Effects of *Bordetella pertussis* toxin pretreatment on the antiarrhythmic action of ischaemic preconditioning in anaesthetized rats

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1 *Bordetella pertussis* toxin, which catalyses the ADP-ribosylation of certain guanine nucleotide binding proteins (G proteins), thus functionally uncoupling them from associated receptors, was examined to determine whether it modified the antiarrhythmic effect of ischaemic preconditioning in anaesthetized rats.

2 *Pertussis* toxin (25 µg kg⁻¹, i.p., 48 h prior to heart isolation) attenuated the negative chronotropic effect of acetylcholine (ACh) in rat isolated Langendorff perfused hearts. ACh (10 µM) reduced heart rate by 4% in hearts taken from *pertussis* toxin-treated animals, compared to a reduction of 57% in hearts taken from animals treated only with vehicle.

3 In anaesthetized rats, ischaemic preconditioning (a single 3 min occlusion of the left main coronary artery followed by 10 min reperfusion) had a pronounced antiarrhythmic effect during a subsequent 30 min period of regional myocardial ischaemia. Compared to hearts receiving only a 30 min period of left coronary occlusion, there was a reduced mortality (67% and 0% for control and preconditioned groups, respectively; $P < 0.01$) and decreased incidences of ventricular tachycardia (VT) and ventricular fibrillation (VF). Pretreatment with *pertussis* toxin (25 µg kg⁻¹, i.p., 48 h previously) did not modify the arrhythmias associated with a 30 min period of regional myocardial ischaemia, neither did it modify the reduction in mortality (from 56% to 0%; $P < 0.05$) associated with preconditioning. Furthermore, the decrease in total ventricular premature beat count induced by preconditioning seen in controls (from 427 ± 130 to 95 ± 45) was also seen in *pertussis* toxin-treated rats (from 252 ± 190 to 57 ± 25).

4 These results suggest that receptor coupling to *pertussis* toxin-sensitive G proteins is not necessary for the antiarrhythmic effect of ischaemic preconditioning in this model.

Keywords: Preconditioning; antiarrhythmic; *pertussis* toxin-sensitive G proteins; myocardial ischaemia; coronary artery occlusion; rat isolated hearts

Introduction

Ischaemic preconditioning is a phenomenon whereby a short period of myocardial ischaemia and reperfusion renders the heart more tolerant to a subsequent more prolonged ischaemic episode (Murry *et al.*, 1986). This cardioprotection is characterized by a reduction in infarct size (Murry *et al.*, 1986), an improvement in post-ischaemic function (Asimakis *et al.*, 1992) and attenuation of arrhythmias associated with ischaemia and reperfusion (Vegh *et al.*, 1992). The mechanisms underlying preconditioning have yet to be fully elucidated. In most species studied, it is thought that some of the cardioprotective effects of preconditioning are mediated by adenosine receptors (Liu *et al.*, 1991; Schwarz *et al.*, 1992; Auchampach & Gross, 1993), possibly of the A₁ subtype. In rats this does not appear to be the case since the improvement in post-ischaemic functional recovery (Asimakis *et al.*, 1993; Cave *et al.*, 1993) and the infarct size limiting (Liu & Downey, 1992) and antiarrhythmic (Piacentini *et al.*, 1992) effects associated with preconditioning occur independently of adenosine receptor stimulation. It remains possible that in the rat heart the initial hormonal response differs from that seen in other species but thereafter employs a similar underlying signal transduction system. In this regard, in both rat (Speechly-Dick *et al.*, 1994) and rabbit (Ytrehus *et al.*, 1994) models, induction of cytosolic protein kinase C to its activated membrane state has been shown to occur as a consequence of the preconditioning stimulus.

It has been demonstrated that the infarct size-limiting effect of preconditioning in rabbits is partly dependent

(Thornton *et al.*, 1993b) on *pertussis* toxin-sensitive guanine nucleotide binding proteins (G proteins). However, conflicting results surround the involvement of such G proteins in rats. We have found previously that the application of a single preconditioning cycle exerted an antiarrhythmic effect which was *pertussis* toxin-sensitive in crystalloid perfused rat isolated hearts (Piacentini *et al.*, 1993). However, the antiarrhythmic and infarct size-limiting effects of multiple preconditioning cycles in blood perfused rat hearts (Lawson *et al.*, 1993) and anaesthetized rats (Liu & Downey, 1993), respectively, appear to be *pertussis* toxin-insensitive. These opposing results may be a consequence of the different experimental models and preconditioning protocols employed and of the end-points measured. Thus, since the main differences in the models used to date are the presence of blood and the application of differing numbers of preconditioning cycles, the aim of this study was to examine whether the antiarrhythmic effect of a single preconditioning cycle, which is *pertussis* toxin-sensitive in an *in vitro* model, is also *pertussis* toxin-sensitive in an *in vivo* anaesthetized rat model.

