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Involvement of tyrosine kinase in peroxynitrite-induced preconditioning in rat isolated heart

Selen Söylemez[a], A. Tuncay Demiryürek[a,b,*], İlker Kanzık[a]

[a] Department of Pharmacology, Faculty of Pharmacy, Gazi University, Ankara, Turkey
[b] Department of Pharmacology, Faculty of Medicine, University of Gaziantep, Gaziantep, Turkey

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Abstract

We have investigated the role of tyrosine kinase in the antiarrhythmic effects of peroxynitrite preconditioning in rat isolated heart by using a tyrosine phosphatase inhibitor, sodium orthovanadate, and tyrosine kinase inhibitors, genistein and tyrphostin. Rat hearts were preconditioned by peroxynitrite administration at 1 μM for 3 min, which was followed by 10-min washout and 30 min of ischemia. None of the hearts had ventricular fibrillation in the peroxynitrite preconditioning group (from 64%, $n=11$, to 0%, $n=11$). Neither sodium orthovanadate (10 μM) nor genistein (50 μM) or tyrphostin (100 μM) alone showed any effects on arrhythmias. Peroxynitrite preserved its beneficial effects on arrhythmias (to 0% ventricular fibrillation, $n=7$) during sodium orthovanadate infusion (for 23 min) prior to 30 min of an ischemic period. On the other hand, genistein or tyrphostin treatment significantly reversed the protective effects of the peroxynitrite preconditioning (to 71% ventricular fibrillation, $n=14$, genistein and, to 75% ventricular fibrillation, $n=8$, tyrphostin). These results suggest that the tyrosine kinase pathway plays a significant role in peroxynitrite-induced preconditioning in rat isolated heart.

Keywords: Peroxynitrite; Preconditioning; Tyrosine kinase; Genistein; Tyrphostin

1. Introduction

Brief episodes of ischemia–reperfusion in the heart exert powerful endogenous cardioprotection against a subsequent prolonged ischemic insult, which has been known as ischemic preconditioning (Murry et al., 1986). The exact mechanism responsible for preconditioning has not been fully elucidated despite intensive investigation. There is growing evidence that free radicals, adenosine, bradykinin, nitric oxide (NO) and opioids may play a pivotal role in ischemic preconditioning (Schulz et al., 2001). Although much is known about the mediators of ischemic preconditioning, the cellular signaling leading to cardioprotection has remained elusive.

There is considerable experimental evidence to suggest that, during the early reperfusion period, peroxynitrite formation occurs parallel to superoxide formation. A marked increase in peroxynitrite formation was detected during the early period of reperfusion (Wang and Zweier, 1996). Although high concentrations of peroxynitrite appear to be responsible for most of the NO-mediated toxicity, in recent years, it has become apparent that low levels of peroxynitrite can also mediate beneficial effects (Beckman and Koppenol, 1996). Peroxynitrite relaxes various arteries including coronary arteries through stimulation of cGMP (Liu et al., 1994a,b; Wu et al., 1994). Peroxynitrite produces *S*-nitrosothiols, which stimulate guanylate cycles and release NO (Wu et al., 1994; Moro et al., 1995). Peroxynitrite has been found to be cardioprotective in micromolar concentrations both in vivo and in vitro (Lefer et al., 1997; Nossuli et al., 1997, 1998). Lefer et al. (1997) were the first to describe the cytoprotective effects of low concentrations of peroxynitrite against myocardial ischemia–reperfusion injury in isolated rat heart perfused with rat neutrophils. We have shown that peroxynitrite also exerts beneficial effects on ischemia–reperfusion-induced arrhythmias in rat isolated heart (Altug et al., 1999). We have also presented evidence that peroxynitrite is able to precondition the heart and contribute to the antiarrhythmic effect of ischemic preconditioning (Altug et al., 2000, 2001). This observation is further supported by the determination of peroxynitrite

* Corresponding author. Department of Pharmacology, Faculty of Medicine, University of Gaziantep, 27310 Gaziantep, Turkey. Tel.: +90-342-360-6060/7740; fax: +90-342-360-1617.
E-mail address: tn.atd@angelfire.com (A.T. Demiryürek).

