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ORIGINAL CONTRIBUTION

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Postconditioning and intermittent bradykinin induced cardioprotection require cyclooxygenase activation and prostacyclin release during reperfusion

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Abstract Postconditioning (PostC), obtained with brief intermittent cycles of ischemia alternating with reperfusion applied after the ischemic event, has been shown to reduce infarct size. Recently, we have shown that PostC triggering includes B_2 receptor activation and its downstream pathway. Moreover, we showed that BK intermittent infusion induces a cardioprotection similar to PostC. The aim of this study was to investigate the involvement of cyclooxygenase-(COX)-derivated prostaglandins, such as prostacyclin (PGI_2) pathway in the cardioprotective action mediated by intermittent BK infusion.

Isolated rat hearts underwent 30 min ischemia and 120 min reperfusion. Myocardial damage was evaluated using nitro-blue-tetrazolium staining. The production of metabolite of PGI_2, 6-keto-PGF1α, was evaluated with EIA assay on the samples collected during reperfusion. The perfusion pressure and the left ventricular pressure were monitored. In Control hearts, the infarct size was 64% ± 4% of risk area. PostC reduced significantly the infarct size (28% ± 4% $P < 0.001$ Vs. Control). BK intermittent protocol to mimic PostC, attenuated infarct size (40% ± 2% $P < 0.01$ Vs. Control). The BK-intermittent and PostC protections were abolished with COX-inhibition. Intermittent BK and PostC enhanced the release of prostacyclin metabolite, 6-keto-PGF1α, in the late phase of reperfusion (i.e., 6-keto-PGF1α peaked 30 min after protective maneuvers). Also the stable PGI_2 analogue, Iloprost, given in the early reperfusion reduced infarct size and improved post-ischemic heart function. In conclusion, protection by PostC and intermittent BK requires COX activation and PGI_2 release during late reperfusion. These data suggest that COX must not be inhibited to have PostC protection. This finding should be kept present by future clinical studies on PostC.

Key words bradykinin B_2 receptors – ischemia/reperfusion – postconditioning – prostacyclin – rat

Introduction

It has been reported that Postconditioning (PostC), i.e., *repetitive cycles* of reperfusion and coronary occlusion following an ischemic insult, cause massive salvage of the myocardium similar to that of ischemic preconditioning (IP) [35, 36, 38]. Moreover, PostC involves signal transduction pathways

which are similar to those seen in IP [12, 16, 23–26, 31–40].

Virtually in all of the species in which different PostC protocols have been tested they have been protective, including humans [e.g., 4, 31, 32, 38], with the exception of recent works conducted on a rodent model [5]. In this respect, it is intriguing that PostC protection is present in wildtype mice [2, 13] and it is not lost in connexin 43-deficient mice [13]; yet an age-related loss of PostC protection in mice has been described, which seems to be related to a reduced level of signal transducer and activator of transcription 3 (STAT3) with increasing age [2]. Remote ischemic PostC has been also induced by cycles of few minutes of ischemia/reperfusion applied to a distal artery territory (femoral or renal artery) [15, 40]. Importantly, the protection by postconditioning represent a long-term protective effect and not a mere attenuation of event involved in early reperfusion injury [21]. Therefore, postconditioning has impressive clinical potential and to precisely clarify the involved pathway signaling is of paramount importance.

Recently, we have demonstrated that the intermittent infusion of bradykinin (BK) at the beginning of reperfusion can trigger PostC-like cardioprotection against infarct size via B_2 BK receptors activation [24].

Cyclooxygenase enzymes (COX) are constitutively expressed in most tissues, including myocardium [10, 11]. It is reported that B_2 BK receptors activation results in de novo synthesis of prostacyclin (PGI_2) [34], which has been demonstrated to attenuate ischemia–reperfusion injury [4]. In particular, Berti et al. [1] and Rossoni et al. [28] have reported that both indomethacin and aspirin aggravated ischemia-induced ventricular dysfunction in perfused rabbit hearts and this was associated with inhibition of PGI_2 production in the cardiac tissue. Yet, either administration of the stable PGI_2 analogue Iloprost [7] or prostaglandin E_1 [8] during ischemia have been seen to attenuate myocardial stunning in open chest dogs.

It seems that PGI_2 is produced by jeopardized myocardium [33] and that the rate of its formation increases particularly during the first 5–10 min of reperfusion declining thereafter [1, 6]. Endogenous prostaglandins are also involved in ischemic preconditioning [10].

Therefore, we hypothesized that intermittent BK may enhance PGI_2 release during reperfusion and that COX activation is involved in PostC.

To verify the role of COX pathway in the cardioprotection by PostC and intermittent BK, we studied the effect of COX inhibition during PostC maneuvers and during the intermittent infusion of BK. Moreover, we studied the effects of the administration of the stable PGI_2 analogue Iloprost during early reperfusion. Finally, we measured the release of 6-ketoPGF1α, a PGI_2 metabolite, which is used to evaluate the activation of B_2 BK receptor, during reperfusion.

Materials and methods

Male Wistar rats (n = 105; body weight 450–550 g) received humane care in compliance with Italian law (DL-116, 27 Jan. 1992) and 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).