Methods

Animal preparation

Male Sprague-Dawley rats (240–340 g) were anaesthetized with sodium pentobarbitone (60 mg kg⁻¹, i.p.) and maintained under anaesthesia with sodium pentobarbitone (3 mg, i.v.) as required. Following anaesthesia, the right femoral vein and artery were cannulated (with heparinized catheters) for the administration of additional anaesthetic and for the

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measurement of arterial blood pressure, respectively. A pre-calibrated steel thermistor probe was inserted into the rectum to measure core temperature which was maintained at 37–38°C with the aid of a heating lamp.

To allow for artificial respiration, a tracheostomy was performed. The rats were then prepared for coronary artery ligation by the method described by Clark *et al.* (1980). The chest was opened using a left thoracotomy performed between the fourth and fifth ribs approximately 3 mm from the sternum. Artificial respiration with room air was immediately initiated with a respirator (C.F. Palmer, U.K., 48 strokes min^{-1} , 1.5 ml 100 g^{-1} animal weight). The two ribs above the thoracotomy were sectioned and the pericardium incised to allow access to the heart. The heart was then exteriorised by application of gentle pressure to either side of the ribcage and a 6/0 silk suture on a curved taper needle (Mersilk, Ethicon) was placed under the left main coronary artery immediately below the tip of the left atrial appendage. The heart was then replaced with the thoracic cavity and the animal was allowed to stabilize for 15 min before onset of experimental protocols. Any animal in which this procedure caused persistent arrhythmias (i.e. more than a few premature ventricular ectopic beats after manipulation of the heart) or a sustained fall in mean arterial blood pressure below 70 mmHg was excluded from the study. Although direct measurement of area at risk, the size of which may contribute to arrhythmia severity, was not made in these studies, we know from our previous studies that ligation of the coronary artery at this site results in approximately 40–45% of the free left ventricular wall being ischaemic (Garjani *et al.*, unpublished). When animals were to be subjected to the preconditioning protocol, the ends of the suture were threaded through a length of polyethylene tubing during the stabilization period. This then formed a snare which, when pressed against the heart, occluded the coronary vessels rendering the associated area ischaemic. Reperfusion could then be allowed by loosening this snare. When ischaemia without reperfusion was performed, the ligature was tied with a single knot. Successful ligation was assumed for each individual animal by the presence of regional cyanosis, a slight drop in arterial blood pressure and characteristic electrocardiographic changes, notably an increase in the height of the R-wave.

Systemic arterial blood pressure was monitored from the catheter inserted into the right femoral artery, connected to a Spectramed P23KL transducer. The electrocardiogram (ECG) was recorded from standard limb leads placed under the skin. Heart rate was calculated from the ECG over a 10 s period and expressed as beats min^{-1} . Both the ECG and mean arterial blood pressure were recorded continuously with a Gould Easygraf Recorder, model TA240.

Isolated hearts

Rats were anaesthetized as outlined above, heparinized with 100 units of heparin sulphate (i.p.) and the hearts were rapidly removed and transferred to a Langendorff apparatus where retrograde perfusion at a constant flow (10 ml min^{-1}) was started. The perfusate was a modified Krebs-Henseleit solution containing (in mmol l^{-1}): NaCl 118, KCl 3.2, CaCl_2 2.52, MgSO_4 1.66, NaHCO_3 26.88, KH_2PO_4 1.18, glucose 5.55, and sodium pyruvate 2.0, adjusted to pH 7.4, aerated with 95% O_2 /5% CO_2 , filtered and maintained at 37°C. Fine platinum subdermal electrodes (Grass type E2) were placed on the right atrium and the apex of the left ventricle allowing an epicardial electrocardiogram to be recorded, using the metal aortic cannula as a reference electrode. The ECGs were recorded continuously on a Grass model 79 recorder. Heart rate (beats min^{-1}) was calculated from the ECG trace over a 10 s period. Hearts were allowed to stabilize for 30 min before experimental protocols began.

Experimental protocols

Toxin from *Bordetella pertussis* (Sigma) was diluted with normal saline to give a final concentration of $10 \mu\text{g ml}^{-1}$. Rats received either *pertussis* toxin ($25 \mu\text{g kg}^{-1}$, i.p.) or an equivalent volume of the vehicle (a saline solution containing 10% glycerol and 10 mM sodium phosphate). Experimental protocols, both *in vivo* and *in vitro*, were initiated 48 h after the *pertussis* toxin or vehicle pretreatment.

