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Short communication

Genistein, an inhibitor of tyrosine kinase, prevents the antiarrhythmic effects of preconditioning

Zahra Fatehi-Hassanabad, James R. Parratt *

Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW, UK

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Abstract

The possible involvement of tyrosine kinase in the signal transduction processes associated with the antiarrhythmic effects of ischaemic preconditioning was assessed by the administration, prior to the preconditioning stimulus, of the tyrosine kinase inhibitor genistein (5 mg/kg i.v.) to pentobarbitone anaesthetised male rats. The hearts were then removed, perfused by the Langendorff technique and preconditioned by a single brief (3 min) coronary artery occlusion prior to the 30 min test ischaemic insult. Preconditioning reduced ventricular arrhythmias during occlusion (e.g., premature ventricular beats from 333 ± 60 in controls to 52 ± 5 in preconditioned hearts; $P < 0.05$) but this protective effect was not observed in the hearts of rats given genistein (272 ± 37 premature beats). A similar abolition of the protective effects of preconditioning could be demonstrated in hearts perfused in vitro with genistein (100 μM). This suggests the involvement of tyrosine kinase activation in this powerful form of endogenous cardioprotection. © 1997 Elsevier Science B.V.

Keywords: Genistein; Arrhythmias, ventricular; Preconditioning; Tyrosine kinase

1. Introduction

Brief periods of myocardial ischaemia, induced by coronary artery occlusion, markedly reduce the severity of ventricular arrhythmias that occur during a subsequent more prolonged period of ischaemia both in vivo (Vegh et al., 1990, 1992 and recently reviewed by Vegh and Parratt, 1996) and in vitro (Lawson et al., 1993, Piacentini et al., 1993). The precise mechanisms remain unclear but seem to involve the release, by the preconditioning stimulus, of a variety of 'endogenous myocardial protective substances' such as adenosine, bradykinin and nitric oxide (Parratt, 1993) and the subsequent activation of signal transduction pathways which include, in some species at least, protein kinase C (recently reviewed by Cohen et al., 1996).

The administration of bacterial endotoxin, or a non-toxic derivative of the lipid A component of the endotoxin molecule, also markedly reduces arrhythmia severity (Wu et al., 1996; Vegh et al., 1996) and parallels have been drawn between the mechanisms of this form of protection and that induced by ischaemic preconditioning (Parratt and Szekeres, 1995). There is some evidence that activation of tyrosine kinase is involved in the cellular effects of endotoxin (Marczin et al., 1993; Paul et al., 1995) and some of these (e.g., nitric oxide synthase induction, Paul et al., 1995), are markedly reduced by the tyrosine kinase inhibitor genistein. In the present experiments we have examined whether the antiarrhythmic effects of ischaemic preconditioning are also modified by genistein. We have given genistein both in vivo and in vitro and determined how this influences the responses, in rat isolated perfused hearts, to a single preconditioning stimulus.

2. Materials and methods

Male Sprague Dawley rats (250–300 g) were anaesthetised with sodium pentobarbitone (60 mg/kg; i.p.) and given, by i.v. injection into the penile vein, either genistein (Sigma, 5 mg/kg) or vehicle (distilled water and DMSO in a final concentration 0.5% v/v; total volume 0.3 ml). Heparin (500 U) was then given, also i.v. A thoracotomy was performed and a suture loosely placed around the left coronary artery where it emerged from under the left atrium. The hearts were rapidly removed and perfused, according to Langendorff, using retrograde perfusion at a constant flow of 7.5 ml/min (Piacentini et al., 1993). The

* Corresponding author. Tel.: (44-141) 558-2858; Fax: (44-141) 552-2562.

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perfusate was a modified Krebs–Henseleit solution containing (in mmol/l); 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. The perfusate was adjusted to pH 7.4, aerated with a mixture of oxygen (95%) and carbon dioxide (5%), filtered and maintained at 37°C. Perfusion pressure (mmHg) was monitored via a sideport above the aorta. Fine platinum electrodes were placed on the right atrium and on apex of the left ventricle to allow the recording of epicardial electrograms.

