs (glibenclamide) on the protection conferred by IPC. A model of Langendorff-perfused rat heart was utilized in

this study, and ischemia-induced arrhythmias were chosen as the main end-point of injury.

Methods

Animals

Male Wistar rats (250-300 g body weight), fed a standard diet and tap water *ad libitum,* were employed. All studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No 85-23, revised 1996).

Perfusion technique

Rats were anesthetized (sodium pentobarbitone, 60 mg/kg, i.p.) and given heparin (500 IU, i.p.). Hearts were rapidly excised, placed in ice-cold perfusion buffer, cannulated *via* the aorta and perfused in the Langendorff mode at a constant perfusion pressure of 70 mm Hg and at 37 ^{o}C. The perfusion solution was a modified Krebs-Henseleit buffer gassed with 95 % O_2 and 5 % CO_2 (pH 7.4) containing (in mM): NaCl 118.0; KCl 3.2; $MgSO_4$ 1.2; $NaHCO_3$ 25.0; NaH_2PO_4 1.18; $CaCl_2$ 2.5; glucose 11.1. The solution was filtered through a 5 μm porosity filter (Millipore) to remove contaminants.

An epicardial electrogram (EG) was registered by means of two stainless steel electrodes attached to the apex of the heart and an aortic cannula and continuously recorded (Mingograph ELEMA-Siemens, Solna, Sweden). Heart rate was calculated from the EG. Coronary flow was measured by a timed collection of coronary effluent. Left ventricular pressure was measured by means of a latex water-filled balloon inserted into the left ventricle *via* the left atrium (adjusted to obtain end-diastolic pressure of 5-7 mm Hg) and connected to a pressure transducer (P23 Db Pressure Transducer, Gould Statham Instruments, USA). Left ventricular developed pressure (LVDP, systolic minus diastolic pressure), maximum rates of pressure development and fall (+dP/dt and –dP/dt) as the indexes of contraction and relaxation, as well as the heart rate and coronary flow were used to assess cardiac function.

Arrhythmias were measured in accordance with The Lambeth Conventions (Walker *et al.* 1988). In this study we analyzed the incidences of ventricular tachycardia (VT) and fibrillation (VF) as well as their duration. VT was defined as a run of four or more consecutive ectopic beats. VF lasting more than 2 min was considered as sustained. The severity of arrhythmias was quantified by a scoring system, where hearts with premature ventricular beats only were given a score of 1, bigeminy/salvos a score of 2, VT a score of 3, transient VF a score of 4 and a score of 5 was ascribed to the hearts with sustained VF. The number corresponded to the most severe type of arrhythmia observed in each heart, and scores were used for group analysis of their severity.

Experimental protocols

After 30 min equilibration, all hearts were randomly assigned to the following protocols:

1. Test ischemia (n=38)
After additional 15 min perfusion, the hearts were subjected to a test ischemic challenge as described previously (Ravingerova *et al.* 2000). Regional ischemia was induced by a ligature placed loosely around the left anterior descending coronary artery close to its origin. Both ends of the suture were threaded through a traction-type plastic occluder. Coronary occlusion was induced by traction of the suture against the outer cannula and clamping. After 30 min, the ligature was released to permit reperfusion. The efficacy of occlusion and reperfusion was confirmed by a fall in coronary flow of about 40 % at the onset of ischemia and its recovery upon reperfusion. Further verification was performed by dye trapping/exclusion technique with Sulphan Blue dye and measurement of the ischemic zone size (Ravingerova *et al.* 1995).

2. Ischemic preconditioning (n=18)
After equilibration, the hearts were subjected to one cycle of ischemic preconditioning consisting of 5 min ischemia and 10 min reperfusion, prior to the test ischemia.

3. Preconditioning with norepinephrine (NE-PC, n=15).
Norepinephrine (NE; 1 μM) was used to reproduce ischemic preconditioning and administered in a manner mimicking IPC (5 min perfusion, 10 min washout, prior to test ischemia).