Following the stabilization period, anaesthetized rats were randomised to receive one of two protocols outlined in Figure 1. The preconditioning cycle consisted of a 3 min occlusion of the left main coronary artery and 10 min reperfusion which was then followed by a 30 min period of coronary artery occlusion (Vegh *et al.*, 1992). Rats subjected to this preconditioning regimen (vehicle $n=9$; *pertussis* toxin $n=9$) were compared to rats receiving a single 30 min period of ischaemia (vehicle $n=9$; *pertussis* toxin $n=9$). The myocardium was not reperfused after the 30 min period of ischaemia.

Isolated hearts (vehicle $n=5$; *pertussis* toxin $n=4$) were allowed to stabilize for 30 min and then each heart was given two 5 min infusions of acetylcholine (1 and $10 \mu\text{M}$) each separated by 30 min drug-free perfusion.

Arrhythmia analysis

Ventricular arrhythmias were analysed during periods of ischaemia and reperfusion by (i) counting the total number of ventricular premature beats (VPBs), which included single ventricular premature beats, couplets and triplets (termed salvos), and ventricular tachycardia (defined as four or more consecutive premature beats), in accordance with the Lambeth Conventions (Walker *et al.*, 1988), and (ii) determining the incidences of ventricular tachycardia (VT), ventricular fibrillation (VF) and mortality.

Statistical analysis

Except for the incidences of VT and VF, all results are expressed as means with the standard error of the means (s.e.mean). To compare the number of VPBs the Kruskal-Wallis non-parametric test was used, followed, when appropriate, by Dunn's multiple comparison procedure with an experimentwise error rate of 0.05 (Hollander & Wolfe, 1973). For comparisons of incidences of events, the Fisher Irwin

Figure 1 Forty eight hours after receiving either *pertussis* toxin ($25 \mu\text{g kg}^{-1}$) or its vehicle, rats were anaesthetized and randomised to one of two protocols. All rats were subjected to a 30 min occlusion of the left coronary artery. Rats in the preconditioned group were first subjected to a preconditioning cycle of 3 min ischaemia and 10 min reperfusion. Non-preconditioned rats were allowed a further 13 min period of stabilization before being subjected to the 30 min occlusion period. For each of the four groups, $n=9$.

(Chi² with Yates' correction) test was used. For normally distributed data, i.e. mean arterial blood pressure and heart rate, comparisons between groups were made by ANOVA followed by Tukeys *post hoc* test. Direct comparisons between two groups, when necessary, were made with Student's *t* test (unpaired, two tailed). For comparisons at different time points within a single group, a repeated measure ANOVA was carried out, followed if $P < 0.05$, by Dunnett's *post hoc* test. When necessary, Student's *t* test (paired, two tailed) was used for comparisons at two different time points, within a single group. The differences in the duration of ventricular tachycardia between groups was analyzed by the Mann Whitney U-test.

Results

Haemodynamic effects of pertussis toxin pretreatment in anaesthetized rats before and after coronary artery ligation

Pretreatment with *pertussis* toxin resulted in a reduced mean arterial blood pressure with the result that six rats were excluded from the study with a mean arterial pressure of less than 70 mmHg after the stabilization period. However, these rats were replaced immediately to ensure that *n* numbers were equal in each group. The mean arterial pressure of the *pertussis* toxin-treated rats was approximately 10 mmHg lower than in vehicle-treated rats (81 ± 4 and 92 ± 6 mmHg for *pertussis* toxin and vehicle treated rats, respectively). As a consequence of the preconditioning occlusion, pressure initially decreased in both vehicle and *pertussis* toxin treated groups. After 10 min reperfusion, whilst pressure recovered to pre-occlusion levels (92 ± 7 mmHg) in the vehicle-treated rats, recovery was not complete in the *pertussis* toxin-treated rats (77 ± 5 mmHg). Similarly, blood pressure dropped sharply upon onset of the sustained ischaemic episode in all four treatment groups, recovering gradually over the sustained occlusion period (Table 1).

Baseline heart rates were similar for rats pretreated with vehicle and *pertussis* toxin and remained stable throughout the preconditioning occlusion and reperfusion period in both treatment groups. During the sustained 30 min ischaemic period there was a slight increase (not significant) in the heart rates measured in vehicle-treated rats, whether preconditioned or not, which was not apparent in rats which had been pretreated with *pertussis* toxin (Table 1).

Arrhythmias following coronary artery occlusion in control and preconditioned anaesthetized rats

A 30 min occlusion of the left coronary artery in anaesthetized rats not previously subjected to the preconditioning protocol resulted in the development of severe arrhythmias with the majority of animals succumbing to terminal VF (Figure 2). The distribution of ventricular premature beats (VPBs) was similar in both vehicle- and *pertussis* toxin-treated rats, although the latter displayed greater ectopic activity (total VPB count 2022 ± 797 compared to 730 ± 671 in vehicle treated controls; $P > 0.2$). This apparent difference is due to small *n* numbers of rats surviving the 30 min occlusion ($n = 3$ and 4 for vehicle and *pertussis*-treated, respectively) and *pertussis*-treated rats surviving for a longer time and experiencing longer bursts of VT (43 ± 37 and 134 ± 61 s for 9 out of 9 vehicle and 4 out of 9 *pertussis*-treated rats respectively).

