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Effects of a Protocol of Ischemic Postconditioning and/or Captopril in Hearts of Normotensive and Hypertensive Rats

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Abstract

Brief periods (a few seconds) of cyclic coronary occlusions applied early in reperfusion induce a cardioprotection against infarct size, called postconditioning (PostC) in which B_2-bradykinin receptors play a pivotal role. Since angiotensin-converting enzyme (ACE) inhibitors reduce degradation of kinins, we studied the effects of PostC on infarct size and postischemic myocardial dysfunction in both normotensive (WKY) and spontaneously hypertensive rats (SHR) acutely or chronically treated with the ACE inhibitor Captopril.

Isolated hearts from SHR and WKY rats were subjected to the following protocols: *i*) ischemia for 30-min and 120-min reperfusion (I/R); *ii*) I/R+PostC protocol (5-cycles 10-s I/R); *iii*) pretreatment with Captopril for 4-weeks before to subject the hearts to I/R with or without PostC maneuvers. Some SHR hearts were treated with Captopril during the 20- or 40-min early reperfusion with or without PostC maneuvers. Cardiac function was assessed *in vivo* with echocardiography. Left ventricular pressure and infarct size were measured *ex vivo*.

Chronic Captopril significantly reduced left ventricular hypertrophy in SHR, and reduced infarct size in both WKY and SHR hearts. PostC maneuvers significantly reduced infarct size in WKY, but not in SHR hearts. Yet, PostC slightly improved postischemic systolic function in untreated SHR. Captopril given in reperfusion was unable to limit I/R injury in SHR hearts.

Data show that PostC protection against infarct size is blunted in SHR and that PostC is unable to add its protective effect to those of chronic Captopril, which *per se* reduces cardiac hypertrophy and heart susceptibility to I/R insult.

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Introduction

It is well known that ischemic preconditioning (IP) limits the severity of ischemia/reperfusion (I/R) damages; however the need of pretreatment limits its clinical usefulness [8,10,11,17,25,41,44,45,53,62]. In the last two decades researchers intensively investigated whether or not pharmacologically and/or mechanically modified reperfusion could reduce reperfusion injury [3,16-20,22,37-43,46-48,54-57,65-67]. In 2003 Vinten-Johansen's group [66] was able to limit infarct size with stuttering reperfusion immediately after an infarcting ischemia, that is ischemic postconditioning (PostC).

Although pre and postconditioning have been shown to protect the myocardium from lethal I/R injury, numerous experimental studies revealed that the cardioprotective effects of preconditioning have been altered in the presence of some pathological factors such as diabetes, hypercholesterolemia, hyperglycemia, hypertension, obesity, etc. Very few studies have investigated the modulator role of various pathological conditions in postconditioning-mediated myocardial protection [for reviews see 4,11,16-18,39,41]. In their very recent review Granfeldt et al. [16] summarize the available data on "PostC in models of comorbidities", which do not include studies in the presence of hypertension and/or cardiac hypertrophy.

The need to conduct studies in models with comorbidity is now recognized by all the authors who study cardioprotection [4,16,29]. Actually, little is known about the effects of PostC on the infarct size and recovery of post-ischemic function in hypertensive animals. To the best of our knowledge the recent work by Fantinelli & Mosca [10] is the only study on PostC and hypertension. The authors report that PostC was as effective as IP in improving the post-ischemic dysfunction of hearts isolated from spontaneously hypertensive rats (SHR). In such a study SHR and normotensive Wistar Kyoto (WKY) rats were subjected to 20 min global ischemia, but the effects on infarct size were not determined. However, there is the need to consider myocardial

protection as a whole, including not only the limitation of mechanical recovery during reperfusion, but also the extent of infarct size and myocardial contracture [48,54].

Both cardioprotection by IP and PostC can be triggered by several autacoids, including bradykinin (BK) [5,11,18,32,33,39-42,51,57,60-62]. The role of BK and others kinins in IP has been extensively studied. We have shown a role for endogenous BK in PostC via B_2-BK receptor activation [40,43]. A role for these receptors in PostC has been confirmed in other models including a knockout mice model [41,59].

Angiotensin-converting enzyme (ACE) inhibitors are widely used as antihypertensive drugs and have been shown to prolong the survival of patients after cardiac infarction [e.g. 13,35,49,50,52,63]. Besides to reduce angiotensin II formation, ACE inhibitors (ACE-I) reduce the degradation of kinins thus prolonging their activities [14,35,58]. This prolonged activity on B_2 receptors renders this treatment particularly interesting in this context, as interfering with B_2 receptors [2,11,14,35 and references therein] they might alter PostC response. Yet ACE-I has been shown to reduce cardiac hypertrophy and to be cardioprotective *per se* [11,35,49,50,58]; however, the interaction between ACE-I and endogenous cardioprotection by PostC is unknown [11].

We hypothesized that the ACE-I Captoril, increasing kinin levels and reducing left ventricular hypertrophy (LVH), may differently influence the cardioprotective effect of PostC depending on whether they are given in an acute or chronic regime in hypertensive or normotensive conditions.

Therefore the objectives of the present study are threefold: *1)* to compare the effects of a protocol of PostC against infarct size in SHR and WKY rats; *2)* to determine whether or not chronic treatment with an ACE-I (Captopril) can reduce LVH and infarct size in SHR; and *3)* to

determine whether acute or chronic treatment with Captopril can influence the effects of PostC in hypertensive rat hearts.

Material methods

Animals

The experiments were conducted in accordance with the Italian law and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised in 1978 and 1996).

Experiments were conducted in age-matched SHR (n=44) and WKY male rats (n=22), which were originally derived from Charles River Breeding Farms, Wilmington, Mass (USA). All animals were identically housed under controlled lighting and temperature conditions with free access to standard rat chow and tap water for 4 weeks. A subgroup of WKY (n= 12) and of SHR (n = 14) received 300 mg/l of Captopril, an ACE-I, in the drink water [1] (see below "*Chronic treatment with Captopril"*).