Adrenergic modulations

To elucidate the role of adrenergic receptors in the mechanisms of preconditioning, propranolol (10 μM) and prazosin (2 μM) were used for blocking β- and α_1-adrenoceptors, respectively, and were applied during preconditioning protocols 15 min prior to the ischemic test. To assess the role of β-receptors stimulation alone, isoproterenol (0.1 μM) was administered in the same way as norepinephrine in the protocol of NE-PC. The desired amounts of drugs were dissolved in the perfusion buffer immediately before use. The glassware and tubing were

protected from light. To clarify the role of G-proteins in postreceptor signaling mechanisms, in a separate group of experiments, animals were pretreated with Pertussis toxin (islet activating protein from *Bordetella pertussis*, 25 μg/kg, i.p.) 48 h prior to experiments for inactivation of G_i proteins by ADP ribosylation of the α subunit of Gi proteins. The above procedure prevents association of Gi proteins with its receptors and has been reported to abolish the antiarrhythmic protection afforded by IPC in the isolated rat heart (Piacentini *et al.* 1993). To evaluate the role of K(ATP) channels in the mechanisms of IPC, glibenclamide (10 μM) was used for blocking these channels and applied 5 min before and throughout IPC. All drugs were from SIGMA (St Louis, USA).

Statistics

Data were expressed as means ± S.E.M. The one-way analysis of variance (ANOVA) and a subsequent Student-Newman-Keuls test were used for comparison of differences in normally distributed variables among groups. Non-Gaussian distributed variables (incidences of VT and VF) were compared using Fisher's Exact test. Differences were considered significant when $P<0.05$.

Table 1. Preischemic functional parameters of isolated rat hearts before experimental interventions

Group	n	HR	CF	LVDP	+dP/dt	-dP/dt
C	38	300 ± 15	13.6 ± 1.5	94 ± 6	3320 ± 180	1960 ± 160
IPC	18	308 ± 10	12.4 ± 0.8	90 ± 7	2886 ± 237	1820 ± 152
NE-PC	15	290 ± 9	13.0 ± 0.5	92 ± 8	2860 ± 280	1888 ± 200
IPC+Prop	12	305 ± 7	10.2 ± 1.0	88 ± 7	2924 ± 166	1864 ± 100
IPC+Praz	12	310 ± 8	12.0 ± 1.2	78 ± 10	3157 ± 158	1906 ± 60
Iso-PC	10	295 ± 12	11.6 ± 0.4	79 ± 12	3160 ± 230	1989 ± 57
NE-PC+PT	10	288 ± 14	10.1 ± 1.7	80 ± 5	3024 ± 96	1916 ± 76
C + PT	9	297 ± 5	11.0 ± 0.7	82 ± 8	2965 ± 106	1854 ± 188
IPC + G	12	287 ± 10	9.8 ± 2.0	76 ± 12	3110 ± 87	1827 ± 210
C + G	9	307 ± 6	10.0 ± 1.8	73 ± 8	3004 ± 109	1800 ± 200

C – controls; IPC – ischemic preconditioning; NE-PC – preconditioning with norepinephrine; IPC+Prop – IPC plus propranolol; IPC+Praz – IPC plus prazosin; Iso-PC - preconditioning with isoproterenol; NE-PC+PT – NE-PC plus pertussis toxin; C + PT – controls with pertussis toxin; IPC + G – IPC plus glibenclamide; C + G – controls with glibenclamide. Data are means ± SEM (n – number of rats in each group). HR - heart rate (beats/min); CF - coronary flow (ml/min); LVDP - left ventricular developed pressure (mm Hg); +dP/dt and –dP/dt – maximum rates of pressure development and decline, respectively (mm Hg/s).

Results

Cardiac function before interventions

There were no significant differences in the control preischemic values for LVDP, +/-dP/dt, heart rate and coronary flow among the experimental groups after an equilibration period (Table 1).

Susceptibility to ischemia-induced arrhythmias and the effect of ischemic preconditioning

In this model of regional ischemia, occlusion of LAD coronary artery produced an ischemic zone (area at risk) amounting to approximately 43 % of total ventricular mass. There were no differences in the size of ischemic zone among the groups. Severe ventricular arrhythmias peaked after about 10 to 20 min of ischemia. In the control non-preconditioned hearts, VT was observed in all the hearts (Fig. 1), and 70 % of the hearts exhibited VF. One cycle of IPC did not change the temporal profile of arrhythmias, but successfully suppressed their incidence. The incidence of VT was decreased to 33 % and VF was totally abolished (Fig. 1; $P<0.05$). Not only the incidence, but the duration of arrhythmias was also affected by IPC. The total duration of both VT and VF was significantly shorter in the preconditioned hearts than in the controls (Table 2).

