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PRECLINICAL RESEARCH

Multiple, Brief Coronary Occlusions During Early Reperfusion Protect Rabbit Hearts by Targeting Cell Signaling Pathways

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OBJECTIVES An in situ model was used to test whether and how multiple occlusions at reperfusion can protect rabbit myocardium.

BACKGROUND Recently it was demonstrated that postconditioning in dogs salvaged ischemic myocardium.

METHODS Control hearts underwent 30-min regional ischemia/3-h reperfusion, whereas in experimental hearts four postconditioning cycles of 30-s occlusion/30-s reperfusion starting 30 s after release of the index coronary occlusion were added in the presence or absence of various cell signaling antagonists.

RESULTS Postconditioning decreased infarction from 35.4 ± 2.7% of the risk zone in control hearts to 19.8 ± 1.8% ($p < 0.05$). Six cycles did not result in greater protection. If postconditioning cycles were begun after 10 min of reperfusion, protection was no longer evident. Either the non-selective K_{ATP} channel closer glibenclamide or the putatively selective mitochondrial K_{ATP} channel antagonist 5-hydroxydecanoate administered 5 min before reperfusion blocked the protection afforded by postconditioning, indicating involvement of the mitochondrial K_{ATP} channel. PD98059, a mitogen-activated protein/extracellular-signal regulated kinase (MEK) 1/2 and therefore extracellular-signal regulated kinase (ERK) inhibitor, and N^{ω}-nitro-L-arginine methyl ester, an antagonist of nitric oxide synthase, infused shortly before reperfusion also aborted the protection afforded by postconditioning. Combined ischemic postconditioning and preconditioning resulted in significantly greater protection than either alone.

CONCLUSIONS Multiple, short, regional coronary occlusions immediately after prolonged myocardial ischemia are an effective cardioprotective intervention in the rabbit, and the mechanism of protection involves activation of ERK, production of nitric oxide, and opening of mitochondrial K_{ATP} channels. These observations suggest that a similar approach could be applied in the cardiac catheterization laboratory to protect reperfused myocardium after primary angioplasty in patients with acute myocardial infarction. (J Am Coll Cardiol 2004;44: 1103–10) © 2004 by the American College of Cardiology Foundation

Ischemic preconditioning is acknowledged to be a robust cardioprotective intervention that salvages ischemic myocardium in experimental animals and probably in humans (1). However, as its name implies, preconditioning must be applied before an ischemic event to be protective, thus limiting its utility. Ischemic preconditioning is not useful for patients presenting to the hospital after onset of their myocardial infarction. Although thrombolysis, emergency angioplasty, or revascularization surgery can effect reperfusion with documented salvage of ischemic myocardium, these procedures are seldom instituted early enough to eliminate infarction. Furthermore, it is thought that reperfusion itself, although required for salvage, actually contributes to the injury (2–6). Therefore, an intervention is needed that can supplement the reperfusion strategy and attenuate reperfusion injury and/or otherwise limit necrosis in the heart.

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A pharmacologic approach has met with varying success. The effectiveness of adenosine administered at reperfusion is very controversial (7–10), and we have been unable to document any protection in our rabbit model (11). On the other hand, the adenosine A_1/A_{2A} agonist AMP579 when administered at reperfusion has consistently reduced infarction in all animal heart models tested (11–15). A structurally similar A_1/A_2 adenosine agonist 5′-(*N*-ethylcarboxamido) adenosine or NECA was equally protective, and the effect was dependent on phosphatidylinositol 3 (PI3)-kinase, extracellular-signal regulated kinase (ERK), and nitric oxide (NO) (16). We recently reported that CGX-1051, a peptide

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Abbreviations and Acronyms

ERK	= extracellular-signal regulated kinase
5-HD	= 5-hydroxydecanoate
L-NAME	= N^{ω}-nitro-L-arginine methyl ester
NO	= nitric oxide
NOS	= nitric oxide synthase

isolated from the venom of the cone snail, could also salvage myocardium when administered shortly before the onset of reperfusion (17). Several other agents appear to be protective when given to isolated heart models. These include bradykinin (18), cardiotrophin-1 (19), insulin (20), and transforming growth factor-beta-1 (21). Protection with these compounds is generally dependent on PI3-kinase and ERK. However, none of these agents has been validated in an in situ model, which would be required before clinical testing could be considered.

Very recently Zhao et al. reported a most improbable observation (22). They noted that several brief coronary occlusions after a 60-min occlusion in dogs significantly reduced infarct size. This postconditioning protocol has clinical appeal. Because urgent angioplasty is rapidly becoming the principal reperfusion treatment for acute myocardial infarction, it would not be implausible to produce several brief coronary occlusions immediately after the occluded artery is opened. Thus we wondered if ischemic postconditioning could be demonstrated in a second animal model, how its protection compared with that of ischemic preconditioning, and if elements of the signaling pathway leading to protection were at all similar to those already documented for pharmacologic agents that protect when infused at reperfusion.

METHODS

All procedures were approved by the Institutional Animal Care and Use Committee and were in accordance with recommendations published in the *Guide for the Care and Use of Laboratory Animals* (National Academic Press, Washington, DC, 1996).