Experimental procedures

Isolated heart perfusion: the methods were similar to those previously described [38,40,42-48]. In brief, each animal was anesthetized. The chest was opened 10 min after heparin treatment and the left ventricular wall was rapidly pierced with a needle connected to an electromanometer to measure left ventricular pressure (LVP) *in vivo*. The used needle made a hole in the heart wall, which allowed drainage of the thebesian flow throughout the experiments. After measuring for few seconds LVP, the heart was excised, ice-weighed and 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 (9 ml/min/g) was imposed with a proper constant-flow pump [38,40,42-48]. A polyvinyl-chloride balloon was placed into the left ventricle and connected to an

electromanometer for recording of left ventricular pressure (LVP). The balloon was filled with saline to achieve an end-diastolic LVP of 5 mmHg. Coronary perfusion pressure (CPP), coronary flow and LVP were monitored to asses the preparation conditions. The hearts were electrically paced at 280 bpm and kept in to a temperature-controlled chamber (37°C). This heart rate is adequate for 6 month-old rats [38,40,42-48].

Experimental protocol

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 30 min of global no-flow ischemia. A period of 120 min of reperfusion followed the 30 min ischemia in all groups (see below). Ischemia and reperfusion were obtained just stopping and restarting the perfusion pump. Pacing was discontinued on initiation of ischemia and restarted after the third minute of reperfusion in all groups [23,38,40,42-48].

Control Experiments (Groups 1-4, Fig 1A)

After stabilization, the hearts of WKY rats (WKY_I/R; Group 1, n=5) and SHR hearts (SHR_I/R, Group 2, n=5) were exposed to 30 min ischemia and then to 120 min reperfusion only. In Group 3 (WKY_PostC; n=5) and Group 4 (SHR_PostC; n=5) the hearts underwent PostC protocol; this consisted of five cycles of 10 s reperfusion and 10 s global ischemia at the beginning of 120 min reperfusion [23,38,40,42-48,67].

Chronic treatment with Captopril (Groups 5-8, Fig 1B)

As said, to test the effects of chronic treatment with ACE-I, animals (5 month-old) of both strains (14 SHR and 12 WKY rats) were treated with Captopril (300 mg/l) in the drinking water for 4 weeks [1]. Two days after the end of treatment the hearts of these animals were exposed to protocols as those of the Control animals. In particular, hearts of Group 5 (WKY_Captopril/pre+I/R, n= 6) and hearts of Group 6 (SHR_Captopril/pre+I/R, n=7) underwent

30 min ischemia and then to 120 min reperfusion only; in Group 7 (WKY_Captopril/pre+PostC, n= 6) and Group 8 (SHR_Captopril/pre+PostC, n= 7) the hearts, immediately after the 30 min ischemia, underwent a protocol of PostC.

Acute treatment with Captopril (Groups 9-12, Fig 1C)

Since 20 min Captopril enhanced sub-threshold cardioprotective effects of preconditioning [8,30], we also tested the activity of ACE-I in reperfusion in hearts from SHR only: Captopril (200 μM) was infused for 20 or 40 min during early reperfusion, i.e. immediately after the 30 min ischemia, with or without the PostC maneuvers. In particular, the 20 min Captopril infusion was performed at the beginning of the 120 min reperfusion in Group 9 (I/R+20Captopril, n=5) and Group 10 (I/R+20Captopril+PostC, n= 5); the 40 min Captopril infusion was also performed at the beginning of the 120 min reperfusion, with or without PostC, in Group 11 (I/R+40Captopril, n=5) and Group 12 (I/R+40Captopril+PostC, n=5), respectively.

Assessment of myocardial injury

At the end of the experiment, i.e., directly after 120 min reperfusion, each heart was rapidly removed from the perfusion apparatus and the left ventricle (LV) was dissected into 2–3 mm circumferential slices. Following 15 min of incubation at 37°C in 0.1% solution of nitro blue tetrazolium in phosphate buffer [34,38,40,42-48], unstained necrotic tissue was carefully separated from stained viable tissue by an independent observer who was not aware of the nature of the intervention. The weights of the necrotic and non-necrotic tissues were then determined, and the necrotic mass was expressed as a percentage of total left ventricular mass.

Assessment of ventricular function ex vivo

The volume of the intraventricular balloon was adjusted to obtain a left ventricular end-diastolic pressure (LVEDP) of 5 mmHg during the stabilization period [34,38,40,42-48]. Changes in LVEDP, developed LVP (dLVP), and dP/dt values induced by various protocols were

continuously monitored. The difference between LVEDP before the end of the ischemic period or reperfusion and during preischemic conditions was used as an index of the extent of contracture development. During I/R contracture development was defined as an increase in intrachamber pressure of 4 mmHg above preischemic LVEDP values [34]. Maximal recovery of dLVP during reperfusion was compared with respective preischemic values.

Echocardiographic measurement in vivo

To determine the effect of chronic treatment with the Captopril [1], we measured the diastolic and systolic chamber sizes and increases in LV systolic wall stress in a subgroup of anesthetized [15,31,36] animals of both groups (SHR, n= 10 and WKY, n=8). The measures were obtained in a blinded fashion at time 0 and after 4 weeks using a two-dimensional-targeted M-mode echocardiography (*Esaote Medica, Mod Megas, Genova, Italy)* and a 10-*MHz transducer (Esaote Medica, Italy)*. The parameters measured were end-diastolic internal diameter, LV end-systolic internal diameter, and posterior wall thickness in diastole (DT) and systole. LV shortening fraction (SF) and ejection fraction (EF) were calculated as previously described [31] for an assessment of *global* systolic chamber function. The percent thickening of the LV posterior wall (PWT%) from diastole to systole [24] was used as an index of *regional* systolic myocardial function [15,31,36].

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. Functional data (Figs 3-5) were compared with repeated measures ANOVA (RMAOVA, time/group)[27]. A *t* test with Bonferroni correction was also used to compare the last-time points of functional data (Figs 2-5)[27]. A P value <0.05 was considered statistically significant.

Results

Animal characteristics (in vivo) and baseline functional parameters of isolated (ex vivo) rat hearts (Table 1).