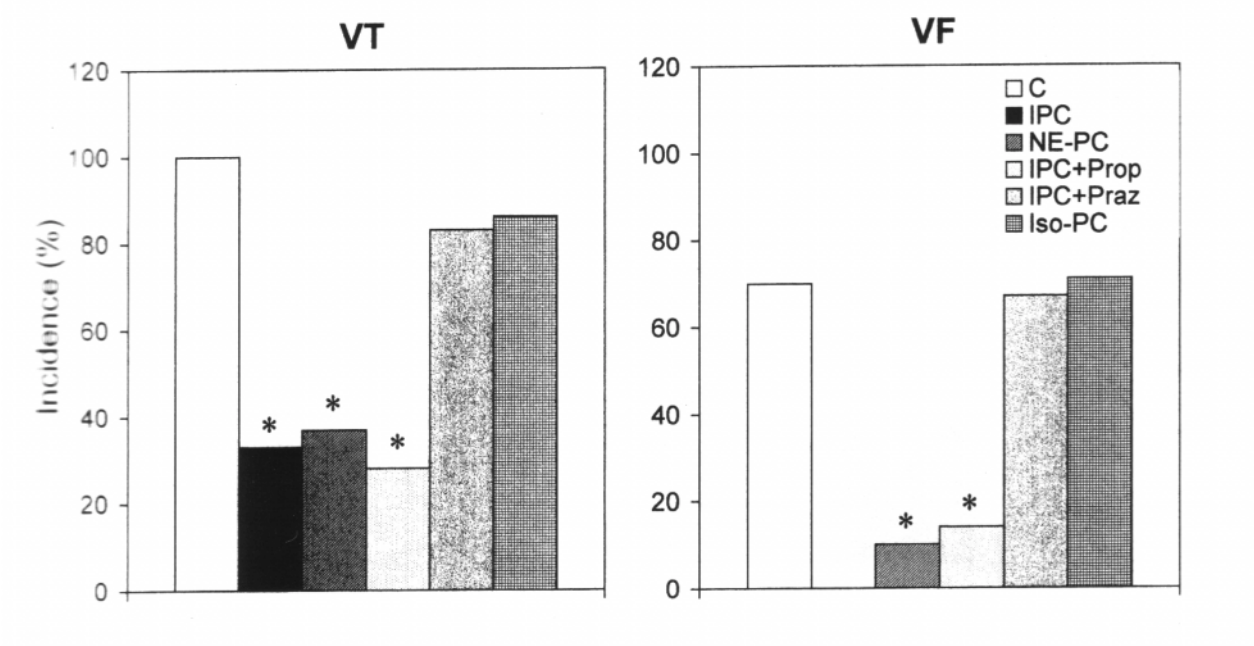

Fig. 1. *Effect of preconditioning and adrenergic modulations on susceptibility to ischemic arrhythmias in isolated rat hearts. C - control hearts subjected to test 30 min ischemia; IPC – hearts subjected previously to ischemic preconditioning; NE-PC – hearts subjected previously to preconditioning with norepinephrine; IPC + Prop – IPC and propranolol; IPC + Praz – IPC and prazosin; Iso-PC – preconditioning with isoproterenol. VT - ventricular tachycardia, VF - ventricular fibrillation. Data are % of incidence evaluated by means of Fisher's Exact test.*
**- P<0.05; vs non-preconditioned controls. Number of experiments per group is indicated in Table 1.*

Effect of preconditioning with norepinephrine on susceptibility to arrhythmias

Short administration of NE induced similar antiarrhythmic protection as classical IPC and reduced the incidence of VF and VT to 10 % and 37 %, respectively (Fig. 1, P<0.05), as well as shortened the duration of tachyarrhythmias (Table 2).