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formation during brief ischemic periods of preconditioning (Csonka et al., 2001). However, signal transduction pathways of peroxynitrite-induced preconditioning are not known. There is evidence that tyrosine kinase is involved in the antiarrhythmic effects of ischemic preconditioning in anaesthetised rats (Fatehi-Hassanabad and Parratt, 1997). It has also been shown that peroxynitrite can activate tyrosine kinase (Li et al., 1998; Mallozzi et al., 1999) and inhibit protein tyrosine phosphatases, resulting in an increase in tyrosine phosphorylation (Takakura et al., 1999). Thus, it might be hypothesized that tyrosine kinase activation might be involved in peroxynitrite preconditioning in rat heart. Therefore, the aim of this study was to determine the role of the tyrosine kinase pathway, using selective tyrosine kinase inhibitors, genistein and tyrphostin, and a tyrosine phosphatase inhibitor, sodium orthovanadate, in peroxynitrite preconditioning in rat isolated heart.

2. Materials and methods

The investigation conforms 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).

2.1. Peroxynitrite synthesis

Peroxynitrite was synthesised in our laboratory from acidified nitrite and hydrogen peroxide (H_2O_2) according to the method of Beckman et al. (1994). Briefly, an aqueous solution of 0.6 M sodium nitrite was rapidly mixed with an equal volume of 0.6 M H_2O_2 containing 0.7 M HCl and immediately quenched with the same volume of 1.2 M NaOH. All reactions were performed on ice. Excess H_2O_2 was removed by addition of manganese dioxide (MnO_2) powder to the peroxynitrite solution. The mixture was then shaken for 5 min and MnO_2 was removed by passage over a cellulose acetate disposable filter. The final concentration of peroxynitrite was determined spectrophotometrically ($\varepsilon_{302} = 1670\ M^{-1}\ cm^{-1}$) as described previously (Altug et al., 1999, 2000, 2001). Fresh dilutions were made with Krebs–Henseleit solution without glucose and sodium pyruvate just before use and the pH of these solutions was adjusted to 8.4 by addition of an appropriate volume of 0.1 N NaOH (Nossuli et al., 1997). The stock solutions were aliquoted and stored at −20 °C for a week.

2.2. Preparation of isolated hearts

Male Sprague–Dawley rats from Başkent University (Ankara) weighing 200–300 g were used in this study. The Sprague–Dawley rats were allowed to acclimatize in cages for at least 10 days after transportation. They had standard laboratory diet and a 12:12-h light/dark cycle at 25 °C and were supplied with standard laboratory diet and tap water ad libitum. The rats were anaesthetized with an i.p. injection of thiopental (60 mg kg^{-1}). After induction of anaesthesia, the abdomen was opened and heparin (500 U) was given through the femoral vein. After 3 min of heparin injection, the abdominal aorta was cut to reduce the blood volume in the heart. Then the thorax was opened and the heart was quickly excised and put into a Petri dish containing an ice-cold, daily prepared, Krebs–Henseleit solution of the following composition (mM): NaCl 118; KCl 3.2; KH_2PO_4 1.18; $MgSO_4$ 1.66; $NaHCO_3$ 26.88; $CaCl_2$ 2.52; glucose 5.55; sodium pyruvate 2.0 (Piacentini et al., 1993). The pH of the solution was adjusted to 7.4. Then the heart was perfused retrogradely via the aorta by means of a modified Langendorff apparatus at a constant flow of 8–10 ml min^{-1} which was determined according to animal weight using the formula: flow (ml min^{-1}) = $7.43x^{0.56}$ (x is the heart weight), heart weight = $0.0027y + 0.6$ (y is the body weight), at 37 °C with the Krebs–Henseleit solution gassed with 95% O_2 and 5% CO_2 (Piacentini et al., 1993). A loose ligature was immediately placed round the left anterior descending coronary artery; both ends of the ligature were then passed through a short piece of polythene tubing (1 mm i.d. and 1.5 mm long) to form a snare. Following a stabilization period of 15 min, the snare around the left anterior descending coronary artery was tightened and held in place with a small clip. An increase in coronary perfusion pressure indicated successful ligation; likewise, a decrease in perfusion pressure indicated successful reperfusion. The electrocardiogram (ECG) was recorded throughout the experiment by two electrodes placed on the right atrium and apex, using a computerized data acquisition system (TDA95, Commat, Ankara, Turkey) (Altug et al., 1999, 2000, 2001). Coronary perfusion pressure was measured via a pressure transducer and recorded continuously by the same data acquisition system.