New Zealand White rabbits of either gender weighing between 1.5 and 2.6 kg were anesthetized with intravenous sodium pentobarbital (30 mg/kg). Throughout the experiment, additional anesthesia was administered as needed (5 to 15 mg pentobarbital/15 min). A heating pad maintained rectal temperature between 38.5°C and 39.5°C. Animals were intubated through a tracheotomy and ventilated with 95% O_2/5% CO_2 with the aid of a mechanical ventilator (MD Industries, Mobile, Alabama). Arterial pH and oxygen and carbon dioxide tensions were maintained within the physiologic range (blood gas analyzer ABL 5, Radiometer, Copenhagen, Denmark). Catheters filled with heparinized saline (10 U/ml) were placed into the left carotid artery to monitor arterial blood pressure and to withdraw blood samples and into the right jugular vein to administer drugs. After left thoracotomy, a major branch of the left coronary artery was surrounded with a suture to form a snare. The rabbits were allowed to stabilize for 20 min after surgery before the protocols were begun.

Protocols. Hearts of 13 experimental groups experienced 30 min of regional ischemia (Fig. 1), whereas hearts of four other groups experienced 45 min of regional ischemia (Fig. 2). In all hearts, reperfusion after the occlusion lasted for 3 h. Control hearts had only this occlusion and reperfusion. For ischemically preconditioned rabbits, 5-min occlusion/10-min reperfusion immediately preceded the long ischemia. In postconditioning

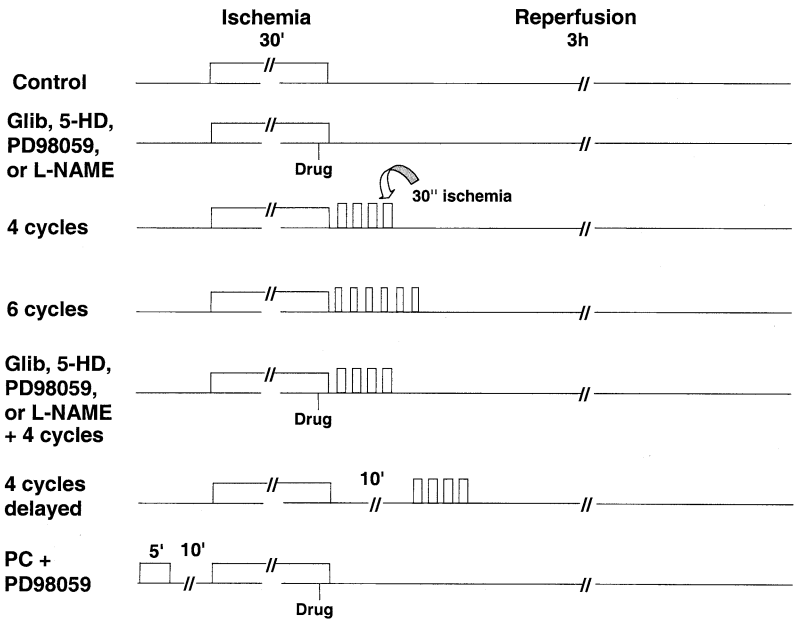

Figure 1. Experimental protocols for animal groups exposed to 30-min coronary artery occlusions. Glib = glibenclamide; 5-HD = 5-hydroxydecanoate; L-NAME = N^{ω}-nitro-L-arginine methyl ester; PC = ischemic preconditioning.

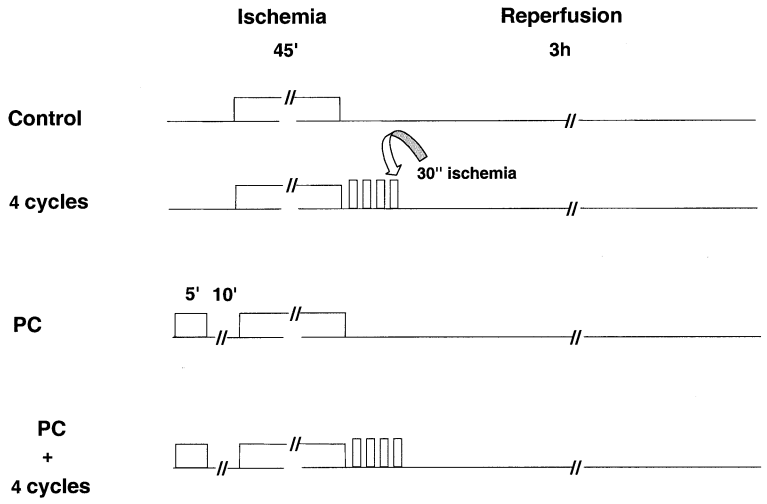

Figure 2. Experimental protocols for animal groups exposed to 45-min coronary artery occlusions. PC = ischemic preconditioning.

rabbits, four or six cycles of 30-s occlusion/30-s reperfusion started 30 s after release of the indicated long ischemia. All drugs, glibenclamide (0.3 mg/kg), 5-hydroxydecanoate (5-HD) (5 mg/kg), PD98059 (0.3 mg/kg), and N^{ω}-nitro-L-arginine methyl ester (L-NAME) (10 mg/kg), were given as intravenous boluses just 5 min before the onset of reperfusion. These doses have previously been used by us (17) and others (23–26) to selectively block the target. In four additional groups, each of these four tool drugs was infused alone just before reperfusion in the absence of any postconditioning stimulus to determine the effect of the drugs themselves. In one last group, four cycles delayed, the postconditioning cycles were delayed for 10 min after the onset of reperfusion.