As can be seen in Table 1, six-month-old SHR are leaner than age-matched WKY animals. As expected untreated SHR had greater heart hypertrophy indices (heart weight/body weight and LV/body weight ratios) and showed higher *in vivo* dLVP than WKY. Four weeks Captopril treatment reduced dLVP and avoided cardiac hypertrophy in SHR. This is also in agreement with echocardiographic data reported in Table 2 and Fig. 3 (see below).

In isolated (*ex vivo*) hearts, the imposed coronary flow of 9 ml/min/g achieved a significantly higher CPP (105±8 mmHg) in the hearts of untreated SHR than in those of Captopril pretreated SHR (85±8 mmHg) and those of WKY rats (untreated: 65±7 mmHg, and Captopril treated: 58±2 mmHg, p= NS *vs* each other). These data suggest that SHR had higher vascular resistances, which were reduced by Captopril pretreatment.

Diastolic LVP was similar in SHR and WKY regardless of ACE-I due to imposed ventricular volume. However, hearts of untreated SHR showed a higher baseline dLVP, which was significantly ($p<0.05$) reduced in Captopril pretreated SHR from 84±4 *to* 62±6 mmHg. Also maximum rate of decrease (dP/dt_{min}) and increase (dP/dt_{max}) of LVP resulted slightly, but not significantly, higher in hearts of untreated SHR than in those of WKY and those of Captopril pretreated SHR. All these *ex vivo* functional values observed in six month old animals are similar to those reported by Ebrahim et al. [8] for hearts of 7-8 month old WKY and SHR.

Echocardiography parameters (Table 2, Figure 2)

In table 2 we report the echocardiographic parameters measured in anesthetized SHR and WKY rats in basal conditions (time 0) both in the Captopril treated and untreated animals.

As can be seen in Fig. 2 A, during the 4 weeks of observation in both Captopril treated and untreated WKY diastolic thickness increased by about 25% ($p<0.05$ with respect to basal condition). While in untreated SHR diastolic thickness increased by 62±2% during the period of observation ($p< 0.001$), in SHR treated with Captopril the diastolic thickness increased similarly to WKY, i.e. by 28±7% only ($p<0.01$ with respect untreated SHR).

Also regional and global systolic functional parameters (i.e. PWT%, EF and SF) increased during the 4 weeks in all animals ($p< 0.05$ for all), with the exception of PWT% in Captopril treated WKY. Importantly, Captopril treatment attenuated the increase of these parameters in SHR which otherwise showed greater increase than untreated animals (Fig. 2).

Control Experiments (Groups 1-4, Figure 3)

Infarct size

Total infarct size, expressed as a percentage of left ventricular mass, was 47±6% and 70±11% in WKY_I/R (Group 1) and SHR_I/R (Group 2) respectively ($p<0.05$).

The PostC maneuvers reduced significantly the infarct size in the WKY, but not in SHR. In particular, in WKY_PostC (Group 3) infarct size was 31±7%, i.e. -30% of WKY_I/R ($p<0.05$). In SHR_PostC (Group 4) infarct size was 56±7%, i.e. there was a *non-consistent* reduction of infarct size that did not reach the statistical significance (Fig. 3, panel A).

Cardiac parameters

Baseline values of the considered parameters are reported in Table 1.

The percent variations in *CPP* are reported in Fig. 3, panel B. All groups showed a marked increase in CPP ($p< 0.001$ *vs* baseline). In particular in SHR the percent CPP increase was slightly higher ($p<0.05$) than WKY. This increase was not influenced by PostC in both strains.

Diastolic function is represented by the level of LVEDP during ischemia and reperfusion (Fig. 3, panel C). A striking difference was observed between WKY and SHR in terms of

contracture development during reperfusion with a significantly higher level for SHR hearts. Moreover, a contracture limitation by PostC was observed in the hearts of the two strains ($p< 0.05$ for both, RMAOVA). Yet, the last time points were not statistically different between I/R and PostC in both strains.

Systolic function is represented by percent variation with respect to baseline level of dLVP (Fig. 3, panel D). At the end of reperfusion the recovery of developed LVP was 48±10% of baseline level in WKY_I/R. The hearts of the SHR_I/R present a marked limitation of dLVP recovery; in fact at the end of reperfusion dLVP was 14±3% of baseline level ($p<0.001$ with respect to WKY). PostC significantly improved the dLVP recovery in both WKY and SHR (RMAOVA). In fact at the end of reperfusion the recovery were 64±12% and 31±9% of baseline levels in WKY_PostC and SHR_PostC, respectively ($p= <0.05$ with respect to each corresponding I/R group) (Fig 3, panel D). This is in line with the data of Fantinelli & Mosca [10].

Chronic treatment with Captopril (Groups 5-8, Figure 4)

Infarct size

Pre-treatment with Captopril markedly and significantly reduced the damage induced by I/R in both WKY and SHR ($p< 0.01$ *vs* control untreated I/R for both strains). However, PostC was ineffective in further reducing infarct size in both Captopril pretreated strains. In fact, infarct size was 27±8% and 22±5% in WKY_Captopril/pre+I/R and WKY_Captopril/pre+PostC, respectively. Yet, infarct size was 52±9% and 67±6% in SHR_Captopril+I/R and SHR_Captopril+PostC, respectively. It is intriguing to note that PostC actually enhanced, albeit not significantly, the infarct size in Captopril pretreated SHR.

Cardiac parameters

Baseline values of the considered parameters are reported in Table 1. The percent variations in CPP are reported in Fig 4, panel B. Captopril pretreated SHR showed a marked increase in CPP, which was significantly ($p<0.05$) hastened by PostC. In Captopril pretreated WKY the postischemic CPP increase was minimal (P= NS *vs* baseline) both in I/R and PostC. Thus both RMAOVA and last-time points analysis revealed a significant difference between SHR and WKY hearts. Notably, the CPP values of treated and untreated WKY hearts were also significantly different (compare Fig 4B and Fig 3 B).