2.3. Experimental protocol

After the surgical procedures, all hearts were allowed to stabilize for 15 min prior to the experimental protocol. These protocols are shown in Fig. 1. In the first group of experiments (protocol 1, control, $n = 11$), the hearts were subjected to 30-min left anterior descending coronary artery occlusion. In the second group of experiments (protocol 2, peroxynitrite-induced preconditioning, $n = 11$), the hearts were subjected to 3-min infusion of peroxynitrite at 1 μM concentration followed by 10-min washout and then 30-min occlusion. The concentration of 1 μM was chosen since it had been shown in our previous study (Altug et al., 2000, 2001) that this concentration could precondition the heart. Peroxynitrite was infused into the perfusion solution through the rubber tubing placed just proximal to the heart. Stock solutions of peroxynitrite were kept on ice, and the infusion lines were wrapped in aluminium foil to reduce exposure to light. In

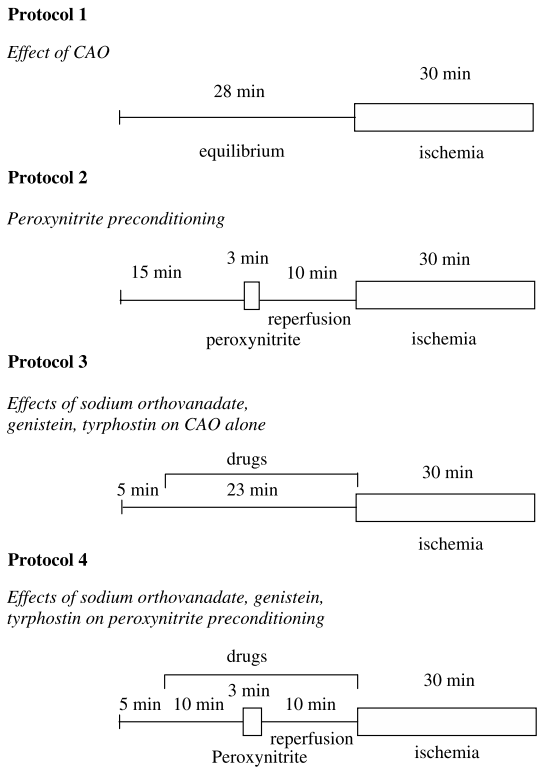

Fig. 1. Experimental protocol for the study. Isolated rat hearts perfused with Krebs–Henseleit solution were subjected to 30-min ischemia (protocol 1). Rat isolated hearts were preconditioned against 30-min ischemia by infusion of 1 μM peroxynitrite for 3 min (protocol 2). Tyrosine phosphatase inhibitor, sodium orthovanadate (10 μM), tyrosine kinase inhibitors, genistein (50 μM) and tyrphostin (100 μM), were added to the perfusate for 23 min prior to the ischemic period (protocol 3) or 10 min prior to peroxynitrite infusion (protocol 4) and kept there until the start of the 30-min occlusion (total 23 min).

protocol 3, sodium orthovanadate (10 μM, $n=10$) (Shah et al., 1995), genistein (50 μM, $n=9$) (Baines et al., 1998; Akaishi et al., 2000) or tyrphostine (100 μM, $n=11$) (Akaishi et al., 2000) was infused alone. For the last series of experiments (protocol 4), the hearts were preconditioned with peroxynitrite as in protocol 3, but received sodium orthovanadate ($n=7$), genistein ($n=14$) or tyrphostine ($n=8$) for 23 min, starting 10 min prior to peroxynitrite infusion and continuing until the start of the 30-min occlusion period.