Although VPBs occurring as a consequence of the preconditioning occlusion were few (8 ± 2 for both vehicle- and *pertussis* toxin-treated groups), there was a significant increase in the total VPB count in the intervening reperfusion

Figure 2 The incidences of Ventricular tachycardia (VT) and ventricular fibrillation (VF) occurring in anaesthetized rats during a 30 min occlusion of the left coronary artery. Values obtained from rats subjected only to a 30 min occlusion (open and solid columns for vehicle- and *pertussis*-toxin treated rats, respectively) are compared to the incidences in rats first subjected to the preconditioning cycle (cross-hatched and stippled columns for vehicle- and *pertussis* toxin-treated, respectively). The preconditioning cycle consisted of a 3 min occlusion and 10 min reperfusion. For each of the four groups, $n = 9$. ** $P < 0.05$ compared to same treatment control.

Table 1 Blood pressure and heart rate in anaesthetized rats, pretreated with either *pertussis* toxin or its vehicle, during a 30 min occlusion of the left coronary artery

Group	n	Pre-occlusion	Occlusion time course (min)				
			1	3	10	20	30
MABP (mmHg)							
Vehicle-treated							
Control	9	90 ± 7	67 ± 7	76 ± 7	85 ± 16 (4)	87 ± 11 (3)	95 ± 8 (3)
Preconditioned	9	92 ± 7	81 ± 5	91 ± 7	93 ± 8	91 ± 7	93 ± 8
<i>pertussis</i> toxin-treated							
Control	9	82 ± 5	72 ± 4	76 ± 5	89 ± 6 (7)	78 ± 7 (4)	81 ± 11 (4)
Preconditioned	9	77 ± 6	74 ± 5	77 ± 7	76 ± 10	76 ± 6	75 ± 5
Heart rate (beats min⁻¹)							
Vehicle-treated							
Control	9	419 ± 16	426 ± 11	433 ± 11	456 ± 29 (4)	452 ± 30 (3)	453 ± 26 (3)
Preconditioned	9	419 ± 19	427 ± 18	433 ± 20	435 ± 21	433 ± 21	427 ± 21
<i>pertussis</i> toxin							
Control	9	392 ± 16	388 ± 16	405 ± 18	402 ± 33 (7)	385 ± 25 (4)	371 ± 28 (4)
Preconditioned	9	420 ± 16	425 ± 16	422 ± 17	421 ± 13	419 ± 17	418 ± 11

n numbers decreased with time as a consequence of animal death. Thus *n* represents the initial number of hearts with the reduced values shown in parentheses where appropriate. MABP = mean arterial blood pressure.

Figure 3 Distribution of arrhythmias over a 30 min period of coronary artery occlusion in anaesthetized rats without (upper panel) and with (lower panel) preconditioning induced by a 3 min occlusion and 10 min reperfusion, before onset of the sustained occlusion. Rats were pretreated with either vehicle (left-hand open columns) or *pertussis* toxin (right-hand solid columns). Each column represents the mean $\pm$ s.e. mean number of ventricular premature beats (VPBs) occurring over each min of the sustained occlusion. All groups had an initial $n = 9$; reductions in the n number as a consequence of terminal VF are shown when they occur.

Table 2 Heart rate responses before and 5 min after the administration of acetylcholine (ACh) to isolated hearts taken from rats pretreated with either *pertussis* toxin ($25 \mu\text{g kg}^{-1}$, i.p.) or its vehicle (10% glycerol in saline)

Pretreatment	n	Heart rate (beats min^{-1})		Pre-drug	ACh (10 μM) 5 min
		Pre-drug	ACh (1 μM) 5 min		
Vehicle	5	367 ± 14	313 ± 48	362 ± 6	$154 \pm 20^{***}$
<i>Pertussis</i> toxin	4	337 ± 23	364 ± 50	332 ± 32	318 ± 26

*** $P < 0.001$ compared to pre-drug value (paired t test).

period in *pertussis* toxin-treated rats compared to vehicle-treated rats (90 ± 48 compared with 2 ± 1 ; $P < 0.05$). Irrespective of whether rats were pretreated with *pertussis* toxin or vehicle, preconditioning the myocardium resulted in attenuation in the number of VPBs and the incidence of VT and VF during a subsequent 30 min ischaemic episode (Figures 2 and 3). All preconditioned rats (i.e. both vehicle- and *pertussis* toxin-treated) survived the ischaemic episode, compared with 67% and 56% mortality in vehicle- and *pertussis* toxin-treated non-preconditioned rats respectively ($P < 0.05$ for both) and the total VPB count during occlusion was low (98 ± 45 and 57 ± 25 VPB for vehicle- and *pertussis* toxin-treated preconditioned animals respectively).