Risk zone and infarct size. After completion of studies, all hearts were excised, suspended by the aortic root from a Langendorff apparatus, and perfused with 0.9% saline. The coronary snare was retightened, and 2- to 9-μm fluorescent microspheres (Duke Scientific, Palo Alto, California) were infused to delineate the area at risk as the nonfluorescent region. Hearts were frozen, cut into 2-mm transverse slices, incubated for 20 min in 1% triphenyltetrazolium chloride (pH 7.4, 37°C), and then immersed in 10% formalin. The borders between fluorescent and nonfluorescent regions were marked under ultraviolet light to identify the risk zone. Infarct and risk zone areas were planimetered, and volumes calculated. Infarct size is presented as a percent of risk zone.

Materials. All drugs were obtained from Sigma Chemical Co. (St. Louis, Missouri). Glibenclamide and PD98059 were dissolved in dimethyl sulfoxide. The 5-HD and L-NAME were dissolved in 0.9% saline.

Statistics. Data are expressed as mean ± SEM. One-way analysis of variance combined with Tukey's post hoc test was used to test for differences in infarct size and baseline hemodynamics between groups. Temporal differences in hemodynamic variables in any given group were analyzed with one-way repeated measures analysis of variance, and Tukey's post hoc test was used to examine differences between measurements at any given time point and baseline observations. Infarct sizes were plotted against risk zone volumes for all groups, and regression lines for groups with interventions were compared with the regression line for the control group by analysis of covariance with Bonferroni's correction for multiple comparisons. For all tests, $p < 0.05$ was considered significant.

RESULTS

Hemodynamics. No differences in basal heart rate or systolic or diastolic pressure were noted among the experimental groups (Table 1). No substantial changes in heart rate and blood pressure were observed after administration of drugs except for an unexpected increase in blood pressure after L-NAME administration in hearts destined to be postconditioned. Blood pressure declined modestly during the coronary occlusion in virtually all groups, with little recovery during reperfusion.

Infarct size. There was no significant difference in body weight, heart weight, or risk zone volume between the groups (Table 2). In control hearts (30-min occlusion), infarct size was 35.4 ± 2.7% of the risk zone (Fig. 3). Four postconditioning cycles starting 30 s after release of the 30-min coronary occlusion significantly decreased infarction (19.8 ± 1.8%, $p < 0.05$ vs. control). Six cycles did not result in greater protection (19.8 ± 1.6% infarction). However, if four postconditioning cycles were started 10 min after release of the 30-min occlusion, there was no longer any protection (34.5 ± 1.7% infarction). Either glibenclamide, a non-selective adenosine triphosphate-sensitive potassium (K_{ATP}) channel closer, or 5-HD, a selective mitochondrial K_{ATP} channel antagonist, blocked the protection of four cycles (32.8 ± 2.3% and 35.8 ± 4.8% infarction, respectively), indicating that the opening of mitochondrial K_{ATP} channels was involved in the protective mechanism (Fig. 4). PD98059, a mitogen-activated protein/extracellular-signal