Isolated heart perfusion

The methods were similar to those previously described [22–25]. In brief, each animal was anesthetized. The chest was opened 10 min after heparin treatment and the heart was rapidly excised. Isolated rat hearts were weighed, attached to the perfusion apparatus and retrogradely perfused with oxygenated Krebs–Henseleit buffer (127 mM NaCl, 17.7 mM $NaHCO_3$, 5.1 mM KCl, 1.5 mM $CaCl_2$, 1.26 mM $MgCl_2$, 11 mM D-glucose and gassed with 95% O_2 and 5% CO_2). A constant flow was adjusted with a proper pump to obtain a typical coronary perfusion pressure (PP) of 80–85 mmHg during the initial part of stabilization. Thereafter the same flow level (9 ± 1 ml/min/g) was maintained throughout the experiment.

A small hole in the left ventricular wall allowed drainage of the thebesian flow, and a polyvinyl-chloride balloon was placed into the left ventricle and connected to an electromanometer for the recording of left ventricular pressure (LVP). The balloon was filled with saline to achieve an end-diastolic LVP of 5 mmHg. Coronary perfusion pressure, coronary flow and LVP were monitored to asses the preparation conditions. LVP was used to calculate the positive (dP/dt_{max}) and negative (dP/dt_{min}) first derivative of pressure during systole and diastole, respectively.

The hearts were electrically paced at 280 bpm and kept in a temperature-controlled chamber (37°C).

All chemicals were purchased from Sigma (USA).

Experimental protocols (Fig. 1)

Each heart was allowed to stabilize for 20 min. After the stabilization period, hearts were subjected to a specific protocol, which included in all groups a 30 min of global no-flow ischemia. A period of 120-min reperfusion followed the 30 min ischemia in all groups (see below). Pacing was discontinued on initiation of ischemia and restarted after the third minute of reperfusion in all groups [16, 22–25].

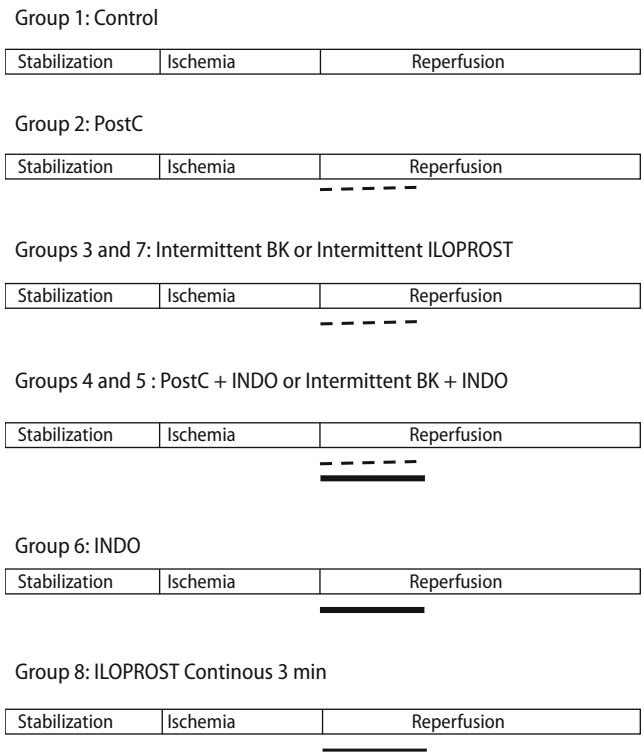

Fig. 1 Experimental design. The isolated, Langendorff-perfused hearts were stabilized for 20 min, and then subjected to 30 min of normothermic global ischemia followed by 120 min of reperfusion. *PostC* postconditioning; *BK* Bradykinin; *INDO* Indomethacin; *ILOPROST* analogous of prostacyclin. For further explanation see text

Experimental protocols are described in Fig. 1.

After stabilization the hearts of the *Control* group (Group 1, $n = 20$) were exposed to 30 min ischemia and then to 120 min reperfusion only.

In Group 2 (*PostC* group; $n = 20$) after the 30 min ischemia, the hearts immediately underwent a protocol of PostC. This consisted of five cycles of 10 s reperfusion and 10 s global ischemia [23–25].

In Group 3 the intermittent infusion of BK (*Intermittent BK + intermittent buffer*; $n = 15$) was performed after 30 min ischemia, this intermittent protocol consisted of five cycles of 10 s of oxygenated/non-oxygenated buffer with BK (100 nM) [24, 27].

To evaluate the role of COX pathway hearts were co-infused with an inhibitor of COX, *Indomethacin* (INDO, 10 μM) [27] during the initial 3 min of reperfusion, either during the PostC maneuvers (Group 4, $n = 10$) or during intermittent BK protocol (Group 5, $n = 10$). To determine the effects of the antagonist, in Group 6 (*INDO group*, $n = 10$) indomethacin was only infused during the initial 3 min of reperfusion. Moreover, in Group 7 Iloprost (12 nM), an analogous of PGI_2 [8, 10], was infused intermittently in lieu of BK (*Intermittent ILOPROST + intermittent buffer*; $n = 10$) after the 30 min ischemia, as in Group 3; in Group 8 Iloprost (12 nM) was infused in continuous (*Continuous ILOPROST*; $n = 10$) for 3 min immediately after the 30 min ischemia.