2.4. Parameters measured

For all the groups, heart rate was measured from the recordings of ECG and the incidences of arrhythmias were registered, in accordance with the Lambeth conventions (Walker et al., 1988), as ventricular tachycardia, ventricular fibrillation and ventricular ectopic beats. Ventricular ectopic beats are defined as a discrete and identifiable premature QRS complex. Ventricular tachycardia was diagnosed as four or more consecutive ventricular ectopic beats. Ventricular fibrillation was diagnosed when the ECG recording showed chaotic activity with an amplitude less than that of the normal ECG. Irreversible ventricular fibrillation was defined as ventricular fibrillation, which did not reverse within the last 10 min of the experiment. The onset and duration of arrhythmias were also measured. The arrhythmia score for these experiments was calculated by using the previously published scale (Demiryurek et al., 2002). The following values were given:

1. 0–50 ventricular ectopic beats with no ventricular tachycardia or ventricular fibrillation over the observation period,
2. 50–500 ventricular ectopic beats only,
3. More than 500 ventricular ectopic beats, or one episode of spontaneously reversible ventricular tachycardia or ventricular fibrillation,
4. Spontaneously reversible ventricular tachycardia and/or ventricular fibrillation for 2–30 episodes,
5. Spontaneously reversible ventricular tachycardia and/or ventricular fibrillation for more than 30 episodes,
6. Occurrence of irreversible ventricular fibrillation.

2.5. Materials

Sodium orthovanadate, genistein, tyrphostin and sodium nitrite were purchased from Sigma (St. Louis, MO, USA). Hydrogen peroxide was purchased from Merck (Darmstadt, Germany). Peroxynitrite was freshly prepared and used and was kept on ice away from light during infusion. Genistein and tyrphostin were dissolved in dimethyl sulfoxide (DMSO). The final concentration of the solvent was less than 0.03%. Addition of an appropriate concentration of the solvent caused no significant change in hemodynamic variables.

Table 1
Effects of peroxynitrite (1 μM, 3 min)-induced preconditioning on ischemic (30 min) arrhythmias, and effects of sodium orthovanadate (10 μM), genistein (50 μM) and tyrphostin (100 μM) (total 23 min) on peroxynitrite-induced preconditioning

	n	Total VEBs	Percent VT	Percent total VF	Percent irr. VF
Control	11	363 ± 84	100 (11)	64 (7)	55 (6)
Peroxynitrite	11	138 ± 42[a]	64 (7)	0 (0)[a]	0 (0)
Sodium orthovanadate	10	344 ± 73	100 (10)	40 (4)	20 (2)
Sodium orthovanadate + Peroxynitrite	7	159 ± 49	71 (5)	0 (0)[a]	0 (0)
Genistein	9	438 ± 47	100 (9)	67 (6)	33 (3)
Genistein + Peroxynitrite	14	413 ± 38[b]	100 (14)[b]	71 (10)[b]	43 (6)
Tyrphostin	11	403 ± 66	100 (11)	46 (5)	27 (3)
Tyrphostin + Peroxynitrite	9	373 ± 34[b]	100 (9)	67 (6)[b]	44 (4)

VEBs, ventricular ectopic beats defined as discrete and identifiable premature QRS complexes; VT, ventricular tachycardia; VF, ventricular fibrillation; irr. VF, irreversible ventricular fibrillation. Numbers in parentheses are the number of hearts that exhibited that particular type of arrhythmia.

[a] $P<0.05$ compared to control group.

[b] $P<0.05$ compared to peroxynitrite group.

Table 2
Effects of sodium orthovanadate (10 μM), genistein (50 μM) and tyrphostin (100 μM) infusion (for 23 min) on the time of onset of first arrhythmias, duration of ventricular tachycardia and arrhythmia scores in rat isolated hearts