Diastolic function: Also in this case some differences were observed between WKY and SHR in terms of contracture development during reperfusion (Fig 4, panel C). In particular, in the absence of PostC, LVEDP revealed an initial sharp increase in SHR. Yet, at the end of reperfusion there were no differences between SHR_Captopril+I/R and WKY_Captopril+I/R. PostC did not affect the already low contracture level in the hearts of WKY, but enhanced it in SHR. In fact, at the end of reperfusion in SHR_Captopril+I/R and SHR_Captopril+PostC the LVEDP levels were 37±10 and 63±12mmHg, respectively. Only the SHR_Captopril+PostC contracture was higher than that of WKY (RMAOVA).

Systolic function is represented in Fig 4 panel D. Compared to untreated, the hearts of Captopril pretreated animals show a better recovery of dLVP during reperfusion in both strains (RMAOVA, compare Figs 3D and 4D). This improvement is in line with the limited infarct size after ACE-I pretreatment. Paradoxically, PostC reduced the dLVP recovery in WKY ($p< 0.05$). Yet in SHR+Captopril PostC had non-significant effect on dLVP. This apparent paradox supports a dichotomy between the effects on stunning and on necrosis by PostC [48].

Acute treatment with Captopril in SHR hearts (Groups 9-12, Figure 5)

In Fig. 5 for comparative purpose are also reported data of Groups 2 and 4 (Control I/R and PostC in SHR).

Infarct size

The treatment with Captopril in reperfusion was not able to reduce the I/R injury both in the presence and in the absence of PostC maneuvers. In fact, in I/R+20Captopril and I/R+40Captopril infarct sizes were similar being 65±1% and 70±7%, respectively. In the PostC Groups (I/R+20Captopril+PostC and I/R+40 Captopril+PostC) infarct sizes were also similar (61±10% and 56±15%, respectively).

Cardiac parameters

Baseline values of the considered parameters are reported in Table 1. The percent variations in CPP are reported in Fig 5, panel B. All groups showed a marked increase in CPP, which was not influenced by acute Captopril. Yet, in both groups treated with acute ACE-I a significant reduction of CPP is induced by PostC maneuvers (RMAOVA).

Diastolic function is represented in Fig. 5, panel C. In these SHR hearts Captopril infusion and PostC do not significantly influence the marked contracture development in reperfusion.

Systolic function is represented by dLVP in Fig 5 panel D. Due to the high infarct size, dLVP recovery is markedly impaired in all Groups. Yet, PostC slightly, but not significantly improved dLVP recovery in I/R+20 Captopril+PostC group. As said, only in untreated SHR PostC improved slightly, but significantly ($p<0.05$), the dLVP recovery.

Discussion

We have shown here that infarcts were larger in SHR than WKY hearts subjected to ischemia/reperfusion and that a postconditioning protocol which is able to induce cardioprotection against infarct size in normotensive WKY is not protective in SHR hearts. Moreover, the ischemic PostC protocol does not add protective effects to the protection provided by chronic

Captopril treatment. In SHR also the simultaneous treatment with acute Captopril and PostC does not trigger cardioprotection.

Limitation of the study

Although, we cannot rule out that ischemic PostC protocol exists also for hypertensive animals, our study suggests that a PostC protocol that is ideal for hearts of normotensive animals is not working in hypertensive animals before and after treatment with Captopril. In experimental animal studies a number of factors (i.e. species, age, gender, temperature and duration of index ischemia) contribute to the outcome obtained with a postconditioning algorithm. The outcome may range from beneficial to null or deleterious effects [4,39,54 and references there in]. Here, we used a single PostC protocol which was effective in hearts from normotensive rats, but did not check whether or not this stimulus was submaximal in hypertensive rats. However, it is not easy to ascertain whether or not increasing or reducing the numbers and/or the duration of postconditioning I/R cycles would be protective [22,54]. Actually, reducing the “additive ischemia” (cumulative coronary re-occlusions during PostC) from 2 to 1% of index ischemia in aging mice fully reestablished the protection [3,4]. Yet, in porcine hearts an increase in “additive ischemia” was effective [39,54].

Although concentrations of Captopril similar to those we used has been already used in previous studies [1,8] a limitation of our study is the use of single doses of Captopril in both acute and chronic experiments. However, ACE inhibitors are not clean drugs which may have side effects. For instance SH-groups containing ACE-I, including Captopril, scavenge non-superoxide reactive oxygen species [9], enalapril interferes with ADMA [7], other ACE-I (ramiprilat and perindoprilat) have been shown to have *outside-in signaling* increasing casein kinase 2 (CK2), c-Jun N-terminal (JNK) and MAP kinase kinase 7 (MKK7) activity [14,24]. All these side effects may interfere with the cardioprotection, especially increasing the drug concentration; for that

reason in acute experiments we tested the same concentration of Captopril in a longer period of time. Further studies assessing kinin levels are necessary to investigate the adequate use of ACE-I, especially in the case of acute myocardial ischemia. Nevertheless, the reasons for the infarct size reduction by chronic Captopril, the lack of protective effect of PostC and/or acute Captopril in SHR hearts remain speculative since the scope of mechanistic insight in the present study is limited.

Implication of the findings

We confirm that the *chronic* application of an ACE inhibitor (Captopril) markedly reduces LVH progression and infarct size in SHR hearts. Yet appreciable reductions in terms of infarct size and post-ischemic vascular resistances are observed in hearts of chronically Captopril pretreated WKY. In both WKY and SHR, ACE-I pretreatment induces a post-ischemic improvement of systolic function, but interferes with PostC protection. In fact, after chronic Captopril treatment infarct size is either not further reduced in WKY or even slightly (not significantly) worsened in SHR by PostC. Notably, while PostC slightly improves post-ischemic systolic function of untreated SHR, it is ineffective in heart of ACE pretreated SHR. *Acute* Captopril treatment in reperfusion has no significant effects in terms of infarct size and cardiac post-ischemic function in SHR hearts with and without PostC. CPP is the only parameter affected by PostC in acutely treated SHR hearts. Our data suggest that acute and chronic ACE inhibitor treatment may trigger different cellular mechanisms.

As to the lack of an acute effect of Captopril on infarct size, our results are in agreement to those of Ebrahim et al. [8], who showed that the acute application of Captopril with and without *preconditioning* did not protect the ischemic-reperfused heart against infarct size in aging SHR. We cannot exclude that the antioxidant side effect of Captopril avoids PostC triggering of protection against infarct size, while favoring vasodilatation.