Assessment of myocardial injury

Infarct areas were assessed at the end of the experiment as previously described [22–25]. In brief, immediately after reperfusion each heart was rapidly removed from the perfusion apparatus and the left ventricle (LV) was dissected into 2–3 mm circumferential slices. Following 20 min of incubation at 37°C in 0.1% solution of nitro-blue tetrazolium in phosphate buffer, unstained necrotic tissue was carefully separated from stained viable tissue by an independent observer. The necrotic mass was expressed as a percentage of total left ventricular mass. In fact, though in this model the whole heart underwent normothermic ischemia, only the LV had a fixed volume and pre-load; therefore only the LV mass was considered as a risk area [22–25].

6-keto-PGF1α assay

In the hearts of Group 1, Group2 and Group 3, the PGI_2-releasing capacity of the isolated heart was measured by evaluating the concentration of its stable metabolite 6-keto-PGF1α in perfusate samples, collected before ischemia and after 3, 5, 10, 30, 60, 90 and 120 min of reperfusion. The amount of 6-keto-PGF1α released was determined with an enzyme-immunoassay kit (Amersham International plc, UK).

Statistical analysis

All data are expressed as means ± SEM One-way ANOVA and Newman–Keuls multiple comparison test (for post-ANOVA comparisons) have been used to compare infarct size. One-way ANOVA has been also used to compare area under the curve of 6-keto-PGF1α release. Two-way ANOVA (variables treatment groups and time point) and one-way ANOVA for multiple measure (post test Newman–Keuls multiple comparison test) have been used for the analysis of PP and LVP data. A P value <0.05 was considered statistically significant.

Results

Infarct size (Fig. 2)

Total infarct size, expressed as a percentage of left ventricular mass, was 64% ± 4% in Control Group 1. PostC (Group 2) significantly ($P < 0.001$) reduced

infarct size to 28% ± 4% of risk area. This high infarct size in Control group and the strong protective effect of PostC are features of the constant flow model of isolated rat heart, as suggested in our previous work [23].

In intermittent BK, Group 3, the infarct size was 40% ± 2%; a value similar to that obtained with PostC and significantly ($P < 0.01$) lower than that of Control Group 1. The cardioprotective effect of PostC and that of intermittent-BK was abolished during the co-infusion with the COX-inhibitor, INDO. In fact, the infarct areas of Group 4 (PostC + INDO, 65% ± 2%) and Group 5 (intermittent BK + INDO, 57% ± 6%) were similar to that of the Control Group 1.

The treatment with INDO alone did not significantly change infarct size, which was 57 ± 6, i.e., similar to that of Control Group 1, and significantly higher than that of intermittent BK ($P < 0.05$) and PostC ($P < 0.001$) groups (Fig. 2).

Iloprost reduced infarct size both when infused intermittently (Group 7, Infarct size 39 ± 6, $P < 0.01$ Vs. Control) and in continuous for three min (Group 8, infarct size 42 ± 7, $P < 0.05$ Vs. Control).

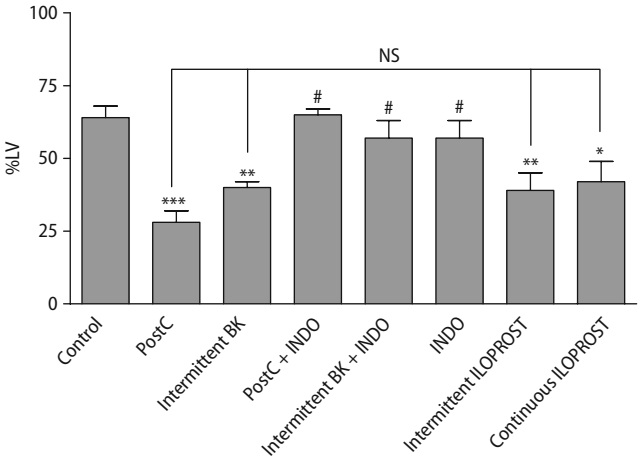

Fig. 2 Infarct size. The amount of necrotic tissue is expressed as percent of the left ventricle (*LV*), which is considered the risk area. *PostC* postconditioning; *BK* Bradykinin; *INDO* Indomethacin; *ILOPROST* analogous of prostacyclin. *NS* not significant; ***$P < 0.001$, **$P < 0.01$ and *$P < 0.05$ Vs. Control; #$P < 0.001$ Vs. PostC and NS Vs. Control. For further explanation see text

Perfusion pressure and left ventricular pressure (Table 1; Figs. 3, 4, 5)

Perfusion pressure is represented as percent variation with respect to baseline level (Fig. 3). Perfusion pressure increased in all groups during reperfusion. Two-way ANOVA revealed that there is not interaction between the considered variables (treatment groups and time point), whereas both treatment groups and time point affect the results ($P < 0.01$ for both). The post ANOVA comparison revealed that the only group that showed a significantly ($P < 0.01$) blunted increase in pressure (i.e., a reduced post-ischemic vasoconstriction) was the Intermittent Iloprost group (Group 7).