	n	Time of onset of first arrhythmias (s)	Duration of VT (s)	Arrhythmia scores
Control	11	21.7 ± 6.2	19.6 ± 6.3	3.8 ± 0.4
Peroxynitrite	11	65.6 ± 17.6[a]	6.8 ± 3.7	1.6 ± 0.4[a]
Sodium orthovanadate	10	25.6 ± 5.9	22.2 ± 8.3	3.3 ± 0.3
Sodium orthovanadate + Peroxynitrite	7	47.1 ± 9.4	9.8 ± 8.0	2.0 ± 0.3[a]
Genistein	9	30.4 ± 8.8	20.3 ± 4.4	3.7 ± 0.3
Genistein + Peroxynitrite	14	25.8 ± 4.1[b]	29.4 ± 6.8	3.9 ± 0.3[b]
Tyrphostin	11	24.3 ± 5.1	16.9 ± 3.7	3.5 ± 0.2
Tyrphostin + Peroxynitrite	9	24.1 ± 4.3[b]	15.2 ± 3.4	3.9 ± 0.3[b]

VT, ventricular tachycardia.
[a] $P < 0.05$ compared to control group.
[b] $P < 0.05$ compared to peroxynitrite group.

2.6. Statistical analysis

Values are presented as means ± standard error of the mean or as the percentage incidence. A Fisher's extract chi-square test was used to detect significant differences in the incidence of ventricular tachycardia and ventricular fibrillation between control and drug-treated groups. Statistical comparison of more than two groups was performed with a one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls test. The Mann–Whitney *U*-test was used to detect significant differences between arrhythmia scores. In all tests, *P* values less than 0.05 were considered to be statistically significant.

3. Results

3.1. Peroxynitrite preconditioning

Preconditioning the hearts with 3 min of peroxynitrite (1 μM) suppressed arrhythmias during the 30-min occlusion period. The total number of ventricular ectopic beats was significantly lower than the controls. Although the incidence of ventricular tachycardia was slightly, but not significantly, lower than in the control hearts, no ventricular fibrillation was recorded in the peroxynitrite preconditioning group (Table 1). Preconditioning with peroxynitrite markedly augmented the time of onset of the first arrhythmias and reduced the arrhythmia score. Although the duration of ventricular tachycardia decreased, this reduction did not reach statistical significance (Table 2). Peroxynitrite administration did not cause any significant change in coronary perfusion pressure throughout the experimental protocol (Table 3).

3.2. Effects of sodium orthovanadate, genistein or tyrphostin on occlusion-induced ventricular arrhythmias

Administration of sodium orthovanadate, genistein or tyrphostin on its own did not show any marked effect on the total number of ventricular ectopic beats, incidences of ventricular tachycardia or total ventricular fibrillation during a 30-min occlusion period (Table 1). These drugs alone did not modify the time of onset of first arrhythmias, duration of ventricular tachycardia or the arrhythmia scores (Table 2). None of these values were significantly different from the control values. These drugs did not modify the coronary perfusion pressure (Table 3).

3.3. Effects of sodium orthovanadate, genistein or tyrphostin on peroxynitrite preconditioning

In the presence of sodium orthovanadate, peroxynitrite was capable of producing protective effects. The total number of ventricular ectopic beats appeared to decrease and no ventricular fibrillation or irreversible ventricular fibrillation was observed (Table 1). However, genistein or tyrphostin reversed the beneficial effects of peroxynitrite preconditioning on ischemia-induced arrhythmias. Genistein and tyrphostin increased the incidence of ventricular fibrillation from 0% ($n = 0$) to 71% ($n = 10$) and 67% ($n = 6$), respectively (Table 1).

Table 3
Mean coronary perfusion pressure values (mm Hg) during peroxynitrite infusion and coronary occlusion in rat isolated hearts

	n	Baseline	Peroxynitrite		Washout		Permanent occlusion	
			1 min	3 min	1 min	10 min	1 min	30 min
Control	11	–	–	–	–	34 ± 2	67 ± 2[a]	65 ± 3[a]
Peroxynitrite	11	34 ± 3	31 ± 2	33 ± 3	32 ± 2	31 ± 2	59 ± 3[a]	63 ± 3[a]
Sodium orthovanadate	10	31 ± 2	32 ± 2	32 ± 2	33 ± 2	32 ± 2	62 ± 3[a]	61 ± 3[a]
Sodium orthovanadate + Peroxynitrite	7	34 ± 2	36 ± 2	38 ± 3	37 ± 3	37 ± 3	64 ± 3[a]	67 ± 4[a]
Genistein	9	34 ± 2	35 ± 2	38 ± 3	38 ± 3	37 ± 3	64 ± 3[a]	69 ± 3[a]
Genistein + Peroxynitrite	14	34 ± 2	36 ± 2	37 ± 2	36 ± 3	37 ± 3	65 ± 3[a]	68 ± 4[a]
Tyrphostin	11	33 ± 2	34 ± 2	34 ± 3	35 ± 3	35 ± 3	65 ± 3[a]	69 ± 3[a]
Tyrphostin + Peroxynitrite	9	32 ± 2	35 ± 2	36 ± 3	37 ± 3	37 ± 3	66 ± 3[a]	68 ± 4[a]