As to the effect of *chronic* ACE inhibitor, our results are in agreement to those of several authors, who shoved infarct size reduction after chronic ACE inhibition in hypertensive and non-hypertensive conditions [11,28,49,50]. However, the interaction between ACE-I effects and other cardioprotective intervention is a complex issue because ACE-I promotes regression of LVH and is not clear whether infarct size reduction is due to reduction of LVH, improved vascular function or to a kinin associated cardioprotective effect [11,28,49,50]. Indeed, the benefits of chronic ACE inhibition in preventing cardiovascular events are not clearly related to the potentiation of kinin actions and/or regression of LVH [11]. However, in our experimental conditions infarct size reduction is observed both with (SHR) and without (WKY) LVH regression. Yet, it is theoretically conceivable that the anti-ischemic effects of ACE inhibitors as kinin potentiating therapeutic strategies could be limited in the presence of LVH as suggested by acute treatment. Actually, our experiments suggest that LVH reduction is associate to a limitation of I/R injury, but PostC cardioprotection is not additive in hypertrophied myocardium that has undergone LVH regression.

Besides the side effect above reported, this reduction of PostC protective effect (in both strains) by chronic Captopril are suggestive of interfering effect of ACE-I on B_2 receptors [2,14] and may be of therapeutic importance in clinical setting. Importantly, chronic ACE-I *per se* reduces infarct size *via* LVH regression (SHR) and limiting perfusion pressure increase (WKY). It is known that kallidin-like peptide increases also during ischemia in the effluent of the perfused rat heart. Kinins, such as bradykinin and Arg-kallidin, can act on B_2 receptors and trigger preconditioning in animal hearts [21,25,26,58,60,62] via nitric oxide, cGMP, protein kinase G, mitochondrial K_{ATP} channel and reactive oxygen species signaling [16,32,33,47]. Bradykinin pretreatment also protects human myocardium. Thus bradykinin has been proposed to be used in clinical scenario to attenuate I/R injury [28]. Yet, we had to use bradykinin in an intermittent

manner during early reperfusion in order to trigger PostC [40,43]. It is, thus, not surprising that a continuous ACE-I infusion in reperfusion does not trigger PostC like protection. Indeed, as said, acute Captopril was also ineffective in SHR hearts against infarct size in preconditioning scenario [8].

Different is the case of chronic ACE-I treatment; in this case it is likely that PostC can not had its effect to an already reduced I/R injury, which has been attribute to LVH regression and/or CPP reduction. It is likely that the association of LVH and high vascular resistance can interfere with PostC protection. For, instance we have shown in a previous study that the high level of perfusion pressure during PostC maneuvers may interferes with protective effects [38]. Indeed, the post-ischemic increase of CPP in Captopril pretreated WKY is less marked than in the other groups, suggesting that in this group the protective effect of ACE-I are strictly related to vascular protection and post-ischemic CPP lowering. This is also in line with the observation that ACE-inhibition augments post-ischemic nitric oxide release, potentiates vasorelaxation and mitigates injury caused by ischemia/reperfusion [2,58,64]. In the presence of hypertrophy and acute Captopril, PostC reduces CPP, but is ineffective in reducing infarct size.

The post-ischemic contracture development correlates with infarct size in all the experimental conditions. The lowest level of contracture and infarct sizes are observed in WKY either untreated or Captopril pretreated. PostC reduces contracture when reduces infarct size. Yet, when PostC tends to worsen infarct size also contracture is worsened (e.g. Fig. 4). This is in line with previous studies of our and other groups [34,48 and references therein].

Our systolic functional data in untreated SHR are partially in agreement with the study of Fantinelli & Mosca [10], who report that PostC improves the post-ischemic systolic function of SHR hearts subjected to 20 min global ischemia and 30 min reperfusion. However, these authors did not measure infarct size. Actually, we observed a slight, but significant, effect on systolic

function after 30 min ischemia and 120 min reperfusion in untreated SHR, even when the effect on infarct size was not significant. Still, in chronically Captopril pretreated hearts the recovery of function is greatly improved by Captopril pretreatment *per se*, but not modified by PostC (see Fig 4). Since the chronic administration of ACE-I enhances the defenses against oxidative stress [6,12], we can suggest that the improved post-ischemic recovery of contractility in hearts of SHR pretreated with ACE-I is due to a limited oxidative stress. However, acute Captopril at the dose we used is not able *per se* to improve post-ischemic systolic function (see Fig 5).

In summary, our data demonstrate that a PostC protocol which is effective in limiting infarct size in normotensive WKY is ineffective in SHR hearts. Moreover, chronic Captopril treatment 1*)* in SHR favors LVH regression and infarct size reduction; *2)* in WKY reduces post-ischemic CPP and infarct size, but attenuates infarct-size limiting effects of PostC. Finally, acute Captopril given in reperfusion cannot reduce infarct size and does not recover PostC protection in SHR.

In conclusion, here, we have shown that PostC cardioprotection is blunted in SHR. Besides to confirm that *chronic* ACE-I promotes infarct size reduction and LVH regression in SHR, we suggest that Captopril interferes with kinin-dependent PostC cardioprotection. In fact, *chronic* Captopril reduces LVH, coronary resistance and infarct size, but PostC cardioprotection was not additive. Yet, *acute* Captopril infusion cannot reduce infarct size and does not recover PostC protection in SHR. Hence, Captopril cardioprotective potential in acute coronary syndrome as an adjunct in reperfusion seems limited in previously untreated hypertensive conditions. Our finding may have clinical implications since ACE inhibitors are clinical tools widely used in chronic hypertension and heart failure.

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Figure Legends

Fig. 1. Experimental design.

The isolated, Langendorff-perfused hearts were stabilized for 20 min (Stab), and then subjected to 30 min of normothermic global ischemia (I) followed by 120 min of reperfusion (R). Postconditioning (PostC) protocol (5 cycles 10 s ischemia/reperfusion) is indicated by vertical lines at the beginning of reperfusion period.