Table 1. Hemodynamic Data

Group	Baseline			25′ Ischemia*			30′ Ischemia			5′/15′ Reperfusion†			30′ Reperfusion		
	HR (beats/min)	SBP (mm Hg)	DBP (mm Hg)	HR (beats/min)	SBP (mm Hg)	DBP (mm Hg)	HR (beats/min)	SBP (mm Hg)	DBP (mm Hg)	HR (beats/min)	SBP (mm Hg)	DBP (mm Hg)	HR (beats/min)	SBP (mm Hg)	DBP (mm Hg)
Control	243 ± 11	94 ± 3	76 ± 2				246 ± 11	79 ± 2‡	61 ± 2‡				258 ± 8	85 ± 3‡	67 ± 4‡
4 cycles	253 ± 4	98 ± 2	77 ± 3				245 ± 9	81 ± 3‡	63 ± 3‡	247 ± 10	77 ± 2‡	61 ± 3‡	254 ± 0	81 ± 3‡	62 ± 4‡
6 cycles	256 ± 17	94 ± 6	71 ± 5				241 ± 17	80 ± 4‡	59 ± 4	235 ± 16	76 ± 3‡	57 ± 4‡	240 ± 15	77 ± 4‡	57 ± 3‡
Glibenclamide + 4 cycles	275 ± 15	101 ± 4	81 ± 4	274 ± 17	91 ± 4‡	70 ± 4	268 ± 15	92 ± 4‡	73 ± 4	263 ± 14	86 ± 4‡	64 ± 4‡	270 ± 13	90 ± 4‡	67 ± 4‡
Glibenclamide	285 ± 10	104 ± 5	87 ± 4	292 ± 2	87 ± 6‡	72 ± 4	293 ± 2	92 ± 4‡	72 ± 4				298 ± 6	95 ± 5	75 ± 6
5-HD + 4 cycles	249 ± 13	103 ± 3	88 ± 4	267 ± 7	87 ± 2‡	72 ± 1‡	267 ± 8	86 ± 2‡	70 ± 1‡	261 ± 10	80 ± 2‡	62 ± 3‡	274 ± 4	87 ± 2‡	68 ± 3‡
5-HD	272 ± 4	95 ± 3	80 ± 3	265 ± 16	81 ± 8	63 ± 8	273 ± 13	82 ± 7	65 ± 6				273 ± 22	82 ± 10	67 ± 10
PD98059 + 4 cycles	255 ± 8	98 ± 2	79 ± 2	265 ± 7	81 ± 5‡	62 ± 5‡	261 ± 8	80 ± 4‡	63 ± 4‡	262 ± 10	78 ± 5‡	60 ± 6‡	258 ± 8	84 ± 5‡	62 ± 5‡
PD98059	265 ± 13	102 ± 2	87 ± 2	265 ± 13	83 ± 4‡	68 ± 4‡	268 ± 9	90 ± 3‡	73 ± 2‡				280 ± 8	92 ± 2‡	76 ± 1‡
L-NAME + 4 cycles	274 ± 2	103 ± 3	85 ± 2	270 ± 4	91 ± 4‡	72 ± 4‡	258 ± 11	102 ± 5	87 ± 5	249 ± 6	92 ± 5‡	76 ± 5	245 ± 10‡	108 ± 3	94 ± 4
L-NAME	273 ± 9	103 ± 3	87 ± 4	280 ± 8	98 ± 2	82 ± 3	255 ± 13	103 ± 2	83 ± 4				253 ± 12‡	110 ± 6	95 ± 6
4 cycles delayed	238 ± 9	101 ± 2	82 ± 2				238 ± 10	88 ± 5‡	69 ± 5	268 ± 13	87 ± 5‡	71 ± 7	250 ± 14	89 ± 4	70 ± 4
PC + PD98059	283 ± 5	101 ± 2	82 ± 3				284 ± 6	95 ± 2	78 ± 3	285 ± 10	84 ± 4‡	67 ± 5‡	287 ± 3	89 ± 4‡	74 ± 3
Control (45′)	261 ± 14	100 ± 4	81 ± 5				233 ± 26	81 ± 4‡	63 ± 3‡				256 ± 15	78 ± 3‡	58 ± 2‡
4 cycles (45′)	223 ± 11	97 ± 3	80 ± 2				228 ± 11	74 ± 3‡	56 ± 5‡	230 ± 11	70 ± 3‡	54 ± 4‡	231 ± 6	75 ± 3‡	55 ± 4‡
PC (45′)	262 ± 10	104 ± 4	83 ± 5				254 ± 13	78 ± 5‡	58 ± 5‡				251 ± 13	74 ± 4‡	53 ± 4‡
PC + 4 cycles (45′)	256 ± 10	100 ± 2	78 ± 2				246 ± 5	88 ± 2‡	65 ± 2‡	224 ± 7‡	81 ± 4‡	59 ± 3‡	253 ± 9	93 ± 3	68 ± 4

Values are mean ± SEM. *Measurements made after 25 min of ischemia and just before tool drug administration. †Measurements made after either 5 min (all groups except 1) or 15 min (4 cycles delayed group) of reperfusion in postconditioned groups. ‡$p < 0.05$, statistically significant difference between experimental data and baseline.

Control (45′) = control hearts with index ischemia of 45 min; DBP = diastolic blood pressure; 5-HD = 5-hydroxydecanoate; HR = heart rate; L-NAME = N$^{\omega}$-nitro-L-arginine methyl ester; PC = ischemic preconditioning; PC (45′) = PC hearts with index ischemia of 45 min; SBP = systolic blood pressure; 4 cycles = 4-cycle hearts with index ischemia of 45 min.

Table 2. Infarct Size Data

Group	n	Body Weight (kg)	Heart Weight (g)	Risk Zone Volume (cm^3)	Infarct Volume (cm^3)	I/R (%)
Control	11	2.29 ± 0.07	7.4 ± 0.3	1.18 ± 0.08	0.43 ± 0.05	35.4 ± 2.7
4 cycles	10	2.35 ± 0.07	7.4 ± 0.2	1.23 ± 0.10	0.25 ± 0.03	19.8 ± 1.8*
6 cycles	6	2.18 ± 0.07	7.2 ± 0.1	1.09 ± 0.12	0.22 ± 0.04	19.8 ± 1.6*
Glibenclamide + 4 cycles	6	2.34 ± 0.03	7.0 ± 0.1	1.27 ± 0.04	0.41 ± 0.04	32.8 ± 1.8
Glibenclamide	3	2.26 ± 0.06	7.2 ± 0.1	1.28 ± 0.05	0.42 ± 0.06	32.9 ± 4.7
5-HD + 4 cycles	6	2.30 ± 0.04	7.0 ± 0.1	1.37 ± 0.13	0.51 ± 0.11	35.8 ± 4.8
5-HD	3	2.18 ± 0.04	8.1 ± 0.1	1.38 ± 0.20	0.46 ± 0.08	33.6 ± 0.6
PD98059 + 4 cycles	6	2.28 ± 0.07	6.8 ± 0.2	1.31 ± 0.16	0.60 ± 0.11	44.8 ± 3.1
PD98059	3	2.39 ± 0.03	6.9 ± 0.1	1.15 ± 0.15	0.39 ± 0.08	34.1 ± 3.7
L-NAME + 4 cycles	6	2.36 ± 0.09	7.1 ± 0.1	1.36 ± 0.04	0.62 ± 0.04	45.1 ± 2.3
L-NAME	3	2.33 ± 0.01	7.1 ± 0.1	1.10 ± 0.07	0.40 ± 0.02	37.0 ± 0.6
4 cycles delayed	6	2.44 ± 0.11	6.0 ± 0.3	1.30 ± 0.18	0.52 ± 0.04	34.5 ± 1.7
PC + PD98059	5	2.60 ± 0.05	7.3 ± 0.3	1.25 ± 0.17	0.19 ± 0.08	13.4 ± 3.9*
Control (45′)	6	2.24 ± 0.04	6.9 ± 0.1	1.24 ± 0.08	0.77 ± 0.06	61.7 ± 2.2
4 cycles (45′)	6	2.15 ± 0.05	6.8 ± 0.1	1.32 ± 0.06	0.53 ± 0.07	39.6 ± 4.2†
PC (45′)	6	2.19 ± 0.17	6.9 ± 0.1	1.34 ± 0.07	0.47 ± 0.06†	35.1 ± 4.0†
PC + 4 cycles (45′)	6	2.16 ± 0.13	6.8 ± 0.3	1.26 ± 0.09	0.30 ± 0.06†	22.5 ± 3.1†