Systolic function is represented by develop LVP and dP/dt_{max}, which are reported as percent variation with respect to baseline level in Fig. 4. For both parameters the two-way ANOVA revealed that there is not interaction between the considered variables (treatment groups and time point), whereas both treatment groups and time point affect the results ($P < 0.01$ for both). In reperfusion, the left ventricular function was not statistically different among groups. The post ANOVA comparison revealed that the only group that performed significantly better than Control group ($P < 0.01$) in terms of dP/dt_{max} (Fig. 4B) was the Intermittent Iloprost group.

Diastolic function is represented by diastolic LVP and dP/dt_{min}, which are reported in Fig. 5 as mmHg and as percent variation with respect to baseline level, respectively. For both parameters the two-way ANOVA revealed that there is no interaction between the considered variables (treatment groups and time point), whereas both treatment groups and time point affect the results ($P < 0.01$ for both). The post ANOVA comparison revealed that in reperfusion there were groups that reached higher diastolic LVP (higher

Table 1 Baseline hemodynamic parameters

	PP (mmHg)	LVEDP (mmHg)	Dev LVP (mmHg)	dP/dt_{max} (mmHg/s)	dP/dt_{min} (mmHg/s)
Control (Group 1)	87 ± 4	4.8 ± 0.9	80 ± 3	1950 ± 60	−1,578 ± 130
PostC (Group 2)	82 ± 2	4.2 ± 0.7	78 ± 5	2021 ± 111	−1,788 ± 122
Intermittent BK (Group 3)	86 ± 3	4.0 ± 1.0	75 ± 4	2187 ± 121	−1,679 ± 120
PostC + INDO (Group 4)	89 ± 2	4.7 ± 0.7	70 ± 6	2078 ± 174	−1,580 ± 164
Intermittent BK + INDO (Group 5)	82 ± 2	4.2 ± 0.7	78 ± 5	2019 ± 120	−1,754 ± 144
INDO (Group 6)	86 ± 3	4.3 ± 0.4	75 ± 4	2188 ± 114	−1,689 ± 120
Intermittent ILOPROST (Group 7)	80 ± 2	5.0 ± 0.7	81 ± 3	1965 ± 89	−1,779 ± 94
ILOPROST 3 min (Group 8)	82 ± 2	4.5 ± 0.9	82 ± 3	1987 ± 93	−1,680 ± 85

PP perfusion pressure; *LVEDP* left ventricular end diastolic pressure; *Dev LVP* developed left ventricular pressure; dP/dt_{max} maximum rate of increase of LVP during systole; dP/dt_{min} maximum rate of decrease of LVP during diastole. Other acronyms as text

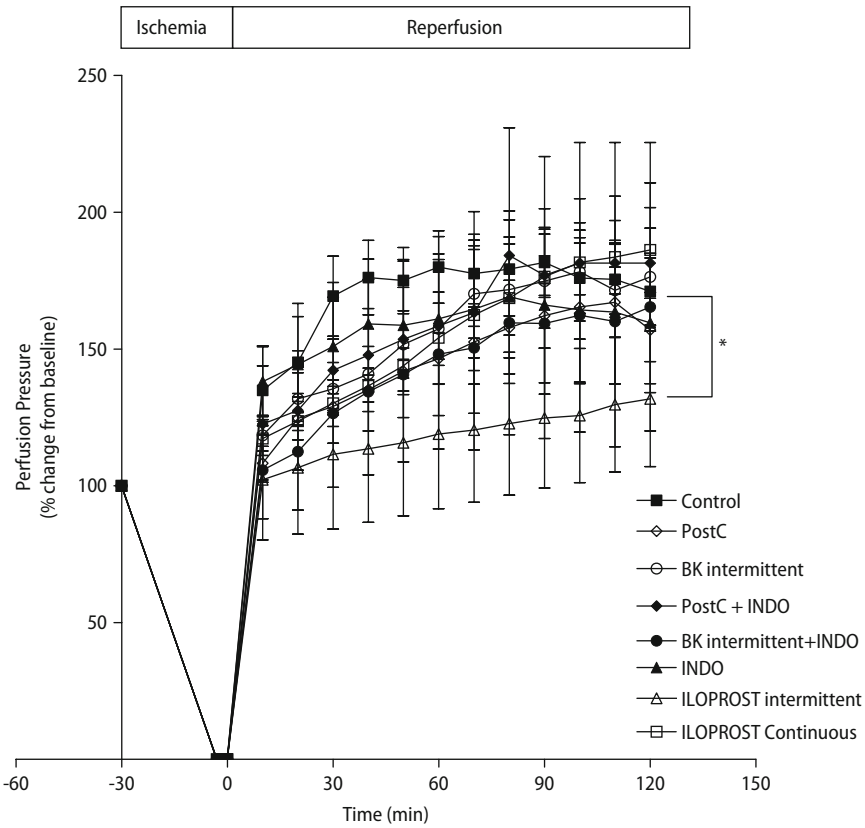

Fig. 3 Perfusion pressure. Percent variation of perfusion pressure with respect to baseline level for each group, during the 30 min ischemia and 120 min reperfusion. Time 0 correspond to the beginning of reperfusion. *PostC* postconditioning; *BK* Bradykinin; *INDO* Indomethacin; *ILOPROST* analogous of prostacyclin. *$P < 0.01$ between Control and Iloprost intermittent groups. For further explanation see text