After a 10 min stabilisation period and about 30 min after the administration of genistein or its vehicle, the coronary artery was occluded and ventricular arrhythmias assessed over a 30 min period; at the end of this time the myocardium was reperfused. Arrhythmias were assessed as the total number of ventricular premature beats, the incidence and duration of ventricular tachycardia (VT) and the incidence of ventricular fibrillation (VF). This assessment and the statistical evaluation, (Piacentini et al., 1993), is based on the guidelines suggested in the 'Lambeth' conventions (Walker et al., 1988). Some hearts were preconditioned by a 3 min occlusion, followed by a 10 min period of reperfusion, prior to the 30 min coronary artery occlusion. This protocol significantly reduces the severity of ischaemia-induced ventricular arrhythmias (Piacentini et al., 1993).

In a separate series of experiments hearts were subjected to a similar preconditioning stimulus but in the presence of genistein (100 μM) in the perfusing fluid. This concentration of genistein prevents the enhancement of phospholipase D and tyrosine kinase activities which normally occurs as a result of preconditioning (Maulik et al., 1996).

At the end of the experiments, the occluded area was assessed by reoccluding the artery and injecting patent V dye into the perfusing fluid; this stains the area not at risk from infarction. The unstained area that was at risk was removed, weighed and expressed as a percentage of the left ventricular wall plus septum.

To compare the number of ventricular premature beats between groups, the Kruskal–Wallis non-parametric test was used. For comparison of incidences of arrhythmias (ventricular tachycardia, ventricular fibrillation) the χ^2 test for independence was used. Differences between groups were considered significant when $P < 0.05$.

3. Results

There was no significant difference between the groups in the mean perfusion pressure before coronary artery occlusion (control and vehicle, 49 ± 3 mmHg; preconditioned and vehicle 43 ± 2 mmHg; control and genistein, given intravenously prior to removal of the hearts, 47 ± 4 mmHg; preconditioned and genistein 48 ± 2 mmHg). Occlusion of the left coronary artery caused similar increases in perfusion pressure (by a mean of 19.2 ± 2.4, 19.9 ± 2.2, 17.9 ± 1.8 and 19.5 ± 2.3 mmHg, respectively). Perfusion pressure returned to preocclusion levels immediately after the snare was released at the end of the 30 min occlusion period.

Coronary artery occlusion led to significant ventricular ectopic activity (Table 1) throughout the entire ischaemic period (Fig. 1). This arrhythmia severity was reduced by preconditioning; no preconditioned heart fibrillated either during occlusion or reperfusion and the duration of ventricular tachycardia during ischaemia was extremely short (Table 1). Ventricular premature beats were reduced throughout the entire occlusion period (Fig. 1). This protective effect of ischaemic preconditioning was not seen in hearts removed from rats to which genistein had been administered prior to removal (Table 1 and Fig. 1).

Similar studies were performed in hearts from normal rats but in the presence of genistein (100 μM) in the Krebs solution. This concentration has been found to inhibit the increase that occurs in various kineses following preconditioning (Maulik et al., 1996). Genistein itself had no effect on the severity of arrhythmias resulting from coronary artery occlusion but, as in the studies in which it had been given in vivo, it was not possible to precondition hearts by a single preconditioning stimulus in the presence of this tyrosine kinase inhibitor (Table 1).