Rat isolated hearts were subjected to peroxynitrite at 1 μM concentration for 3 min followed by 10-min washout period. All groups were then subjected to 30 min of coronary occlusion. Values are given as means ± S.E.M.
[a] $P < 0.05$, significantly different when compared to pre-occlusion values.

Sodium orthovanadate administration in the peroxynitrite-treated group caused a slight, but not significant, increase in the time of onset of first arrhythmias, and markedly reduced the arrhythmia score (from 3.8 ± 0.4, $n = 11$, to 2.0 ± 0.3, $n = 7$), which was similar to the peroxynitrite preconditioning group (1.6 ± 0.4, $n = 11$). On the other hand, in the presence of genistein or tyrphostin, protective effects of peroxynitrite were inhibited, time of onset of first arrhythmias was reduced, and arrhythmia scores were elevated (Table 2). No marked changes were noted in ventricular tachycardia duration between the groups.

4. Discussion

In this study we have shown that exposure to 1 μM concentration of peroxynitrite on its own was capable of producing preconditioning in isolated hearts. Peroxynitrite at this concentration markedly reduced the severity of ischemia-induced arrhythmias. These results confirm our previous reports showing that peroxynitrite can induce preconditioning in rat isolated heart (Altug et al., 2000, 2001). This protective effect was preserved in the presence of a tyrosine phosphatase inhibitor, sodium orthovanadate, and was lost in the presence of tyrosine kinase inhibitors, genistein and tyrphostin. To our knowledge, this is the first experimental evidence that peroxynitrite can activate the tyrosine kinase pathway to initiate a protective response against arrhythmias. There are several studies showing the activation of tyrosine kinases by peroxynitrite, which may support our results. It has been shown that peroxynitrite can upregulate the tyrosine phosphorylation signal through the activation of *src* family tyrosine kinase (Li et al., 1998; Mallozzi et al., 1999), which is shown to be activated during preconditioning (Ping et al., 1999; Hattori et al., 2001). Recently, peroxynitrite was found to induce the dimerization of epidermal growth factor (EGF) receptors, an effect associated with EGF receptor activation (van der Vliet et al., 1998). Stimulation of EGF receptors activates tyrosine kinase-mediated signaling cascades. Peroxynitrite is also capable of inhibiting protein tyrosine phosphatases at micromolar concentrations, resulting in an increase in tyrosine phosphorylation (Takakura et al., 1999). Thus, there is increasing evidence that tyrosine phosphorylation-mediated signaling cascades are susceptible to modulation by peroxynitrite.

Two structurally and functionally distinct tyrosine kinase inhibitors, genistein, which interacts with an ATP binding site (Akiyama et al., 1987), and tyrphostin, which interacts with the substrate binding site (Gazit et al., 1989), both prevented antiarrhythmic effects of peroxynitrite in the present study. Inhibition of tyrosine kinase causes tyrosine dephosphorylation. Similar effects were produced by the two structurally unrelated tyrosine kinase inhibitors confirming that the inhibition was the result of a specific action on tyrosine kinases. In our study, additional support for the involvement of tyrosine kinase in peroxynitrite preconditioning was provided by the ability of sodium orthovanadate to protect the beneficial effects against the severity of arrhythmias. Sodium orthovanadate inhibits the activity of tyrosine-specific phosphatases and enhances the expression of tyrosine kinase-mediated responses (Chao et al., 1992). Therefore, vanadate not only inhibits tyrosine phosphatase activity but also stimulates the activity of tyrosine kinases as demonstrated in Kupffer cells (Chao et al., 1992). Together, these results are the first to provide evidence that the tyrosine kinase pathway is involved in the preconditioning effect of peroxynitrite. Thus, tyrosine phosphorylation appears to be an important component of the signal transduction pathway leading to preconditioning.