contracture) and others that reached lower levels of diastolic LVP (less contracture) than Control group. Again the group that performed significantly better than Control group ($P < 0.01$) in terms of both diastolic LVP (Fig. 4A) and dP/dt_{min} (Fig. 4B) was the Intermittent Iloprost group, which showed less contracture and an increased lusitropism. Yet, the group that performed worst was the INDO group, both in terms of contracture (diastolic LVP) and relaxation (dP/dt_{min}).

6-keto PGF1α release during reperfusion (Fig. 6)

To easily compare the three groups in which 6-keto PGF1α release was measured, in Fig. 6 results are presented as normalized data. In the Control group, the release of 6-keto PGF1α slightly increased during the initial part of reperfusion, then started to decline after 30 min of reperfusion, reaching a value not different from baseline level at the end of reperfusion. On the contrary, in the Intermittent BK group the release of 6-keto PGF1α started to increase after BK-treatment, showing a sharp increase at 30 min reperfusion. Thereafter it slightly declined, and at the end of reperfusion was still higher than baseline. A similar trend was observed in PostC group. The analysis of the area under the curve revealed that the Control curve was significantly lower than that of Intermittent BK curve ($P < 0.05$), but not different from that of PostC curve. The areas under Intermittent BK curve and PostC curve were not statistically different.

Discussion

We have previously shown that cardioprotection triggered by postconditioning involves endogenous activation of B_2 receptors. Using intermittent infusion of BK in the early phase of reperfusion, we triggered cardioprotection akin to postconditioning. We demonstrated that B_2 receptors, nitric oxide, protein kinase G, mitochondrial K_{ATP} channels and reactive oxygen species are also integral to the protection triggered by intermittent BK [24].

In the present study our goals were (1) to show that cardioprotection triggered by postconditioning or intermittent BK involves activation of COX; (2) to show that intermittent BK trigger the release of PGI_2 and (3) to show that PGI_2 analogous can mimic postconditioning.

Here, we show that the cardioprotection induced by PostC or by intermittent BK infusion is dependent