Table 1
The effect of genistein (5 mg/kg in 0.5% (v/v) DMSO by prior intravenous administration), or of genistein (100 μM) in the perfusing fluid, on the arrhythmias resulting from a 30 min occlusion of the left coronary artery in rat isolated perfused hearts

	n	Total VPBs	Arrhythmia incidence		Duration of VT (s)	Reperfusion VT/VF
			VT	Irrev VF		
Control (vehicle)	11	333 ± 60	9/11	1/11	21.6 ± 7.7	2/11
Preconditioning (and vehicle)	11	52 ± 4.7[a]	6/11	0	1.1 ± 0.4[a]	0
Genistein	11	242 ± 37	7/11	1/11	8 ± 2.1	0
Preconditioning and genistein	12	272 ± 37[b]	8/12	1/12	$9.8 + 2.7$[b]	2/12
Control with genistein (100 μM) in the perfusing solution	14	240 ± 26	6/14	2/14	$8.9 + 0.9$	1/14
Preconditioning with genistein (100 μM) in the perfusing solution	12	210 ± 35[b]	7/12	1/12	10.1 ± 1.7[b]	2/12

VPBs = ventricular premature (ectopic) beats; VT = ventricular tachycardia; VF = irreversible fibrillation.
[a] $P < 0.01$ versus control.
[b] $P < 0.05$ versus preconditioning.

