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Exercise induces early and late myocardial preconditioning in dogs

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Abstract

Objective: We tested the hypothesis that exercise induces myocardial preconditioning in dogs. **Methods:** We instrumented dogs with a snare on the anterior descending coronary artery and catheters in the root of the aorta, left ventricular cavity and coronary sinus. After recovering from surgery the dogs were trained to stay in the laboratory and run on a treadmill. Subsequently, they were randomly allocated to five groups: (1) non-preconditioned dogs: under anesthesia, the anterior descending coronary artery was occluded during 1 h and then reperfused during 4.5 h. (2) Early preconditioned dogs: procedure similar to group 1 but the dogs performed exercise on a treadmill for five periods of 5 min each before the coronary occlusion. (3) Late preconditioned dogs: procedure similar to group 2 but 24 h were allowed to elapse between the preconditioning exercise and the coronary occlusion. (4) Early preconditioned dogs plus 5-hydroxydecanoate: procedure similar to group 2 but 5-hydroxydecanoate was administered prior to exercise. (5) Non-preconditioned dogs with 5-hydroxydecanoate: procedure similar to group 1 but 5-hydroxydecanoate was administered at a time equivalent to that in group 4. **Results:** Exercise did not induce myocardial ischemia and the hemodynamics during the experiments did not differ between groups. Exercise immediately before the coronary occlusion decreased the infarct size (percent of the risk region) by 78% ($P<0.05$), an effect that was abolished with 5-hydroxydecanoate. Exercise 24 h prior to coronary occlusion decreased infarct size by 46% ($P<0.05$ vs. non-preconditioned dogs, $P<0.05$ vs. early preconditioned dogs). 5-Hydroxydecanoate by itself did not modify infarct size. These effects could not be explained by changes in collateral flow to the ischemic region. **Conclusions:** Exercise prior to a coronary occlusion induces early and late preconditioning of the infarct size. The early effect is mediated through mitochondrial ATP-sensitive potassium channels.

Keywords: Ischemia; K-ATP channel; Mitochondria; Preconditioning

1. Introduction

Myocardial ischemic preconditioning is a strong endogenous protective mechanism that reduces myocardial infarct size [1], the incidence of arrhythmias [2] and contractile dysfunction [3].

Recently, the preconditioning effect of increasing myocardial O_2 consumption with tachycardia has been described in dogs [4] and pigs [5]. This preconditioning effect is not induced by ischemia [4] but is probably mediated through similar metabolic pathways as ischemic preconditioning because it is suppressed by adenosine antagonists [4] and mitochondrial ATP-dependent potassium channel blockers [6].

Since tachycardia induces myocardial preconditioning, it is reasonable to think that exercise also induces it. Moreover, during exercise, several metabolites that induce preconditioning like α adrenergic agonists, opioids, nitric oxide and bradykinin may participate. Accordingly, the protective effects of exercise could be larger than that of tachycardia alone. Only two studies in rats [7,8] have investigated the preconditioning effect of exercise but the authors did not look for the participation of ATP-dependent potassium channels. We decided to study the preconditioning effect of exercise and the participation in it of mitochondrial ATP-dependent potassium channels. The experiments were conducted in dogs, the same species in which we studied the effect of tachycardia, and using a similar preparation [4] that prevents large hemodynamic changes that may alter the interpretation of the results.

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2. Methods

This investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996).

Seventy-six mongrel dogs were instrumented under aseptic surgery. Briefly, under anesthesia with pentobarbital (30 mg/kg, i.v.) and mechanical ventilation of lungs, the thorax was opened at the 5th intercostal space and sylastic catheters were implanted into the aortic root through its wall, into the coronary sinus through the great coronary vein and into the left atrium through its appendage. An occluder (plastic snare) was implanted around the anterior descending coronary artery immediately distal to the emergence of the first diagonal branch. Dogs were allowed to recover from surgery during 2 weeks and then trained in the laboratory during 2 weeks to allow them to become accustomed to run on a treadmill at a speed of 5 to 9 km/h during 5 min, twice a day. The rest of the day the dogs were kept in comfortable cages at about 22 °C. Seven dogs died during surgery and five dogs died a few days afterwards because of different complications of surgery. Therefore 64 dogs were randomly assigned to five groups (Fig. 1).