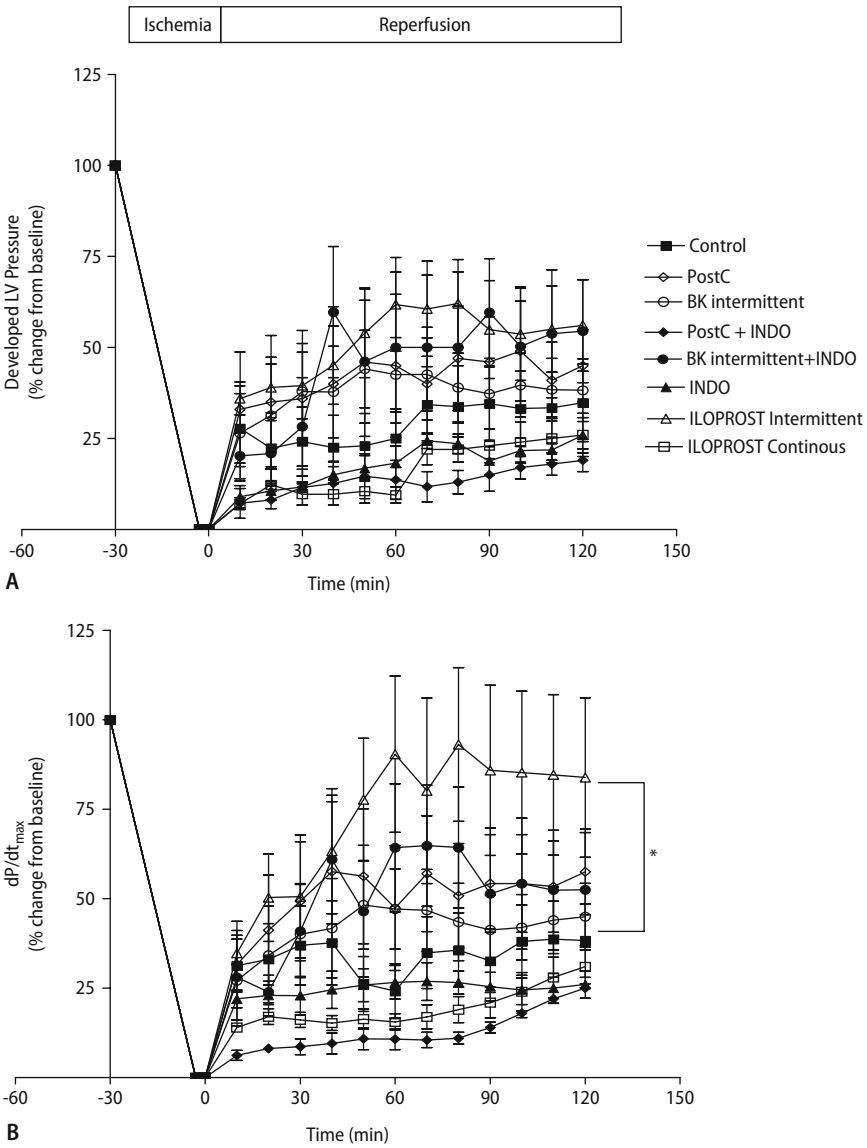

Fig. 4 Systolic function. **A** Percent variation of Developed left ventricular (*LV*) pressure with respect to baseline level for each group, during the 30 min ischemia and 120 min reperfusion. **B** Percent variation of first derivative of LV pressure during systole (dP/dt_{max}) with respect to baseline level for each group, during the 30 min ischemia and 120 min reperfusion Time 0 correspond to the beginning of reperfusion. *PostC* postconditioning; *BK* Bradykinin; *INDO* Indomethacin; *ILOPROST* analogous of prostacyclin. $^*P < 0.01$ between Control and Iloprost intermittent groups. For further explanation see text

on COX activation and augmentation of PGI_2 release in the late phase of reperfusion. Moreover, Iloprost, a stable PGI_2 analogous, given in early reperfusion induced a cardioprotective effect either when infused intermittently or when infused in continuous.

Regardless of the fact that protective stimuli (Intermittent BK or PostC) are applied only for few seconds at the beginning of reperfusion the endogenous release of PGI_2 metabolite is enhanced during the late period of reperfusion. It has been, previously, reported that during reperfusion in non-protected hearts the rate of PGI_2 formation increases particularly during the first 5–10 min of reperfusion declining rapidly thereafter [1, 6]. Here we confirm this finding. We suggest that the potentially protective effect of PGI_2 release cannot operate in naive ischemia/reperfusion because the enhanced production during the first phase of reperfusion rapidly decline in the second phase of reperfusion, when reperfusion injury occurs. On the contrary the PostC maneuvers and intermittent BK triggers the above reported protective cascade [24], and may allow COX activity and PGI_2 release in the late phase of reperfusion, thus preventing reperfusion injury. In other words, we suggest that PostC or Intermittent BK triggers protection, which allows COX activity and subsequent PGI_2 formation.

It is worth of note that the protective effect of Intermittent BK on infarct size seems to be less than that produced by PostC; yet the Intermittent BK seems to increase prostacyclin to a greater level than PostC. Although these differences are not statistically