Values are expressed as mean ± SEM. Statistical significance of difference between experimental group and respective control group: * and †(45′) $p < 0.05$.
n = number of animals in each group; I/R = ratio of infarct to risk zone volume. Other abbreviations as in Table 1.

regulated kinase (MEK) 1/2 and therefore ERK inhibitor, and L-NAME, an antagonist of NO synthase (NOS), also aborted protection (44.8 ± 3.1% and 45.1 ± 2.3% infarction, respectively). For comparison, hearts preconditioned with ischemia were also treated with the same dose of PD98059 just before reperfusion. As seen in Figure 3, the expected protective effect of preconditioning was not affected (13.4 ± 3.9% infarction of the risk zone). None of the four tool drugs administered at reperfusion had any independent effect on infarct size (glibenclamide 42.2 ± 6.2%, 5-HD 33.6 ± 6.0%, PD98059 34.1 ± 3.7%, and L-NAME 37.0 ± 3.6%) (Fig. 4). Infarct sizes were plotted against risk zone volumes for all groups. The regression lines for postconditioned hearts were different from the regression line for control hearts ($p < 0.001$), whereas there was no difference between control hearts and those postconditioned hearts also treated with either PD98059, L-NAME, glibenclamide, or 5-HD (Fig. 5).

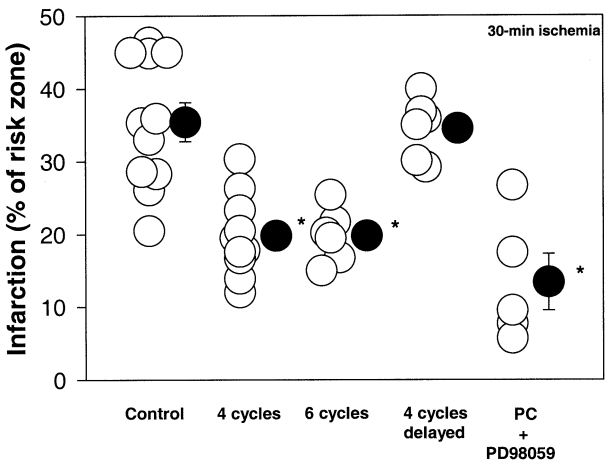

Figure 3. Infarct size in in situ rabbit hearts. All animals had a 30-min coronary occlusion and 3-h reperfusion. **Open circles** represent individual experiments, **closed circles** depict group means with SEM. Both four and six immediate postconditioning cycles protected ischemic hearts, whereas delayed postconditioning elicited no protection. PD98059 did not block the protective effect of ischemic preconditioning. *$p < 0.05$ versus control.

When regional ischemia was extended to 45 min in control hearts, infarction increased to 61.7 ± 2.2% of the risk zone (Fig. 6), and four postconditioning cycles resulted in significant salvage (39.6 ± 4.2% infarction, $p < 0.05$). A single preconditioning cycle of 5-min occlusion/10-min reperfusion before the 45-min ischemia also was protective (35.1 ± 4.0% infarction, $p < 0.05$). The combination of postconditioning and preconditioning resulted in even greater protection than either alone (22.5 ± 3.1% infarction, $p < 0.05$ vs. either ischemic preconditioning or four cycles).

DISCUSSION

A major goal of cardiovascular research is currently the identification of a reliable cardioprotection intervention that can salvage ischemic myocardium. Preconditioning was introduced in 1986 (27), and we know a lot about the signaling pathways leading to the protection afforded by preconditioning (1,28,29), although the identity of its end-effector has been quite elusive. Unfortunately, the clinical value of preconditioning itself is limited. On the other hand, an intervention that could be applied just before or at reperfusion would have great clinical appeal. Although several pharmacologic agents that appear to limit reperfusion injury have been identified (11,12,16,19–21), none of these is available for clinical use. The recent report about repetitive, brief postconditioning ischemia (22), which could easily be produced in patients undergoing angioplasty to open infarct arteries, again raises hope that a simple cardioprotective intervention could produce salutary clinical effects.