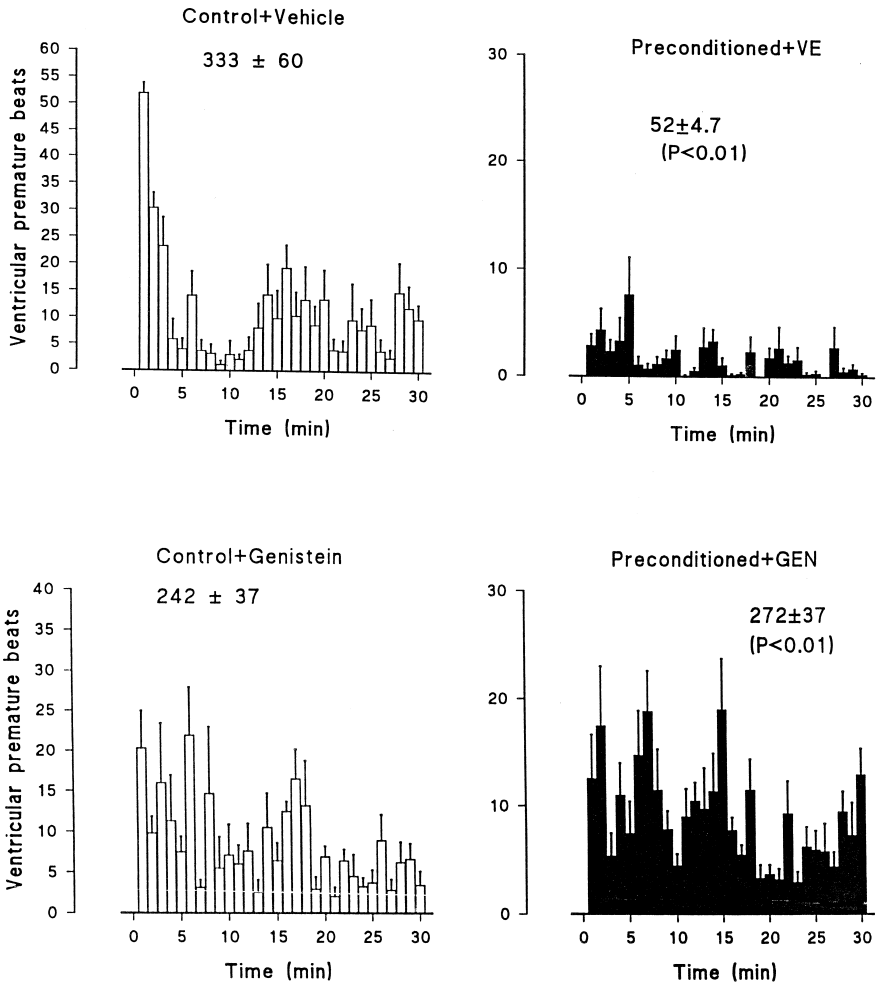

Fig. 1. The distribution of ventricular premature beats during a 30 min occlusion of the left coronary artery in rat isolated hearts. Rats were administered the vehicle for genistein in vivo and the hearts then subjected to coronary artery occlusion in vitro (top left). Prior to their occlusion some hearts were preconditioned in vitro by a single 3 min occlusion of the left coronary artery (top right). Rats were also given genistein (5 mg/kg in 0.5% (v/v) DMSO) in vivo and their hearts were then subjected to coronary occlusion in vitro (bottom left). Some of these hearts were also then subjected to a preconditioning stimulus (bottom right). The results are given as means ± S.E.M. Note that the scale for arrhythmias in preconditioned hearts is different from that in the control groups. The figures indicate the total number of ventricular premature beats over the entire 30 min occlusion period.

There was no significant difference between any of the groups with regard to the area at risk. These were 36.4 ± 2.1% (control plus vehicle), 33.3 ± 0.8% (control plus genistein), 34.6 ± 1.6% (preconditioned plus vehicle), 35.3 ± 1.8% (preconditioned plus genistein), 36.0 ± 1.5% (control plus genistein in the perfusing fluid) and 33.0 ± 2.0% (preconditioned plus genistein in the perfusing fluid).

4. Discussion

Both bacterial lipopolysaccharide (endotoxin) and a non-toxic derivative of the active lipid A component of the endotoxin molecule, perhaps rather surprisingly, protect the heart against damage by a quite different (ischaemic) stimulus (Wu et al., 1996, Vegh et al., 1996). This protection is manifested both by a reduction in myocardial infarct size and a suppression of those life-threatening ventricular arrhythmias that occur soon after coronary artery occlusion. This protection is similar in its time course to the delayed form of protection afforded by ischaemic preconditioning and by cardiac pacing (reviewed by Parratt and Szekeres, 1995).

Because the signal transduction pathways in various cell types incubated with lipopolysaccharide involve activation of tyrosine kinase we investigated whether one particular manifestation of ischaemic preconditioning (arrhythmia suppression) was modified following inhibition of this enzyme. Maulik et al. (1996) have recently shown, in rat isolated hearts, that genistein prevents the enhanced recovery of contractile function that occurs following a period of ischaemia and reperfusion and which is one of the beneficial effects of preconditioning. Activation of mitogen-activated protein (MAP) kinase and MAP kinase activated protein (MAPKAP) kinase 2, which normally occurs in preconditioned hearts, is also prevented by genistein.

Recently Imagawa et al. (1997) have shown, also using genistein, that tyrosine kinase may also be involved in the reduction in infarct size that results from ischaemic preconditioning 24 h after the preconditioning stimulus.

In the present study preconditioning reduced coronary artery occlusion-induced arrhythmias in isolated hearts (a reduction in the total number of ventricular premature beats and in the duration of ventricular tachycardia during the occlusion period; abolition of ventricular fibrillation during both occlusion and reperfusion). This anti-arrhythmic effect of preconditioning was not seen following administration of genistein either in vivo (that is prior to removal of the hearts) or when it was given, in the perfusing fluid, to normal hearts in vitro. These results suggest that ischaemic preconditioning leads, probably by receptor activation, to the early stimulation of tyrosine kinase and that this is an important step leading to the ability of the heart to resist one of the most important consequences of myocardial ischaemia. It seems then that tyrosine kinases are involved in each of the three major manifestations of ischaemic preconditioning — reduction of ischaemic damage, suppression of arrhythmias and enhanced recovery of contractile function following a period of ischaemia.

The down-stream pathways are unclear, but activation of phospholipase C-γ (or D) with the subsequent activation (and translocation) of protein kinase (PK) C or the activation of the ras raf MAP-K pathway (with subsequent gene activation) through GTPase-activity protein, are two of the most likely pathways (reviewed by Wang and McWhirter, 1994). The unravelling of these downstream pathways is important for an understanding of the powerful suppression of life-threatening arrhythmias associated with ischaemic preconditioning.