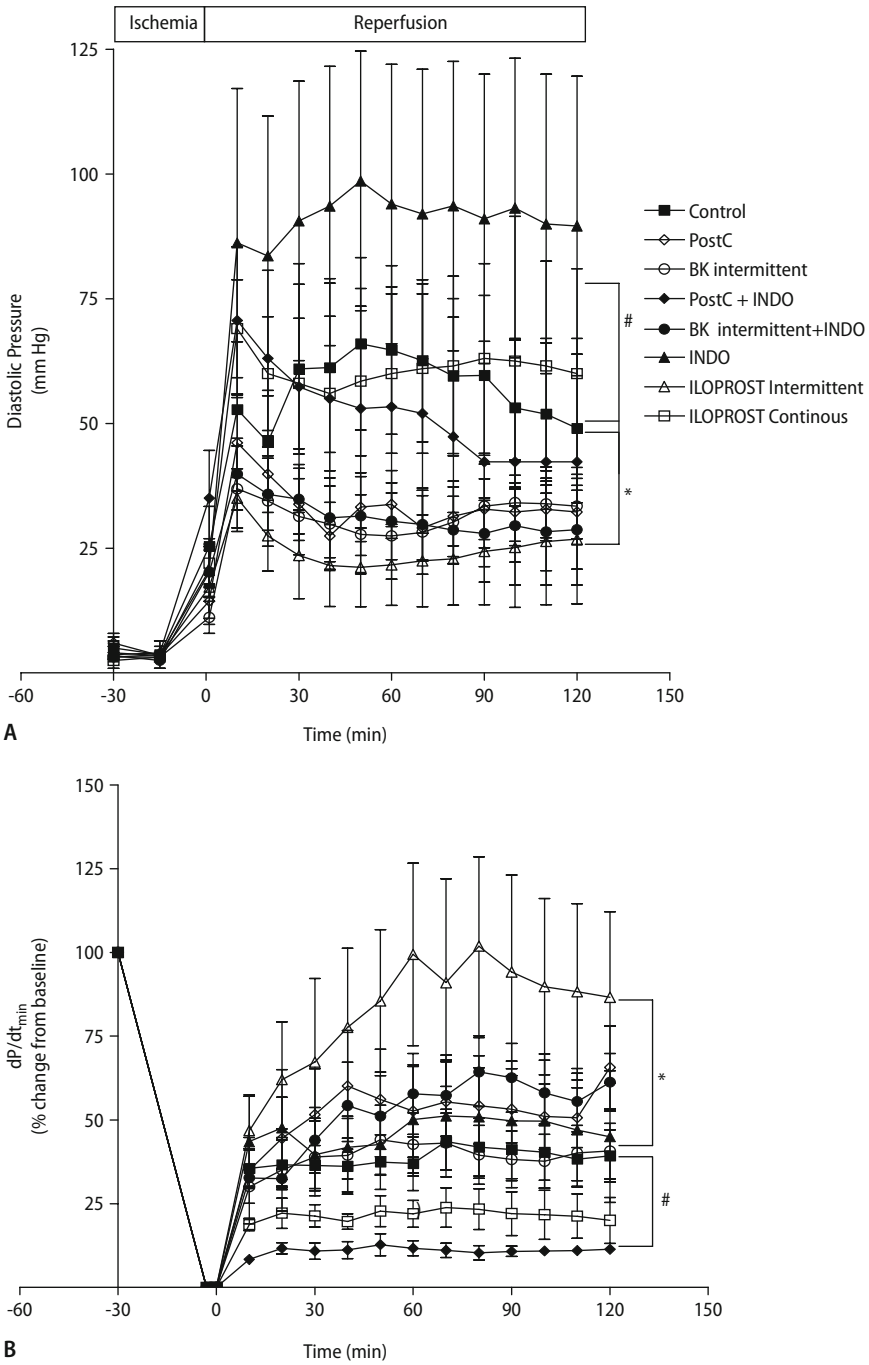

Fig. 5 Diastolic function. **A** LV diastolic (mmHg) during the 30 min ischemia and 120 min reperfusion. **B** Percent variation of first derivative of LV pressure during diastole (dP/dt_{min}) with respect to baseline level for each group, during the 30 min ischemia and 120 min reperfusion Time 0 correspond to the beginning of reperfusion. *PostC* postconditioning; *BK* Bradykinin; *INDO* Indomethacin; *ILOPROST* analogous of prostacyclin. $*P < 0.01$ between Control and Iloprost intermittent groups; $\#P < 0.01$ between Control and INDO groups. For further explanation see text

significant, these data are in line with the fact that other factors (e.g., adenosine and opioids), other than BK and COX, are involved in the PostC-induced protection [e.g., 12, 26, 35, 39].

Moreover, Intermittent BK and Iloprost (intermittently and in continuous) achieved similar degree of protection in terms of infarct size reduction. Once again suggesting that other factors, other than BK and prostaglandin, are involved in the PostC-induced protection.

The fact that Iloprost may induce protection in reperfusion is in agreement with previous studies which have shown a protective role of prostaglandins in reperfusion [7, 8, 10]. With respect to this, it is intriguing that the involvement of COX has been very recently demonstrated in the additive effect of late preconditioning and PostC protection [29]. However, the fact that Iloprost is able to induce protection when infused intermittently and in continuous in the early reperfusion, whereas the endogenous prostaglandin

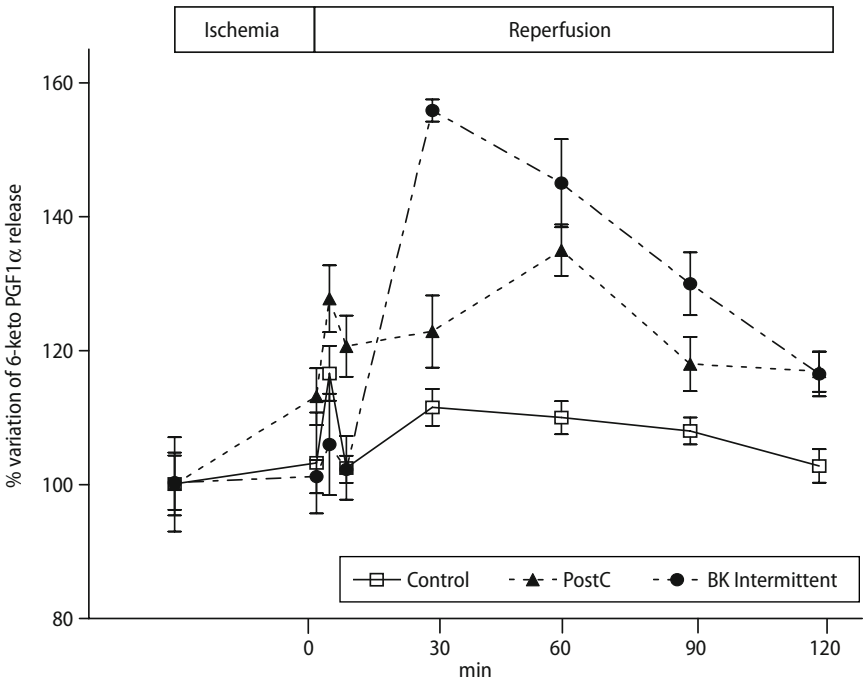

Fig. 6 6-keto PGF1α release. The PGI2-releasing capacity of the isolated heart is estimated by the percent variation of the concentration of its stable metabolite 6-keto-PGF1α in perfusate samples during baseline and reperfusion. Each value is represented as percent with respect to the mean baseline data of each group. Time 0 correspond to the beginning of reperfusion. *PostC* postconditioning; *BK* Bradykinin. For further explanation see text

release is enhanced in the late phase of reperfusion by PostC, suggests different mechanisms of protection by endogenous prostaglandin(s) release triggered by PostC and infusion of exogenous prostaglandin analogue. Differences might be due to molecule stability differences and/or pharmacodynamic features; further studies may clarify the reasons of these differences.