The initial investigation by Zhao et al. (22) demonstrated that three cycles of 30-s occlusion/30-s reperfusion starting

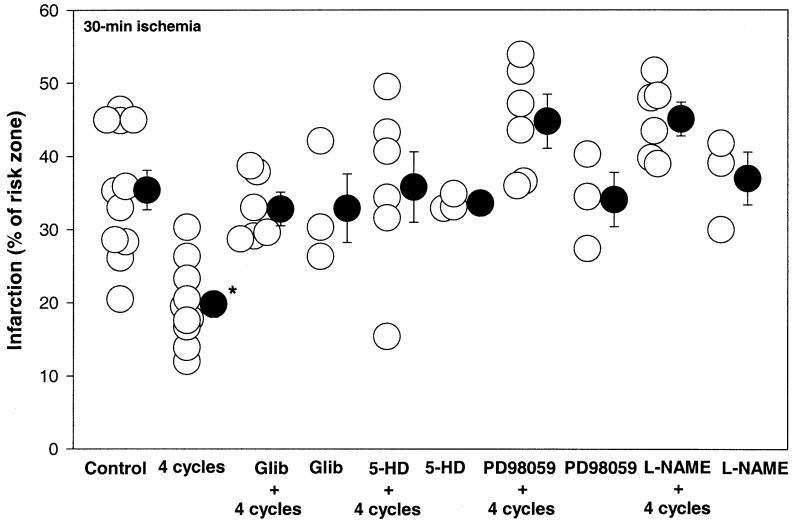

Figure 4. Infarct size in in situ rabbit hearts. All animals had a 30-min coronary occlusion and 3-h reperfusion. **Open circles** represent individual experiments, **closed circles** depict group means with SEM. Multiple cycles of immediate postconditioning limited infarct size, whereas glibenclamide (Glib), 5-hydroxydecanoate (5-HD), PD98059, and N^{ω}-nitro-L-arginine methyl ester (L-NAME) blocked their protection. The tool drugs alone had no effect. *$p < 0.05$ versus other groups.

30 s after release of a 60-min coronary occlusion in open-chest dogs decreased infarction by 40%, equivalent to the effect of preconditioning. In our rabbit model, four cycles decreased infarction by 43%, whereas increasing the number of cycles did not increase the amount of salvaged tissue. We did not determine the minimum number of cycles required to reduce infarction. However, we did note that the timing of this postconditioning ischemia was critical. If the intervention were delayed for only 10 min, protection was lost. Hence, in the rabbit, postconditioning must occur within the first 10 min of reperfusion to be protective. A similar observation was made for AMP579 (30) and CGX-1051 (17) when administered after reperfusion.

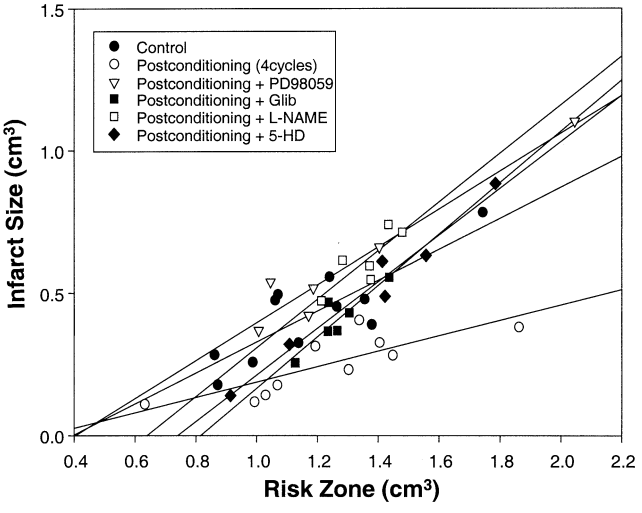

Figure 5. Infarct size plotted against risk zone volume for control hearts and hearts with four cycles of postconditioning alone or after treatment with either PD98059, glibenclamide (Glib), N^{ω}-nitro-L-arginine methyl ester (L-NAME), or 5-hydroxydecanoate (5-HD). Regression lines for the control group and postconditioned hearts treated with PD98059, Glib, L-NAME, or 5-HD were significantly different from that for hearts subjected only to postconditioning ($p < 0.001$).

Because ischemic postconditioning was protective, we wondered if this protection could be added to that expected after ischemic preconditioning. To test for such an additive effect, the index ischemia was prolonged to 45 min to increase infarct size in control hearts. Under these conditions, both ischemic preconditioning and postconditioning decreased infarction from 62% to comparable levels of 35% to 40% of the risk zone. When the same heart was subjected to both forms of cardioprotection, infarct size declined further to 22%, significantly less than either preconditioning or postconditioning alone. Because each form of cardioprotection has a maximal infarct-sparing effect when applied singly in this protocol, their additive effect when combined implies that their mechanisms of action must differ. This is further supported by the different effects of PD98059 on the protection afforded by ischemic preconditioning and postconditioning.