A: *Control Experiments* (untreated animals);

B: *Chronic treatment with CAP* (Captopril, 300 mg/l given in the drinking water for 4 weeks);

C: *Acute treatment with CAP* (Captopril, 200 μM infused for 20 or 40 min during early reperfusion, as indicated by horizontal lines). For further explanation see text.

Fig. 2. Echocardiographic data.

Percent variation of echocardiographic data with respect to mean baseline level for each group, during the 4 weeks of observation. DT, diastolic thickness; PWT%, systolic thickening of the LV posterior wall; SF, shortening fraction; EF, ejection fraction; CAP, Captopril.

* $p < 0.05$ *vs* untreated animals.

Fig. 3. D**ata from hearts of Control groups (Groups 1-4).**

A: *Infarct size (percent of risk area).* The amount of necrotic tissue is expressed as percent of the left ventricle, which is considered the risk area.

B: *Coronary perfusion pressure (CPP).* Percent variation of CPP with respect to baseline level for each group, during the 30 min ischemia and 120 min reperfusion.

C: *Diastolic Function.* Left ventricular end diastolic pressure (LVEDP, mmHg) during the 30 min ischemia and 120 min reperfusion.

D: *Systolic Function.* Percent variation of developed left ventricular pressure (dLVP) with respect to baseline level for each group, during the 30 min ischemia and 120 min reperfusion.

Time -30 correspond to the beginning of ischemia and time 0 to the beginning of reperfusion.

* p< 0.05, ** p< 0.01 SHR *vs* WKY (with and without PostC); # p< 0.05 PostC *vs* I/R in the same strain.

Fig. 4. Data from hearts of c*hronic Captopril treatment groups* (Groups 5-8).

A: *Infarct size (percent of risk area).* The amount of necrotic tissue is expressed as percent of the left ventricle, which is considered the risk area.

B: *Coronary perfusion pressure (CPP).* Percent variation of CPP with respect to baseline level for each group, during the 30 min ischemia and 120 min reperfusion.

C: *Diastolic Function.* Left ventricular end diastolic pressure (LVEDP, mmHg) during the 30 min ischemia and 120 min reperfusion.

D: *Systolic Function.* Percent variation of developed left ventricular pressure (dLVP) with respect to baseline level for each group, during the 30 min ischemia and 120 min reperfusion.

Time -30 correspond to the beginning of ischemia and time 0 to the beginning of reperfusion. CAP, Captopril.

*p< 0.05 (with PostC), ** p< 0.01 SHR *vs* WKY (with and without PostC); # p< 0.05 PostC *vs* I/R in the same strain.

Fig. 5. Data from hearts of acute *Captopril treatment groups* (Groups 9-12).

A: *Infarct size (percent of risk area).* The amount of necrotic tissue is expressed as percent of the left ventricle, which is considered the risk area.

B: *Coronary perfusion pressure (CPP).* Percent variation of CPP with respect to baseline level for each group, during the 30 min ischemia and 120 min reperfusion.

C: *Diastolic Function.* Left ventricular end diastolic pressure (LVEDP, mmHg) during the 30 min ischemia and 120 min reperfusion.

D: *Systolic Function.* Percent variation of developed left ventricular pressure (dLVP) with respect to baseline level for each group, during the 30 min ischemia and 120 min reperfusion.

Time -30 correspond to the beginning of ischemia and time 0 to the beginning of reperfusion. CAP, Captopril.

# $p < 0.05$ PostC *vs* corresponding I/R.

Figure 1

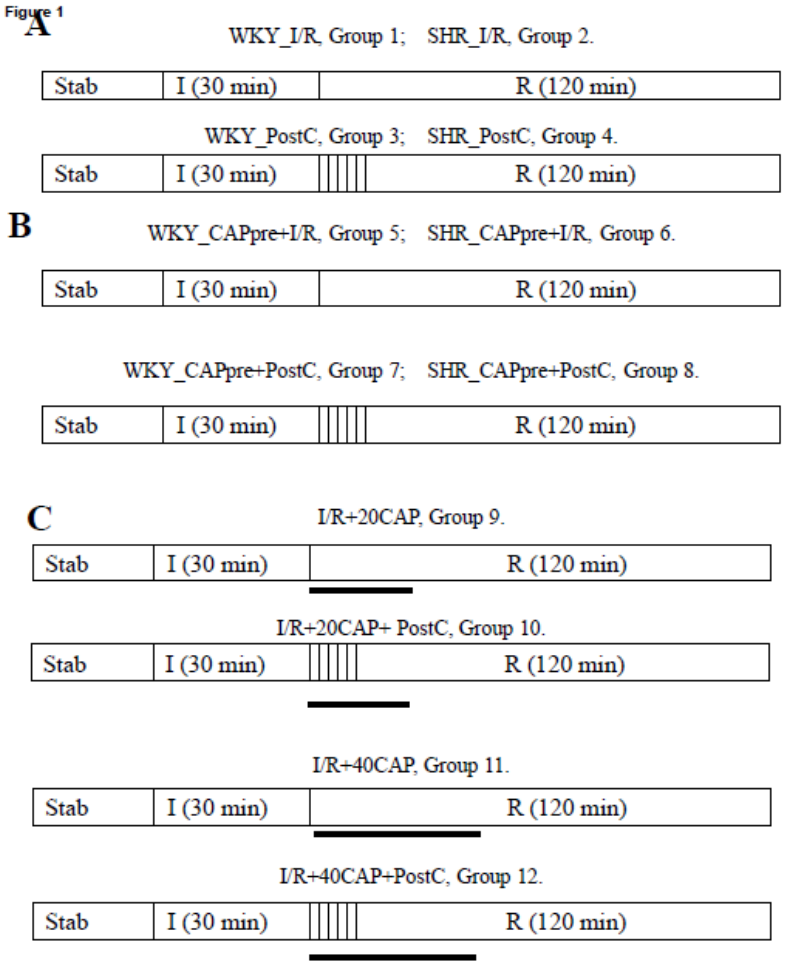
A
WKY_I/R, Group 1; SHR_I/R, Group 2.
Stab
I (30 min)
R (120 min)
WKY_PostC, Group 3; SHR_PostC, Group 4.
Stab
I (30 min)
R (120 min)
B
WKY_CAPpre+I/R, Group 5; SHR_CAPpre+I/R, Group 6.
Stab
I (30 min)
R (120 min)
WKY_CAPpre+PostC, Group 7; SHR_CAPpre+PostC, Group 8.
Stab
I (30 min)
R (120 min)
C
I/R+20CAP, Group 9.
Stab
I (30 min)
R (120 min)
I/R+20CAP+ PostC, Group 10.
Stab
I (30 min)
R (120 min)
I/R+40CAP, Group 11.
Stab
I (30 min)
R (120 min)
I/R+40CAP+PostC, Group 12.
Stab
I (30 min)
R (120 min)