Effects of adrenergic modulations on preconditioning-induced antiarrhythmic protection

Application of propranolol or prazosin alone was tested in separate groups of non-preconditioned hearts (9-10 hearts per group) and did not modify arrhythmogenesis in the protocol of test ischemia (propranolol: VT 77 %, VF 55 %; prazosin: VT 80 %, VF 60 %; P>0.05 vs controls). Propranolol also failed to suppress the protective effect when applied during both preconditioning protocols. On the contrary, prazosin abrogated antiarrhythmic protection afforded by both interventions in a similar way. The effects of propranolol and prazosin on IPC-induced suppression of arrhythmias are shown in Fig. 1. The incidence of VT and VF after IPC in the presence of propranolol were 28 % and 14 %, respectively, whereas prazosin reversed the effect of IPC

Table 2. Effect of ischemic preconditioning (IPC) and preconditioning with norepinephrine (NE-PC) on the duration of ischemia-induced ventricular tachycardia (VT) and fibrillation (VF) in isolated perfused rat hearts

Duration (sec)	**Groups**		
	Control (n = 38)	**IPC (n = 18)**	**NE-PC (n = 15)**
VT	228 ± 45	17.5 ± 11*	80 ± 35*
VF	417 ± 80	0	90 ± 20*

*Data are means ± S.E.M. *P<0.05; preconditioned vs non-preconditioned control hearts*

(VT 83 %, VF 67 %). Substitution of norepinephrine with isoproterenol was not effective and did not reproduce the antiarrhythmic effect of preconditioning (VT 86 %, VF 71 %, Fig. 1). Figure 2 demonstrates the effect of adrenergic modulations on the severity of arrhythmias (arrhythmia score, AS) that corresponded to the above findings. Severity of arrhythmias was significantly lower in both PC protocols than in the controls (1.7±0.4 and 1.8±0.3 for IPC and NE-PC, respectively, vs 4.1±0.2 in the controls; P<0.05). AS was also low in the

propranolol-treated IPC group (2.2±0.6, P<0.05 vs controls), whereas prazosin abolished the effect of IPC and increased the severity of arrhythmias to the score of 4.0±0.8. Pretreatment with isoproterenol did not suppress the severity of arrhythmias as well (AS 4.1±0.7, Fig. 2).