This study has not uncovered the actual mechanism of postconditioning, However, we have identified several elements of its signal transduction pathway. Both ERK and NO are probably involved. Perhaps not surprisingly, these elements are also thought to be involved in the signaling of several pharmacologic agents that appear to be protective when administered at reperfusion (16,18,19,21). Interestingly Komalavilas et al. (31) noted that in vascular smooth muscle cells protein kinase G activation stimulated phosphorylation of ERK 1/2, and, of course, production of NO by NOS leads to protein kinase G activation. Therefore, NO and ERK may be signaling elements in a common pathway. Additionally both glibenclamide and 5-HD blocked the protection of postconditioning, implying a role for mitochondrial K_{ATP} channels. The protection of CGX-

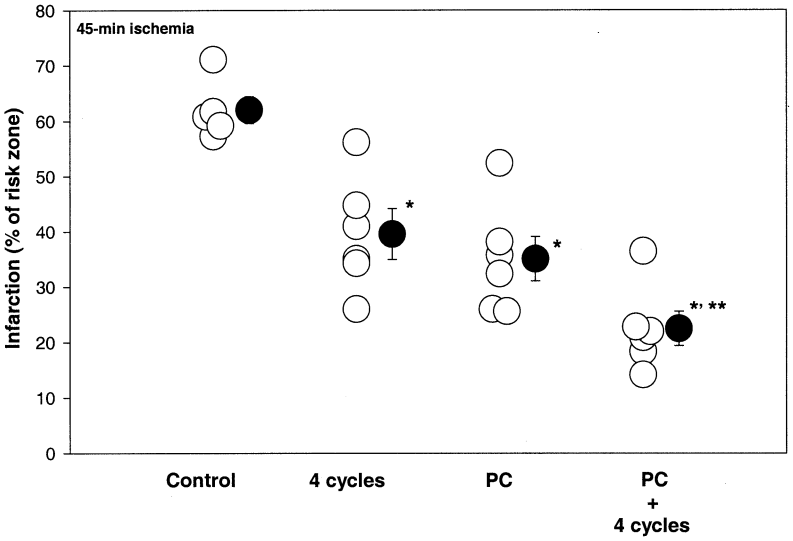

Figure 6. Infarct size in in situ rabbit hearts. All animals had a 45-min coronary occlusion and 3-h reperfusion. **Open circles** represent individual experiments, **closed circles** depict group means with SEM. Four postconditioning cycles resulted in significant salvage. A single preconditioning (PC) cycle of 5-min occlusion/10-min reperfusion before the 45-min ischemia also was protective. The combination of ischemic postconditioning and preconditioning resulted in even greater protection than either alone. *$p < 0.05$ versus control; **$p < 0.05$ versus PC and four cycles.

1051 that was found to limit infarction in the in situ rabbit heart when given at reperfusion was also dependent on mitochondrial K_{ATP} channels (17). Possible involvement of these channels in the protection of other reperfusion agents has not been investigated. Triggering of preconditioning depends on the production of reactive oxygen species by mitochondria after generation of NO and the opening of mitochondrial K_{ATP} channels (29). Thus there are some parallels between preconditioning and postconditioning. However, ERK is not part of preconditioning's signaling (32), as again shown in this study.

Thus, the protection afforded by postconditioning has now been observed in two animal models—dog and rabbit. The timing of the intervention is critical, and its protection can be added to that of preconditioning. The mechanism of the protection is uncertain. It is not yet known if ion fluxes or mitochondrial or cell swelling is affected. An initial report suggests that postconditioning reduces superoxide production by reperfused myocardium (22), but we do not know if that is the cause or the result of the protection. This report not only has confirmed the efficacy of postconditioning but also has revealed that its protection is dependent on cellular signal transduction pathways. In fact, most of these critical signaling entities are also involved in preconditioning. Perhaps more notable, other pharmacologic agents successful at protecting ischemic myocardium when administered at reperfusion use these same pathways leading to activation of the final effector.

This procedure of postconditioning could be safely and readily adapted for clinical use. After initial reperfusion of the infarct artery during primary angioplasty in a patient with an acute myocardial infarction, several brief, low-pressure balloon occlusions of the vessel could be performed before the procedure is completed with subsequent additional dilations to obtain grade 3 Thrombolysis In Myocardial Infarction (TIMI) flow and stenting if required. Current catheterization laboratory protocols would not be expected to routinely postcondition the heart, because of the critical requirement for multiple, brief occlusions in the first seconds of reperfusion. This approach has important clinical potential and should be further explored.

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REFERENCES

1. Cohen MV, Downey JM. Ischemic preconditioning: description, mechanism, and significance. In: Sperelakis N, Kurachi Y, Terzic A, Cohen MV, editors. Heart Physiology and Pathophysiology. 4th edition. San Diego, CA: Academic Press, 2001:867–85.
2. Becker LC, Ambrosio G. Myocardial consequences of reperfusion. Prog Cardiovasc Dis 1987;30:23–44.
3. Farb A, Kolodgie FD, Jenkins M, Virmani R. Myocardial infarct extension during reperfusion after coronary artery occlusion: pathologic evidence. J Am Coll Cardiol 1993;21:1245–53.
4. Vanden Hoek TL, Shao Z, Li C, Zak R, Schumacker PT, Becker LB. Reperfusion injury in cardiac myocytes after simulated ischemia. Am J Physiol 1996;270:H1334–41.
5. Matsumura K, Jeremy RW, Schaper J, Becker LC. Progression of myocardial necrosis during reperfusion of ischemic myocardium. Circulation 1998;97:795–804.
6. Piper HM, García-Dorado D, Ovize M. A fresh look at reperfusion injury. Cardiovasc Res 1998;38:291–300.
7. Olafsson B, Forman MB, Puett DW, et al. Reduction of reperfusion injury in the canine preparation by intracoronary adenosine: importance of the endothelium and the no-reflow phenomenon. Circulation 1987;76:1135–45.
8. Goto M, Miura T, Iliodoromitis EK, et al. Adenosine infusion during early reperfusion failed to limit myocardial infarct size in a collateral deficient species. Cardiovasc Res 1991;25:943–9.
9. Todd J, Zhao Z-Q, Williams MW, Sato H, Van Wylen DGL, Vinten-Johansen J. Intravascular adenosine at reperfusion reduces