Figure 2

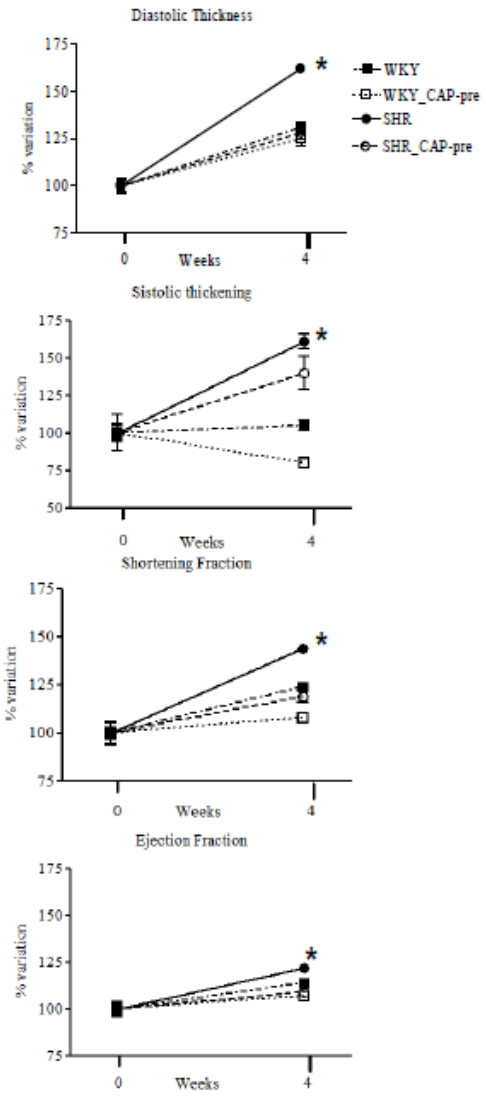
Diastolic Thickness
175
150
125
100
75
% variation
0
Weeks
4
*
WKY
WKY_CAP-pre
SHR
SHR_CAP-pre
Sistolic thickening
175
150
125
100
75
50
% variation
0
Weeks
4
*
Shortening Fraction
175
150
125
100
75
% variation
0
Weeks
4
*
Ejection Fraction
175
150
125
100
75
% variation
0
Weeks
4
*

Figure 3

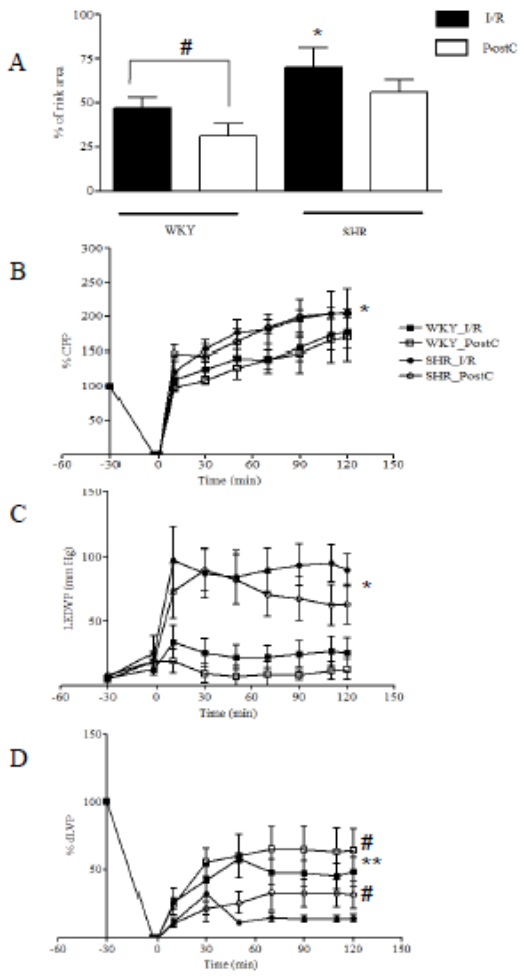
A
I/R
PostC
% of risk area
#
*
WKY
SHR
B
% CFP
WKY_I/R
WKY_PostC
SHR_I/R
SHR_PostC
Time (min)
C
LEDVP (mm Hg)
Time (min)
D
% dLVP
#
**
#
Time (min)

Figure 4

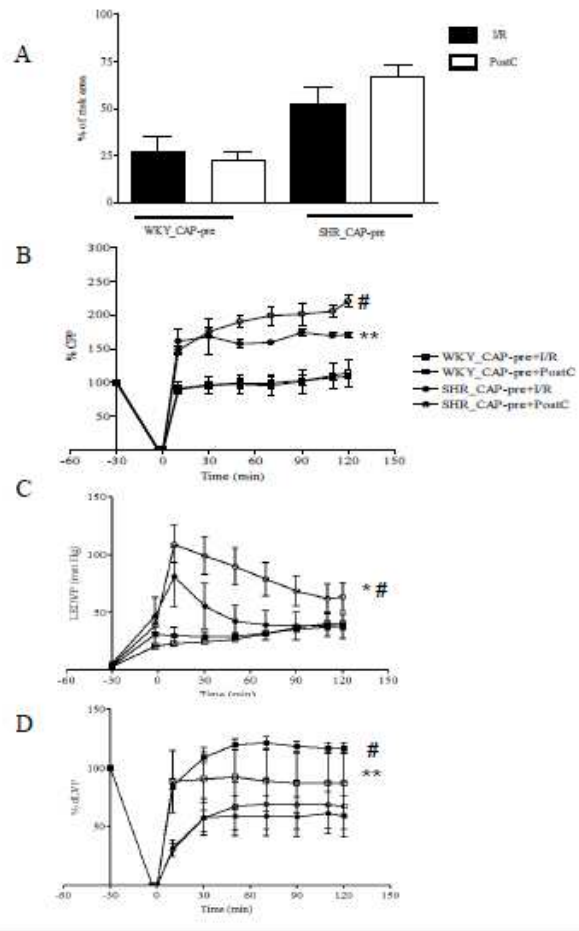
A
I/R
PostC
% of risk area
WKY_CAP-pre
SHR_CAP-pre
B
% CP
Time (min)
WKY_CAP-pre+I/R
WKY_CAP-pre+PostC
SHR_CAP-pre+I/R
SHR_CAP-pre+PostC
C
D
#
**