Maulik et al. (1996) were the first to demonstrate the involvement of protein tyrosine kinase in ischemic preconditioning of isolated working rat hearts. It has been shown in rabbit isolated hearts that inhibition of protein tyrosine kinase with genistein also prevents ischemic preconditioning (Baines et al., 1998). Delayed ischemic preconditioning was also abolished by genistein (Imagawa et al., 1997). It is now increasingly clear that protein tyrosine kinases play a crucial role in mediating ischemic preconditioning in some animal species. Protein tyrosine kinases may act in parallel to (Fryer et al., 1999; Tanno et al., 2000; Vahlhaus et al., 1998) or downstream of (Baines et al., 1998; Ping et al., 1999) protein kinase C in eliciting preconditioning. Although it was not aimed to study the involvement of protein kinase C in the present study, our data may support the observations showing that tyrosine kinase is involved in early or classic preconditioning. The current findings also suggest that ischemic preconditioning and pharmacological preconditioning with peroxynitrite appear to share at least the tyrosine kinase part of the signaling pathway in rat isolated hearts.

Participation of protein kinase C and tyrosine kinase in the preconditioning signal transduction pathway differs from species to species. Although blockade of protein kinase C alone aborts the protection from ischemic preconditioning in rat (Fryer et al., 1999; Tanno et al., 2000) and rabbit hearts (Liu et al., 1994a,b; Baines et al., 1998), the combined inhibition of both protein kinase C and tyrosine kinase is required in pigs (Vahlhaus et al., 1998). Protein kinase C and an unidentified tyrosine kinase may also act via parallel pathways in the rat heart, given that both inhibitors must be present to completely abolish protection from multiple cycles of preconditioning, whereas either is sufficient to block protection from a single cycle of ischemic preconditioning (Fryer et al., 1999). Since genistein or tyrphostin blocked cardioprotective effects of a single administration of peroxynitrite in the present study, our results may support the results of the single cycle experiments showing the blockade of preconditioning with tyrosine kinase inhibitors in rat hearts (Fatehi-Hassanabad and Parratt, 1997; Fryer et al., 1999).

The precise mechanism of how tyrosine kinase activation may eventually lead to a suppression of arrhythmias is not known. Current evidence indicates that activation of tyrosine kinase ultimately leads to the activation of p38 mitogen-activated protein kinase (Maulik et al., 1998). It has been shown that preconditioning protects the heart by activating the p38 mitogen-activated protein kinase pathway during ischemia and that genistein blocks the protection from ischemic preconditioning by preventing the tyrosine phosphorylation of p38 mitogen-activated protein kinase (Nakano et al., 2000). Once active, p38 mitogen-activated protein kinase then stimulates mitogen-activated protein kinase-activated protein kinase-2 in a phosphorylation-dependent manner, which in turn leads to the phosphorylation of low molecular weight heat shock protein 27. In many cells, phosphorylation of heat shock protein 27 leads to the polymerization of actin, which appears to increase the tolerance of the cytoskeleton to stress (Cohen et al., 2000). Although the mechanism of ischemic preconditioning remains elusive, the K_{ATP} channel may be the end-effector, activated by G proteins, protein kinase C, and/or possibly tyrosine kinase (Fryer et al., 1999). The unraveling of these downstream pathways is important for an understanding of the powerful suppression of arrhythmias associated with ischemic and peroxynitrite-induced preconditioning.

In conclusion, administration of peroxynitrite produced preconditioning in isolated heart. This protective effect was preserved in the presence of sodium orthovanadate and was abolished by tyrosine kinase inhibitors, genistein or tyrphostin. These results suggest that the tyrosine kinase signaling pathway plays a significant role in peroxynitrite preconditioning in rat isolated heart.

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