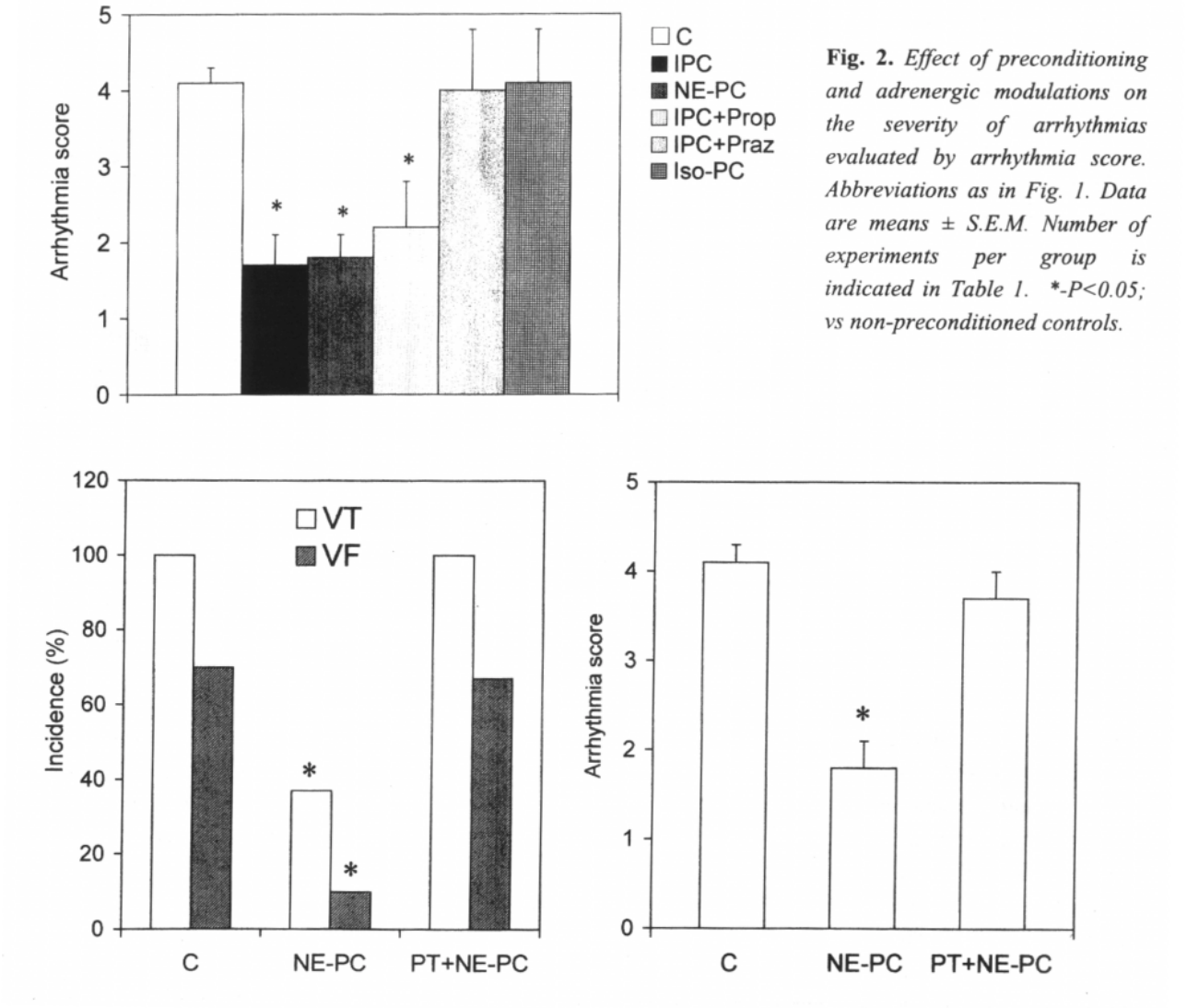

Fig. 2. *Effect of preconditioning and adrenergic modulations on the severity of arrhythmias evaluated by arrhythmia score. Abbreviations as in Fig. 1. Data are means ± S.E.M. Number of experiments per group is indicated in Table 1. *-P<0.05; vs non-preconditioned controls.*

Fig. 3. *Effect of inactivation of Gi proteins by pretreatment of animals with Pertussis toxin (PT) on the incidence of VT and VF (left) and severity of arrhythmias (right) in isolated rat hearts preconditioned with norepinephrine (NE-PC). Data are % of incidence evaluated by means of Fisher's Exact test (left) and means ± S.E.M. (right). Number of experiments per group is indicated in Table 1. *-P<0.05; vs non-preconditioned controls.*

Effect of pertussis toxin pretreatment on the antiarrhythmic effect of preconditioning

Pretreatment of animals with Pertussis toxin did not affect arrhythmogenesis in a setting of test ischemia (not shown) and resulted in a loss of antiarrhythmic protection afforded by short-term pretreatment with NE, so that the incidence and severity of arrhythmias did not differ from those in the control group (VT 100 %, VF 67 %, AS 3.7±0.3,P>0.05 vs controls, Fig. 3).

Effect of K(ATP) blockade on the antiarrhythmic effect of ischemic preconditioning

K(ATP) blockade with glibenclamide did not substantially suppress arrhythmogenesis in the protocol of test ischemia (VT 80 %, VF 20 %, AS 3.1±0.3, Fig. 4). Neither had it any effect on the reduction of arrhythmias induced by IPC (VT 17 %, VF 0 %, AS 1.7±0.3, P<0.05 vs controls, Fig. 4).