Responses to acetylcholine infusions in isolated hearts removed from rats given *pertussis* toxin

In hearts taken from rats pretreated with vehicle there was a marked negative chronotropic response to ACh infusions (Table 2). As a consequence of a 5 min infusion of ACh

(10 μM) there was a significant reduction in heart rate. In contrast, even a high concentration of ACh failed to elicit a reduction in heart rate when hearts were taken from rats pretreated with *pertussis* toxin.

Discussion

Although the cardioprotective effect of preconditioning in rats does not appear to depend upon stimulation of adenosine receptors (Piacentini *et al.*, 1992; Liu & Downey, 1992), it does share some characteristics of preconditioning with other species. For example, in rabbit (Thornton *et al* 1993a; Toombs *et al.*, 1993), dog (Kitakaze *et al.*, 1992; Vegh *et al.*, 1994) and rat (Banerjee *et al.*, 1993; Ravingerova *et al.*, 1994) models, stimulation of α_1 -adrenoceptors prior to sustained ischaemia can mimic the cardioprotective effect of preconditioning. A role for the endogenous release of noradrenaline as a mechanism of preconditioning has thus been proposed in dogs (Kitakaze *et al.*, 1992) and rats

(Banerjee *et al.*, 1993) although the evidence from rabbit studies is conflicting (Toombs *et al.*, 1993; Thornton *et al.*, 1993a). α_1 -Adrenoceptors can be coupled to *pertussis* toxin-sensitive G-proteins in neonatal rat cardiac myocytes (Barratt *et al.*, 1993) while the A_1 adenosine and M_2 muscarinic receptors are also coupled to some of their effectors by *pertussis* toxin-sensitive G-proteins (Birnbaumer, 1990). Stimulation of muscarinic receptors can mimic preconditioning in rabbits (Thornton *et al.*, 1993b) and dogs (Yao & Gross, 1993) and treatment with *pertussis* toxin reduces the infarct size limiting effect in rabbits (Thornton *et al.*, 1993b). Thus, in light of these similarities, we speculated that a G-protein signal transducer system, rather than the receptor type which triggers it, may be focal to the preconditioning phenomenon.

We have found previously, in a rat isolated heart model, that the antiarrhythmic effect of preconditioning was attenuated when hearts were taken from rats pretreated with *pertussis* toxin (Piacentini *et al.*, 1993). However, studies examining the infarct size limiting (Liu & Downey, 1993) and antiarrhythmic (Lawson *et al.*, 1993) effects of preconditioning in anaesthetized rats and blood-perfused rat hearts, respectively, showed that the cardioprotective effects of preconditioning were still apparent after *pertussis* toxin treatment. Two experimental differences could explain this anomaly. Firstly the presence of blood containing a blood-borne, *pertussis* toxin-insensitive, substance might contribute to this cardioprotective effect. This possibility is not excluded by the results of Lawson *et al.* (1993) since in their model rat isolated hearts were perfused with blood from donor rats not treated with *pertussis* toxin. Second, the application of different numbers of preconditioning cycles may influence the eventual outcome. In our previous study we examined the effects of a single preconditioning cycle whereas both Lui & Downey (1993) and Lawson *et al.* (1993) employed three preconditioning cycles. The number of cycles employed is of importance since, although the antiarrhythmic effect is seen after one preconditioning cycle *in vivo* (present study; Vegh *et al.*, 1992), increasing the number of preconditioning cycles appears to increase the protection against ischaemic arrhythmias *in vitro* (Lawson & Hearse, 1993). Furthermore, while a reduction in infarct size is seen in anaesthetized rats after either three (Liu & Downey, 1992; Li *et al.*, 1992) or one (Yellon *et al.*, 1992) preconditioning occlusion, a single cycle gives the maximal improvement in post-ischaemic function (Asikamis *et al.*, 1992). It could be speculated that more than one mechanism of protection determining the degree and type of protection is important in this species. We hypothesized that the *pertussis* toxin-sensitive cardioprotective effect, as a consequence of a single preconditioning cycle, was overridden by a second *pertussis* toxin-insensitive mechanism when multiple preconditioning cycles were applied. In order to minimize experimental differences further we repeated our previous *in vitro* study using an *in vivo* model to investigate this antiarrhythmic effect in the presence of blood. However, the results of this study indicate that application of a single preconditioning cycle was not *pertussis* toxin-sensitive in anaesthetized rats.