infarct size and neutrophil adherence. Ann Thorac Surg 1996;62: 1364–72.
10. Vander Heide RS, Reimer KA. Effect of adenosine therapy at reperfusion on myocardial infarct size in dogs. Cardiovasc Res 1996; 31:711–8.
11. Xu Z, Downey JM, Cohen MV. AMP 579 reduces contracture and limits infarction in rabbit heart by activating adenosine A_2 receptors. J Cardiovasc Pharmacol 2001;38:474–81.
12. Smits GJ, McVey M, Cox BF, Perrone MH, Clark KL. Cardioprotective effects of the novel adenosine A_1/A_2 receptor agonist AMP 579 in a porcine model of myocardial infarction. J Pharmacol Exp Ther 1998;286:611–8.
13. Budde JM, Velez DA, Zhao Z-Q, et al. Comparative study of AMP579 and adenosine in inhibition of neutrophil-mediated vascular and myocardial injury during 24 h of reperfusion. Cardiovasc Res 2000;47:294–305.
14. Baxter GF, Ebrahim Z, Yellon DM. Amp579, an A1/A2A agonist, limits infarct size at reperfusion via a p42/p44 MAPK-dependent pathway (abstr). Circulation 2000;102:II212.
15. Xu Z, Yang X-M, Cohen MV, Neumann T, Heusch G, Downey JM. Limitation of infarct size in rabbit hearts by the novel adenosine receptor agonist AMP 579 administered at reperfusion. J Mol Cell Cardiol 2000;32:2339–47.
16. Yang X-M, Krieg T, Cui L, Downey JM, Cohen MV. NECA and bradykinin at reperfusion reduce infarction in rabbit hearts by signaling through PI3K, ERK, and NO. J Mol Cell Cardiol 2004;36:411–21.
17. Zhang SJ, Yang X-M, Liu GS, Cohen MV, Pemberton K, Downey JM. CGX-1051, a peptide from Conus snail venom, attenuates infarction in rabbit hearts when administered at reperfusion. J Cardiovasc Pharmacol 2003;42:764–71.
18. Bell RM, Yellon DM. Bradykinin limits infarction when administered as an adjunct to reperfusion in mouse heart: the role of PI3K, Akt and eNOS. J Mol Cell Cardiol 2003;35:185–93.
19. Liao Z, Brar BK, Cai Q, et al. Cardiotrophin-1 (CT-1) can protect the adult heart from injury when added both prior to ischaemia and at reperfusion. Cardiovasc Res 2002;53:902–10.
20. Jonassen AK, Sack MN, Mjøs OD, Yellon DM. Myocardial protection by insulin at reperfusion requires early administration and is mediated via Akt and p70s6 kinase cell-survival signaling. Circ Res 2001;89:1191–8.
21. Baxter GF, Mocanu MM, Brar BK, Latchman DS, Yellon DM. Cardioprotective effects of transforming growth factor-β1 during early reoxygenation or reperfusion are mediated by p42/p44 MAPK. J Cardiovasc Pharmacol 2001;38:930–9.
22. Zhao Z-Q, Corvera JS, Halkos ME, et al. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. Am J Physiol 2003;285:H579–88.
23. Yao Z, Gross GJ. A comparison of adenosine-induced cardioprotection and ischemic preconditioning in dogs: efficacy, time course, and role of K_{ATP} channels. Circulation 1994;89:1229–36.
24. Takano H, Tang X-L, Bolli R. Differential role of K_{ATP} channels in late preconditioning against myocardial stunning and infarction in rabbits. Am J Physiol 2000;279:H2350–9.
25. Fryer RM, Hsu AK, Gross GJ. ERK and p38 MAP kinase activation are components of opioid-induced delayed cardioprotection. Basic Res Cardiol 2001;96:136–42.
26. Patel VC, Yellon DM, Singh KJ, Neild GH, Woolfson RG. Inhibition of nitric oxide limits infarct size in the *in situ* rabbit heart. Biochem Biophys Res Commun 1993;194:234–8.
27. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 1986;74:1124–36.
28. Oldenburg O, Cohen MV, Downey JM. Mitochondrial K_{ATP} channels in preconditioning. J Mol Cell Cardiol 2003;35:569–75.
29. Oldenburg O, Qin Q, Krieg T, et al. Bradykinin induces mitochondrial ROS generation via NO, cGMP, PKG, and mitoK_{ATP} channel opening and leads to cardioprotection. Am J Physiol 2003;286:H468–76.
30. Xu Z, Downey JM, Cohen MV. Timing and duration of administration are crucial for antiinfarct effect of AMP 579 infused at reperfusion in rabbit heart. Heart Dis 2003;5:368–71.
31. Komalavilas P, Shah PK, Jo H, Lincoln TM. Activation of mitogen-activated protein kinase pathways by cyclic GMP and cyclic GMP-dependent protein kinase in contractile vascular smooth muscle cells. J Biol Chem 1999;274:34301–9.
32. Kim SO, Baines CP, Critz SD, et al. Ischemia induced activation of heat shock protein 27 kinases and casein kinase 2 in the preconditioned rabbit heart. Biochem Cell Biol 1999;77:559–67.