Group 1: non-preconditioned dogs (NP, $n=12$). The dogs were allowed to rest for 60 min in the laboratory and then they were anesthetized with intravenous pentobarbital (30 mg/kg), and the anterior descending coronary artery was occluded with the plastic snare during 1 h and reperfused during 4.5 h. In order to obtain a stable preparation and comparable hemodynamic conditions between groups, aortic pressure changes were damped by connecting the systemic arterial circulation, through the brachiocephalic trunk, to a large reservoir filled with homologous, heparinized, thermoregulated to 37 °C, and constantly stirred blood. The height of the reservoir was frequently adjusted to maintain a mean arterial pressure of about 100 mmHg in the aortic root during the experiments.

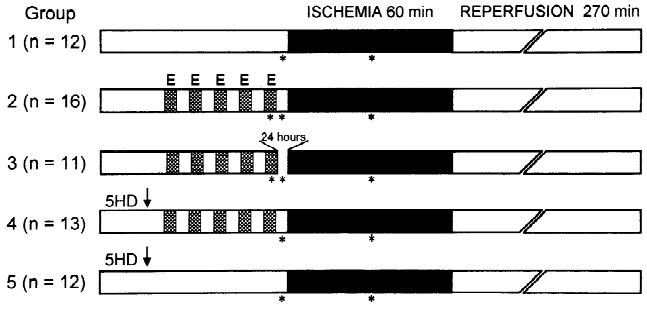

Fig. 1. Experimental design. E, exercise; 5 HD, 5 hydroxydecanoate; NP, non-preconditioned dogs; EP, early preconditioned dogs; LP, late preconditioned dogs; EP+5HD, early preconditioned dogs plus 5HD; NP+5HD, non-preconditioned dogs plus 5HD; *, flow measurement with microspheres.

We showed in previous experiments that this procedure together with the induction of a small infarct (less than 10% of the left ventricular free wall volume) prevented from excessive distension of the ischemic region and severe left ventricular failure [4].

Group 2: early preconditioned dogs (EP, $n=16$). The same procedure as in group 1 was followed but dogs were allowed to run on a treadmill five periods of 5 min each at 6 km/h with intervening 5-min periods of rest. After the last exercise period, the animals rested for 10 min to allow the heart rate and the aortic pressure to recover to basal values prior to inducing the infarction as in group 1.

Group 3: late preconditioned dogs (LP, $n=11$). Similar to group 2 but 24 h were allowed to elapse between the exercise and the coronary occlusion.

Group 4: early preconditioned dogs plus 5-hydroxydecanoate (EP+5HD, $n=13$). Similar to group 2, but mitochondrial ATP sensitive potassium channels were blocked with 5HD. The drug was dissolved in 10 ml of saline and administered intravenously 15 min prior to exercise in a dose of 0.7 mg/kg/min over 10 min.

Group 5: non-preconditioned dogs plus 5HD (NP+5HD, $n=12$). The drug was administered at a time equivalent to the blockade time in group 4. This group was produced as a control to group 4 to discard the effect of the drug.

Aortic pressure and heart rate were continuously measured during rest and exercise. Myocardial flow to the left ventricular wall was measured with the radioactive microspheres technique [9] during the last period of exercise and after recovery in all preconditioned dogs and before the coronary occlusion in non-preconditioned dogs. Myocardial O_2 consumption was calculated as the product of myocardial flow times the coronary arteriovenous difference in O_2 content (between aortic root and coronary sinus blood samples). Collateral flow to the ischemic left ventricular wall, 30 min into the ischemic period, was also measured with the microspheres technique. We used carbonized plastic microspheres (15.5 ± 0.5 μm diameter, New England Nuclear) labeled with ^{46}Sc, ^{85}Sr or ^{57}Co suspended in isotonic saline and 0.01% Tween 80 and ultrasonicated. The microspheres were placed in a plastic chamber (1 ml volume capacity), magnetically stirred and flushed manually with 2 ml of saline into the left atrium in about 20–30 s. A reference flow sample (10 ml/min) was simultaneously obtained from the brachial artery (or aortic root for measurements during exercise) during 1.5 min [9]. After the experiments, transmural pieces of the left ventricular wall were obtained from the center of the ischemic region. Each sample was weighed and its radioactivity (*Cm*) and the radioactivity of the blood collected from the reference samples (*Cr*) were measured in a gamma spectrometer equipped with a multichannel analyzer (Packard Auto Gamma 5500). Regional collateral flow (*Qm*) was calculated as $Qm = Qr \times Cm \times Cr^{-1}$, where *Qr* is the flow rate of the reference sample. Flow values are expressed per gram of tissue. The size of the infarction relative to the risk