Since an identical preconditioning protocol to that used in our *in vitro* study was employed it must be assumed that either the presence of a *pertussis* toxin-insensitive component(s) of blood plays a role in this model, or that intact autonomic nervous control actively contributes to the antiarrhythmic effect. Although it seems unlikely that blood-borne substances play a role in the infarct size limiting effect of

preconditioning in rabbits (Liu *et al.*, 1992), the possibility has not been fully explored in rat models. With regard to the latter possibility, autonomic reflexes are better preserved during ischaemia after preconditioning (Miyazaki & Zipes, 1989).

Differences in the effects of *pertussis* toxin pretreatment between the *in vitro* and *in vivo* models or preconditioning may also lie in dissimilarities in the mechanisms underlying the antiarrhythmic effect of preconditioning in each model as might the application of different exclusion criteria. In the previous *in vitro* study (Piacentini *et al.*, 1993) *pertussis* toxin had an antiarrhythmic effect; *in vivo*, if anything, *pertussis* toxin enhanced arrhythmogenesis. It cannot be excluded that a low VPB count, *in vitro*, masked the antiarrhythmic effect of preconditioning. Furthermore, in the present *in vivo* study, 25% of *pertussis* toxin-treated rats were excluded with a low arterial blood pressure. Since blood pressure before heart isolation was not measured, the same exclusion criteria could not be applied to hearts in the *in vitro* study. The low pressure was probably a consequence of a combination of *pertussis* toxin effects, both related and unrelated to its ability to ADP-ribosylate certain G_s subunits (Nogimori *et al.*, 1984). It is unclear whether the rats with a low blood pressure which had to be excluded were more successfully ADP-ribosylated and, although unlikely, it cannot be excluded that the hearts of the rats included in the study were not sufficiently modified by *pertussis* toxin pretreatment. Unfortunately, since the role of G-protein activation in preconditioning was the process under study, we were unable to determine the extent of inhibition of ACh-induced bradycardia by pretreatment with *pertussis* toxin in our *in vivo* rats, since this would have confounded the subsequent arrhythmia analysis. However, in our earlier study we found that this concentration of *pertussis* toxin consistently attenuated the negative chronotropic effect of acetylcholine (Piacentini *et al.*, 1993). We have demonstrated this once again in this present study by determining the effects of pretreatment, with the same batch of *pertussis* toxin given to the *in vivo* rats, on the bradycardic response to ACh in hearts isolated from a separate group of rats. This indicates that in this series of experiments *pertussis* toxin-sensitive proteins, at least in atrial tissue, were no longer able to couple with associated receptors. We have assumed that the *pertussis* toxin-sensitive G proteins in the ventricular tissue are similarly uncoupled; indeed, successful ADP-ribosylation of G proteins in rat ventricular tissue can be demonstrated after pretreatment with a similar concentration of *pertussis* toxin (Anand-Srivastava, 1989).

The results of this study do not exclude a role for G_i/G_o proteins in the antiarrhythmic effect of preconditioning, since phosphorylation at a non-hormonal site, as a consequence of protein kinase C activation, can modify the activity of these proteins (Pyne *et al.*, 1989). Furthermore, the functional modification of G_i which occurs as a consequence of ischaemia in canine ventricular tissue (Niroomand *et al.*, 1992) is not apparent after preconditioning (Weiss *et al.*, 1993).

In conclusion, we have found that the antiarrhythmic effect of preconditioning in anaesthetized rats is still apparent after treatment with *pertussis* toxin. This indicates that in this model functional coupling of receptors to *pertussis* toxin-sensitive G proteins is not necessary for cardioprotection.

L.P. was supported by a scholarship from the British Heart Foundation.

References

- ANAND-SRIVASTAVA, M.B. (1989). Angiotensin II receptors negatively coupled to adenylate cyclase in rat myocardial sarcolemma. *Biochem. Pharmacol.*, **38**, 489–496.
- ASIMAKIS, G.A., INNERS-MCBRIDE, K. & CONTI, V.R. (1993). Attenuation of postischaemic dysfunction by ischaemic preconditioning is not mediated by adenosine in the isolated rat heart. *Cardiovasc. Res.*, **27**, 1522–1530.
- ASIMAKIS, G.K., INNERS-MCBRIDE, K., MEDELLIN, G. & CONTI, V.R. (1992). Ischemic preconditioning attenuates acidosis and postischemic dysfunction in isolated rat hearts. *Am. J. Physiol.*, **263**, H887–H894.
- AUCHAMPACH, J.A. & GROSS, G.J. (1993). Adenosine A_1 receptors, K_{ATP} channels, and ischemic preconditioning. *Am. J. Physiol.*, **264**, H1327–H1336.