The more robust end-point in analyzing I/R injury is infarct size [9, 20]. Therefore, we focused our attention on myocardial damages evaluated by infarct size. As for preconditioning [9, 20], systolic function (developed LV pressure and dP/dt_{max}) may not be an appropriate end-point to study PostC protection. The improvement of function during reperfusion in preconditioned rat hearts has been attributed to the reduction of adenosine release during this phase [9]. On the contrary, in post-conditioned hearts an accumulation of adenosine has been reported [16]. These differences may explain our findings of an unclear effect on systolic function by PostC treatment. Yet, it is hard to distinguish the impairment of function due to necrosis and/or to stunning of viable tissue. In order to understand the role of PostC on myocardial stunning appropriate studies are required. Moreover, it has been suggested that in rodent models contracture development, rather than systolic function, may be a more appropriate end-point to understand the protective effects of cardioprotective maneuvers [9]. In the present study the only conditions that clearly improves post-ischemic heart performance is the treatment with Intermittent Iloprost, which also improved contracture. On the other hand, the only treatment that clearly worsens diastolic heart performance is Indomethacin treatment. On the base of these effects on contracture, but keeping in mind the model limits above reported, we can argue that COX is involved not only in reducing infarct size, but also in improving heart function recovery of viable tissue. This is also in line with the reduced post-ischemic vasoconstriction (less increase in PP) observed in the present study. Moreover, the beneficial effect by Iloprost is in agreement with the involvement of endogenous prostaglandins in ischemic preconditioning [10], with the anti-stunning effect by Iloprost observed in a canine model of ischemia/reperfusion [8] and with the involvement of COX in late preconditioning, which clearly improves post-ischemic stunning [29].

Differently from previous studies [8, 10, 17, 19, 26, 30, 37], which used BK or other agents in continuous for a long period during reperfusion, we were able to reproduce PostC by means of few seconds (50 s in total) treatment with BK only when given in an intermittent manner in the very early period of reperfusion [24] and we confirm that late PGI_2 release is mandatory to limit reperfusion injury by PostC.

Another novelty of the present study is that the inhibitor, indomethacin, has been given for 3 min only to prevent the triggering of protection by PostC or intermittent BK, confirming that the initial phase of reperfusion is of paramount importance for trig-

gering cardioprotective pathways. Also the stable PGI_2 analogue, iloprost, is able to induce protection when applied for few minutes at the beginning of reperfusion.

Kinins are produced during ischemia reperfusion by the action of a kininogenase activated by the acidosis. Recently, PostC has been seen to induce cardioprotection by maintaining acidosis during the first minutes of reperfusion as reoxygenated myocardium produces reactive oxygen species that activate protective signaling [3]. Bradykinin was believed to be the only kinin acting on the B_2 receptors in rats; however, it has been demonstrated that isolated rat hearts also releases Arg-kallidin, a kallidin-like peptide, which acts on B_2 receptors too [14, 18, 19]. Importantly, Arg-kallidin release increases during ischemia and mediates IP and preconditioning-like protection, via B_2 receptor activation, in isolated rat hearts [18, 19]. Whether the endogenous mediator of PostC protection is BK or Arg-kallidin was beyond the aims of the present study.

In conclusion, the major new findings in this study are: (1) postconditioning triggered cardioprotection involves endogenous activation of COX and release of PGI_2; (2) intermittent BK infusion also triggers the COX activation and induces PGI_2 formation in the late phase of reperfusion; (3) exogenous infusion of the stable PGI_2 analogue, Iloprost, is able to induce cardioprotection when infused in the early reperfusion either continuously or intermittently.

We suggest that protection exerted by postconditioning and intermittent BK is not solely dependent on the activation of the so called RISK (reperfusion injury salvage kinase [12, 24, 25, 38], but it also depends on the late release of PGI_2. That is, during PostC maneuvers the heart may release kinins that accumulate in an intermittent manner and triggers a protective pathway that lead to a protected state, which include COX activation and PGI_2 release.

Clinical implication

One goal in applying reperfusion therapeutic strategies is to pharmacologically mimic postconditioning. It is likely that one drug or maneuver cannot target all of the mechanisms of reperfusion injury, but a combined therapy integrating pharmacological agents (e.g., BK and Iloprost) and maneuvers, such as intermittence of infusion, in the very early phase of reperfusion may provide a broader spectrum approach to attenuate reperfusion injury. From a clinical point of view, it is important that ischemia is not necessary, whereas intermittence of drug infusion is able to trigger PostC. Equally important, COX must not be inhibited to obtain cardioprotection by ischemic postconditioning during reperfusion.

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