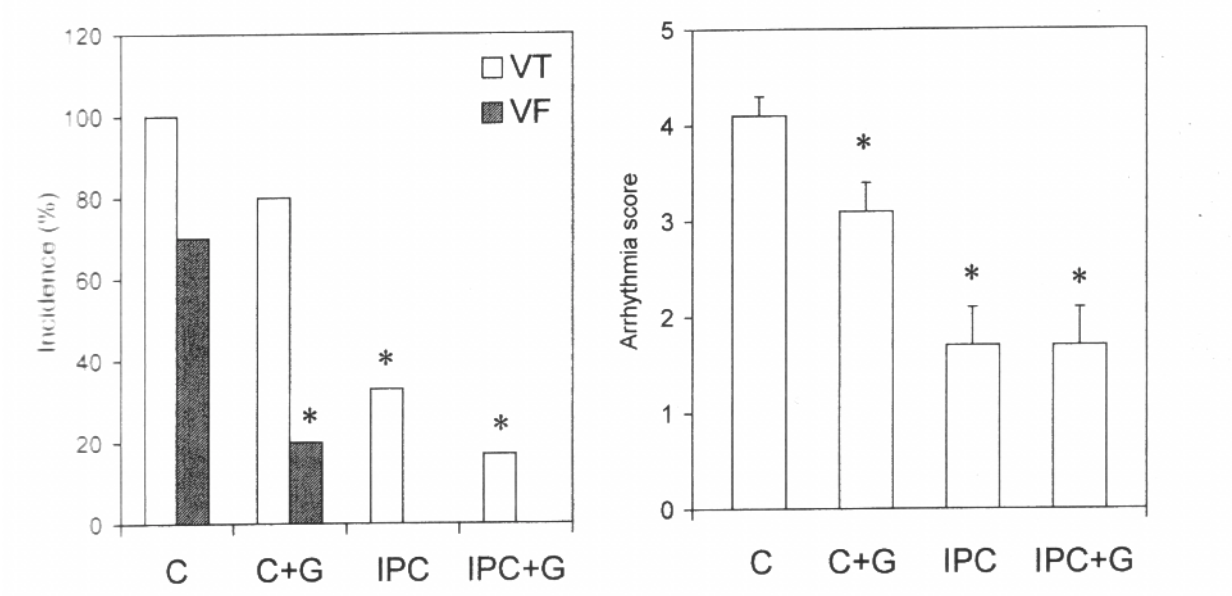

Fig. 4. *Effect of blockade of K(ATP) channels with glibenclamide (G) on the incidence of VT and VF and severity of arrhythmias in isolated rat hearts subjected to ischemic preconditioning (IPC). Data are % of incidence evaluated by means of Fisher's Exact test (left) and means ± S.E.M. (right). Number of experiments per group is indicated in Table 1. *-P<0.05; vs non-preconditioned controls.*

Discussion

The main objective of this study was to demonstrate that short-term stimulation of adrenergic receptors, either by endogenously released or by exogenously administered catecholamines, is involved in the preconditioning-induced antiarrhythmic protection during sustained myocardial ischemia. Ischemic preconditioning by one cycle of ischemia/reperfusion effectively suppressed ischemia-induced arrhythmias in the Langendorff-perfused rat heart, a model, in which regional ischemia elicits a high incidence of severe ventricular arrhythmias (Curtis *et al.* 1998). Short-term exogenous administration of norepinephrine afforded similarly effective protection.

One of the major determinants of arrhythmogenesis is the size of the ischemic area (Curtis 1998). Another factor that might contribute to the reduced arrhythmogenesis is the heart rate (Bernier *et al.* 1989). However, we can disregard both factors since there were no differences in the size of the ischemic area or in the heart rate among the groups.

Since norepinephrine stimulates both α- and β-adrenergic receptors, pharmacological modulations have been used to clarify which particular receptors are involved in this cardioprotection. The antiarrhythmic effect appeared to be due to stimulation of α1-adrenergic receptors, since their blockade suppressed the protective effect of preconditioning. In contrast, β-adrenergic receptors do not appear to be involved in the antiarrhythmic effect of preconditioning. Their blockade neither affected preconditioning-induced protection, nor was β-adrenergic stimulation capable of mimicking preconditioning. The latter is in concert with the proarrhythmic effects of β-receptor stimulation in the normal myocardium, such as the shortening of the refractory period. On the other hand, the effects of α1-adrenergic stimulation (which might be more important under pathological conditions) prolong the refractoriness and action potential duration, as well as increase the conduction velocity and decrease the automaticity, effects which in general are considered to be antiarrhythmic (Wendt and Martins 1990). Short-term stimulation of α1-receptors has been demonstrated to suppress the incidence of reperfusion-induced arrhythmias in a model of global ischemia/reperfusion and to reduce the accumulation of Na^+ and loss of K^+ in the myocardium during ischemia (Tosaki *et al.* 1995). The latter can also account for the suppression of arrhythmias during ischemia as observed in the present study.