- BANERJEE, A., LOCKEWINTER, C., ROGERS, K.B., BREW, E.C., CAIRNS, C.B., BENSARD, D.D. & HARKEN, A.H. (1993). Preconditioning against myocardial dysfunction after ischemia and reperfusion by an α_1 -adrenergic mechanism. *Circ. Res.*, **73**, 656–670.
- BARRATT, S., HONBO, N. & KARLINER, J.S. (1993). Alpha $_1$ -adrenoceptor-mediated inhibition of cellular cAMP accumulation in neonatal rat ventricular myocytes. *Naunyn. Schmied. Arch. Pharmacol.*, **347**, 384–393.
- BIRNBAUMER, L. (1990). G proteins in signal transduction. *Annu. Rev. Pharmacol. Toxicol.*, **30**, 675–705.
- CAVE, A.C., COLLIS, C.S., DOWNEY, J.M. & HEARSE, D.J. (1993). Improved functional recovery by ischaemic preconditioning is not mediated by adenosine in the globally ischaemic isolated rat heart. *Cardiovasc. Res.*, **27**, 663–668.
- CLARK, C., FOREMAN, M.I., KANE, K.A., McDONALD, F.M. & PARRATT, J.R. (1980). Coronary artery ligation in anaesthetized rats as a method for the production of experimental dysrhythmias and for the determination of infarct size. *J. Pharmacol. Methods*, **3**, 357–368.
- HOLLANDER, M. & WOLFE, D.A. (1973). *Non Parametric Statistical Methods*. New York: John Wiley & Sons Inc..
- KITAKAZE, M., TAKASHIMA, S., MORIOKA, T., SATO, H. & MORI, H. (1992). α_1 -adrenoceptor activation limits infarct size by augmenting adenosine production and 5' nucleotidase activity in ischemic preconditioning. *Circulation*, **86** (suppl I), I-213.
- LAWSON, C.S., COLTART, D.J. & HEARSE, D.J. (1993). The antiarrhythmic action of ischaemic preconditioning in rat hearts does not involve functional Gi proteins. *Cardiovasc. Res.*, **27**, 681–687.
- LAWSON, C.S. & HEARSE, D.J. (1993). The antiarrhythmic effect of ischaemic preconditioning: true protection or temporal effect? *J. Mol. Cell Cardiol.*, **25** (suppl. I), S.33.
- LI, Y., WHITTAKER, P. & KLONER, R.A. (1992). The transient nature of the effect of ischaemic preconditioning on myocardial infarct size and ventricular arrhythmia. *Am. Heart J.*, **123**, 346–352.
- LIU, Y. & DOWNEY, J.M. (1992). Ischemic preconditioning protects against infarction in rat heart. *Am. J. Physiol.*, **263**, H1107–H1112.
- LIU, Y. & DOWNEY, J.M. (1993). Preconditioning against infarction in the rat heart does not involve a *pertussis* toxin sensitive G protein. *Cardiovasc. Res.*, **27**, 608–611.
- LIU, G.S., STANLEY, A.W.H. & DOWNEY, J.M. (1992). Ischaemic preconditioning is not dependent on neutrophils or glycolytic substrate at reperfusion in rabbit heart. *Cardiovasc. Res.*, **26**, 1195–1198.
- LIU, G.S., THORNTON, J., VAN WINKLE, D.M., STANLEY, A.W.H., OLSSON, R.A. & DOWNEY, J.M. (1991). Protection against infarction afforded by preconditioning is mediated by A $_1$ adenosine receptors in rabbit heart. *Circulation*, **84**, 350–356.
- MIYAZAKI, T. & ZIPES, D.P. (1989). Protection against autonomic denervation following acute myocardial infarction by preconditioning ischaemia. *Circ. Res.*, **64**, 437–448.
- MURRY, C.E., JENNINGS, R.B. & REIMER, K.A. (1986). Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation*, **74**, 1124–1136.
- NIROOMAND, F., BANGERT, M., BEYER, T. & RAUCH, B. (1992). Reduced adenylyl cyclase inhibition by carbachol and GTP during acute myocardial ischaemia. *J. Mol. Cell Cardiol.*, **24**, 471–475.
- NOGIMORI, K., TAMURA, M., YAJIMA, M., ITO, K., NAKAMURA, T., KAJIKAWA, N., MARUYAMA, Y. & UI, M. (1984). Dual mechanisms involved in development of diverse biological activities of islet-activating protein, *pertussis* toxin, as revealed by chemical modification of lysine residues in the toxin molecule. *Biochim. Biophys. Acta.*, **801**, 232–243.