Figure 5

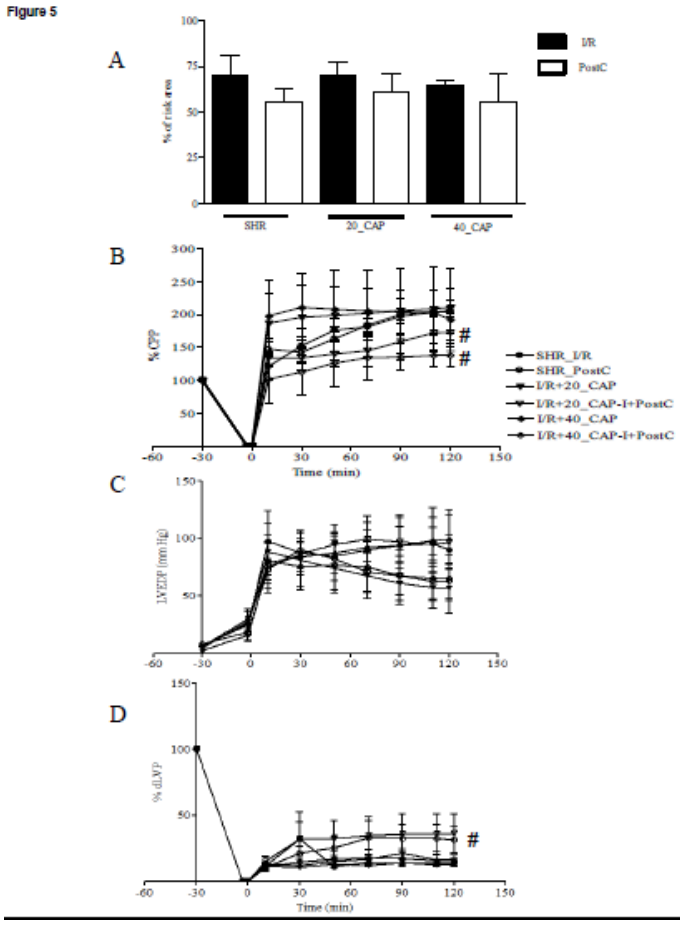
A
I/R
PostC
% of risk area
SHR
20_CAP
40_CAP
B
%CPP
Time (min)
SHR_I/R
SHR_PostC
I/R+20_CAP
I/R+20_CAP-I+PostC
I/R+40_CAP
I/R+40_CAP-I+PostC
C
LVEDP (mm Hg)
D
% dLVP

Table 1

Animal characteristics and baseline functional parameters of isolated (*ex vivo*) rat hearts

	6 months old WKY		6 months old SHR	
	untreated	*4 weeks CAP pretreatment*	*untreated*	*4 weeks CAP pretreatment*
n	*10*	*12*	*30*	*14*
BW (g)	525±13	534±6	381±7**	390±5**
HW (mg)	1763±56	1547±62	1488±41	1454±33
LV weight (mg)	994±37	869±50	1018±26#	910±32*
HW mg/BW g ratio	3.28±0.16	2.89±0.11	3.90±0.09#	3.66±0.11**
LV mg/BW g ratio	1.89±0.07	1.72±0.10	2.67±0.99#	2.29±0.15*
dLVP *in vivo* (mmHg)	99±5	98±4	160±12**	105±7**
CPP *ex vivo* (mmHg)	65±7	58±2	105±8#	85±8 *
LVEDP *ex vivo* (mmHg)	5±1	6±1	6±1	5±1
dLVP *ex vivo* (mmHg)	65±4	56±3	84±4	62±6*
dP/dt_{min} *ex vivo* (mmHg/s)	-1360±228	-1450±115	-1900±206	-1618±154
dP/dt_{max} *ex vivo* (mmHg/s)	1734±300	1800±130	2256±162	1928±143

CAP: (Captopril 300 mg/l in drinking water for 4 weeks); BW: body weight; HW: heart weight, LV weight: left ventricular weight, dLVP: developed left ventricular pressure, LVEDP: left ventricular end diastolic pressure, CPP: coronary perfusion pressure; dP/dt_{min}: maximum rate of decrease of LVP during diastole, dP/dt_{max}: maximum rate of increase of LVP during systole. *p< 0.05 and **p< 0.01 with respect to untreated; #p= 0.05 and ## p< 0.01 with respect to WKY.

Table 2. Baseline values of echocardiographic parameters

	4-5 month old animals			
	Untreated WKY	***CAP pretreated WKY***	***Untreated SHR***	***CAP pretreated SHR***
Body Weight (g)	479±16	490±10	370±11#	383±9#
Diastolic thickness (mm)	**0.17±0.02**	**0.17±0.02**	**0.15±0.01**	**0.16±0.03**
PWT%	**35±8**	**44±5**	**33±3**	**29±2#**
Shortening Fraction %	**39±4**	**45±5**	**36±2**	**40±4**
Ejection Fraction %	**73±4**	**79±5**	**77±6**	**71±2**

CAP pretreated: will receive Captopril (300 mg/l) in the drinking water for 4 weeks.
#=p<0.05 SHR vs WKY.