References

Cohen, M.V., Liu, Y., Downey, J.M., 1996. Activation of protein kinase C is critical to the protection of preconditioning. In: Wainwright, C.L., Parratt, J.R. (Eds.), Myocardial Preconditioning. Springer, Berlin, p. 185.

Lawson, C.S., Coltart, D.J., Hearse, D.J., 1993. 'Dose-dependency' and temporal characteristics of protection by ischaemic preconditioning against ischaemia-induced arrhythmias in rat hearts. J. Mol. Cell. Cardiol. 25, 1391.

Imagawa, G., Baxter, G.F., Yellon, D.M., 1997. Genistein, a tyrosine kinase inhibitor, blocks the second window of protection 48 h after ischaemic preconditioning. Br. J. Pharmacol. 120, 356P.

Marczin, N., Papapetropoulos, A., Catravas, J.D., 1993. Tyrosine kinase inhibitors suppress endotoxin- and IL-10-induced NO synthase in aortic smooth muscle cells. Am. J. Physiol. 265, H1014.

Maulik, N., Watanabe, M., Zu, Y.-L., Huang, C.-K., Cordis, G.A., Schley, J.A., Das, D.K., 1996. Ischemic preconditioning triggers the activation of MAP kinases and MAPKAP kinase 2 in rat hearts. FEBS Lett. 396, 233.

Parratt, J.R., 1993. Endogenous myocardial protective substances. Cardiovasc. Res. 27, 693.

Parratt, J.R., Szekeres, L., 1995. Delayed protection of the heart against ischaemia. Trends Pharmacol. Sci. 16, 351.

Paul, A., Pendreigh, R.H., Plevin, R., 1995. Protein kinase C and tyrosine kinase pathways regulate lipopolysaccharide-induced nitric oxide synthase activity in RAW 264.7 murine macrophages. Br. J. Pharmacol. 114, 482.

Piacentini, L., Wainwright, C.L., Parratt, J.R., 1993. The antiarrhythmic effect of ischaemic preconditioning in isolated rat hearts involves a pertussis toxin sensitive mechanism. Cardiovasc. Res. 27, 674.

Vegh, A., Parratt, J.R., 1996. Ischaemic preconditioning markedly reduces the severity of ischaemia and reperfusion-induced arrhythmias: Role of endogenous myocardial protective substances. In: Wainwright, C.L., Parratt, J.R. (Eds.), Myocardial Preconditioning. Springer, Berlin, p. 35.

Vegh, A., Szekeres, L., Parratt, J.R., 1990. Protective effects of preconditioning of the ischaemic myocardium involve cyclooxygenase products. Cardiovasc. Res. 24, 1020.

Vegh, A., Komori, S., Szekeres, L., Parratt, J.R., 1992. Antiarrhythmic effects of preconditioning in anaesthetised dogs and rats. Cardiovasc. Res. 26, 487.

Vegh, A., Papp, J.Gy., Elliott, G.T., Parratt, J.R., 1996. Pre-treatment with monophosphoryl lipid c (MPL-C) reduces ischaemia-reperfusion-induced arrhythmias in dogs. J. Mol. Cell. Cardiol. 28, abstr. 217.

Walker, M.J., Curtis, M.J., Hearse, D.J., Campbell, R.W., Janse, M.J., Yellon, D.M., Cobbe, S.M., Coker, S.J., Harness, J.B., Harron, D.W., Higgins, A.J., 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.J., Winslow, E., Woodward, B., 1988. The Lambeth conventions; Guidelines for the study of arrhythmias in ischaemia, infarction and reperfusion. Cardiovasc. Res. 22, 447.

Wang, J.W.J., McWhirter, J.R., 1994. Tyrosine-kinase-dependent signalling pathways. Trends Cardiovasc. Med. 4, 264.

Wu, S., Furman, B.L., Parratt, J.R., 1996. Delayed protection against ischaemia-induced ventricular arrhythmias and infarct size limitation by the prior administration of *Escherichia coli* endotoxin. Br. J. Pharmacol. 118, 2157.