- PIACENTINI, L., WAINWRIGHT, C.L. & PARRATT, J.R. (1992). The antiarrhythmic effect of preconditioning, in rat isolated hearts, does not involve A $_1$ adenosine receptors. *Br. J. Pharmacol.*, **107**, 137P.
- PIACENTINI, L., WAINWRIGHT, C.L. & PARRATT, J.R. (1993). The antiarrhythmic effect of ischaemic preconditioning in isolated rat heart involves a *pertussis* toxin sensitive mechanism. *Cardiovasc. Res.*, **27**, 674–680.
- PYNE, N.J., MURPHY, G.J., MILLIGAN, G. & HOUSLAY, M.D. (1989). Treatment of intact hepatocytes with either the phorbol ester TPA or glucagon elicits the phosphorylation and functional inactivation of the inhibitory guanine nucleotide regulatory protein G $_i$. *FEBS Lett.*, **243**, 77–82.
- RAVINGEROVA, T., PYNE, N.J. & PARRATT, J.R. (1994). Adrenergic stimulation protects rat hearts against severe arrhythmias: relevance to ischaemic preconditioning. *J. Mol. Cell Cardiol.*, **26**, CXII, Abstract 467.
- SCHWARZ, E.R., MOHRI, M., SACK, S., ARRAS, M. & SCHAPER, W. (1992). Preconditioning by ischemia lasts for only 30 min and is inhibited by adenosine antagonists. *J. Mol. Cell Cardiol.*, **24** (suppl. I), S.93.
- SPEECHLY-DICK, M.E., BAXTER, G.F. & YELLON, D.M. (1994). Ischaemic preconditioning protects hypertrophied myocardium. *Cardiovasc. Res.*, **28**, 1025–1029.
- THORNTON, J.D., DALY, J.F., COHEN, M.V., YANG, X.M. & DOWNEY, J.M. (1993a). Catecholamines can induce adenosine receptor-mediated protection of the myocardium but do not participate in ischemic preconditioning in the rabbit. *Circ. Res.*, **73**, 649–655.
- THORNTON, J., LIU, G.S. & DOWNEY, J.M. (1993b). Pretreatment with *pertussis* toxin blocks the protective effects of preconditioning: evidence for a G protein mechanism. *J. Mol. Cell Cardiol.*, **25**, 311–320.
- TOOMBS, C.F., WILTSE, A.L. & SHEBUSKI, R.J. (1993). Ischemic preconditioning fails to limit infarct size in reserpinized rabbit myocardium. *Circulation*, **88**, 2351–2358.
- VEGH, A., KOMORI, S., SZEKERES, L. & PARRATT, J.R. (1992). Antiarrhythmic effects of preconditioning in anaesthetized dogs and rats. *Cardiovasc. Res.*, **26**, 487–495.
- VEGH, A., PAPP, G.Y. & PARRATT, J.R. (1994). Intracoronary noradrenaline suppresses ischaemia-induced arrhythmias in anaesthetized dogs. *J. Mol. Cell Cardiol.*, **26**, CXII, Abstract 343.
- WALKER, M.J.A., CURTIS, M.J., HEARSE, D.J., CAMPBELL, R.W.F., JANSE, M.J., YELLON, D.M., COBBE, S.M., COKER, S.J., HARNESS, J.B., HARRON, D.W.G., HIGGINS, A., JULIAN, D.J., LAB, M.J., MANNING, A.S., NORTHOVER, B.J., PARRATT, J.R., RIEMERSMA, R.A., RIVA, E., RUSSELL, D.C., SHERIDAN, D.C., WINSLOW, E. & WOODWARD, B. (1988). The Lambeth Conventions: guidelines for the study of arrhythmias in ischaemia, infarction and reperfusion. *Cardiovasc. Res.*, **22**, 447–455.
- WEISS, A., ZEIFANG, F., RAUCH, B. & NIROOMAND, F. (1993). A functional modification of G $_i$ proteins as the underlying mechanism of ischaemic preconditioning. *Circulation*, **88**, I-631.
- YAO, Z. & GROSS, G.J. (1993). Role of nitric oxide, muscarinic receptors, and the ATP-sensitive K $^+$ channel in mediating the effects of acetylcholine to mimic preconditioning in dogs. *Circ. Res.*, **73**, 1193–1201.
- YELLON, D.M., ALKHULAIFI, A.M., BROWNE, E.E. & PUGSLEY, W.B. (1992). Ischaemic preconditioning limits infarct size in the rat heart. *Cardiovasc. Res.*, **25**, 983–987.
- YTREHUS, K., LIU, G.S. & DOWNEY, J.M. (1994). Preconditioning protects the ischaemic rabbit heart by protein kinase C activation. *Am. J. Physiol.*, **266**, H1145–H1152.

(Received August 17, 1994

Revised October 4, 1994

Accepted October 18, 1994)