Moreover, α1-receptor stimulation either by endogenously released or by exogenous catecholamines (Bankwala *et al.* 1994) can trigger a cascade of adaptive mechanisms in the myocardium. Suppression of NE-PC-induced antiarrhythmic protection by inactivation of Gi proteins further supports the role of G-protein-mediated

signal transduction in postreceptor mechanisms of cardioprotection, in addition to their role in the infarct size-limiting and antiarrhythmic effects of classical IPC (Thornton *et al.* 1993, Piacentini *et al.* 1993). Furthermore, it has been demonstrated that stimulation of α1-receptors by catecholamines preconditions the rat and rabbit heart against contractile dysfunction and myocardial infarction, and that this protection is associated with activation of PKC (Banerjee *et al.* 1993, Tsuchida *et al.* 1994, Mitchell *et al.* 1995). This is in accord with our previous study which demonstrated that the administration of norepinephrine in rats resulted in an immediate subcellular relocalization of PKC to the membrane fraction lasting for up to 4 hours indicating its activation (Wilson *et al.* 1996).

Electrophysiological mechanisms underlying the antiarrhythmic effects might involve alterations in the outward potassium currents. Activation of K(ATP) channels is considered to be one of the mechanisms of cardioprotection in general (Noma, 1983), including protection against arrhythmias related to triggered activity due to enhanced Ca^{2+} influx (Spinelli *et al.* 1991, Tan *et al.* 1993). In our study, the role of sarcolemmal K(ATP) channels has not been confirmed unequivocally, since their blockade with glibenclamide did not modify protective effect of IPC on the incidence and severity of ischemic arrhythmias. A moderate antiarrhythmic effect of glibenclamide on the incidence of VF in the protocol of test ischemia (Fig. 4) can be related to antiarrhythmic properties of the drug and prolongation of action potential duration. Thus, we cannot exclude that suppression of re-entry arrhythmias by glibenclamide could contribute, in a setting of IPC, to the maintenance of the antiarrhythmic potential of preconditioning. In addition, failure of glibenclamide to block the IPC-induced protection can at least be partially explained by its ability to potentiate the release of norepinephrine from sympathetic nerve endings (Oe *et al.* 1999). This might further facilitate the cardioprotective effect of IPC.

On the other hand, recent studies have demonstrated that cardioprotection occurs independently of the shortening of action potential duration, which is the main target of sarcolemmal K(ATP) blockers (Yao and Gross 1994, Hamada *et al.* 1998). Furthermore, we have previously shown that the protection against contractile dysfunction by preconditioning in guinea pig papillary muscle could be abolished by 5-hydroxydecanoate (a more selective inhibitor for mitochondrial K(ATP) channels) that also blocked the infarct size-limiting effect of IPC in both rats and rabbits, without affecting ischemia-induced shortening of action potential duration in rabbits (Ravingerova *et al.* 1998, Munch-Ellingsen *et al.* 2000). In addition, in the study of antiarrhythmic protection induced by chronic hypoxia in rats (Asemu *et al.* 1999), the involvement of mitochondrial K(ATP) channels has also been suggested. It was recently proposed that the mitochondrial K(ATP) channel is 2000-fold more sensitive than the sarcolemmal one to K(ATP) opener diazoxide, which has been shown to mimick the IPC protection, and that it is the most likely end-effector involved with IPC (Garlid *et al.* 1997, Liu *et al.* 1998). The participation of K(ATP) channels in signal transduction mechanisms is supported by the findings that the activation of PKC appears to phosphorylate the sarcolemmal K(ATP) channel (Hu *et al.* 1996). Furthermore, there is some evidence that PKC also facilitates the opening of mitochondrial K(ATP) channels (Sato *et al.* 1998). However, the consequences of their activation and their role in the mechanisms of cardioprotection require further exploration.

In conclusion, we can suggest on the basis of the results of the present study, as well as on our previous observations, that antiarrhythmic protection in the rat heart may be induced by short-term stimulation of α1-adrenergic receptors, either by endogenously released or exogenously applied catecholamines. The protective mechanisms might involve a G-protein-mediated pathway coupled with the activation of PKC, and surface K(ATP) channels do not appear to play a role in cardioprotection in this experimental model.

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