region was measured with the triphenyltetrazolium staining technique [4]. After the experiments were finished, the right coronary artery and the circumflex coronary artery were perfused from the aorta with a solution of Evans blue dye in saline. The anterior descending coronary artery was perfused from the place where it had been ligated, with a solution of 1% triphenyltetrazolium chloride. The perfusions were done simultaneously at a pressure of 90 mmHg. After perfusion the left ventricle was cut into slices of ≈8 mm thickness parallel to the AV grove. The slices were incubated for 10 min in a 1% solution of triphenyltetrazolium. In each slice, the volumes of the non-risk zone, the risk zone and the necrotic zone (stained blue, red and not stained, respectively) were obtained by measuring with planimetry the corresponding areas on the cross surface of the slice and multiplying them by the thickness of the slices. These volumes were added across the slices to obtain the corresponding total volumes of the three regions. The magnitude of the infarction was expressed by the volume of the necrotic region as percent of the volume of the risk region. The risk region was expressed as percent of the total left ventricular volume.

2.1. Statistical analysis

Results are expressed as mean±S.E.M. Differences in the hemodynamic and metabolic variables and infarct sizes between groups were tested with one- or two-way ANOVA with or without repeated measurements as appropriate followed by Student–Newman–Keuls analysis. The effect of coronary collateral flow to the ischemic region on the difference of infarct size between the groups was assessed with ANCOVA. The null hypothesis was discarded with $P<0.05$.

3. Results

Nine dogs were discarded during the experiments because of more than three episodes of ventricular arrhythmias: one dog in groups 4 and 5; two dogs in groups 1 and 3; three dogs in group 2. Seven dogs were discarded from the study because of a collateral flow to the ischemic region greater than 0.2 ml/min/g: two dogs in groups 1 and 4 and one dog in groups 2, 3 and 5. Therefore the results are presented for 48 dogs.

Table 1 shows the hemodynamic and metabolic effects of exercise in preconditioned dogs. During exercise there was a significant increase in heart rate (50%), coronary flow (52%), myocardial O_2 consumption (59%) and coronary arteriovenous difference in lactate content (43%). Lactate concentration in arterial blood during resting of the dogs was 1.03±0.16 μmol/ml. Mean aortic pressure and coronary arteriovenous difference of O_2 content increased each one by 8% during exercise but these changes were not significantly different. No repolarization changes were observed in the ECG during exercise.

Table 2 shows the hemodynamic variables during baseline, ischemia and reperfusion after the animals were anesthetized. There were not significant alterations across these periods nor between groups.

Table 3 shows the net blood volume uptake (from the reservoir) by the dogs' circulation due to the aortic pressure damping system during ischemia and reperfusion. In several dogs there was a small release of blood from the dog circulation towards the reservoir before ischemia (not shown) because the mean aortic pressure in these animals was slightly higher than 100 mmHg (the pressure set up with the reservoir). During ischemia the reservoir had to be raised in small steps to maintain aortic pressure at about 100 mmHg. This meant a relative small uptake of blood, by the animals circulation, not larger than 147 ml in any animal (about 10–15% of the blood volume of these dogs). The uptake was smaller in preconditioned than in non-preconditioned dogs but the difference was not statistically significant. During reperfusion there was an additional uptake of blood not larger than 92 ml in any animal and without differences between the groups.

Fig. 2 shows the effects of exercise on infarct size in all the groups. Exercise decreased infarct size from 23.7±3.0% in the non-preconditioned group to 5.3±1.2% in the early preconditioned group ($P<0.05$) and to 12.7±1.4 in the late preconditioned group ($P<0.05$). The late preconditioning effect was less than the early one ($P<0.05$). The effect of early preconditioning was abolished by the administration of 5HD. The drug by itself did not modify the infarct size. These effects were independent of the collateral flow to the ischemic region (COVARIANCE, at least $P<0.05$, not shown). There were no significant differences between groups in risk region volumes as percent of the left ventricular wall volumes (48±2.3%, 50±4.1%, 46±3.8%, 47±4.2% and 45±3.1%,

Table 1
Hemodynamics and metabolic effects of exercise in dogs ($n=30$)

	HR (c/min)	MAP (mmHg)	CF (ml/min/g)	MVO_2 (ml/min/100 g)	AVO_2 (ml/100 ml)	AVlactate (μmol/ml)
Baseline	101.2±13.6	79.7±7.5	0.46±0.14	7.9±1.8	12.0±4.0	0.42±0.08
Exercise	152.0±12.7*	86.4±7.5	0.70±0.17*	12.6±2.8*	13.0±4.0	0.60±0.08*

Values are mean±S.E. HR, heart rate; c/m, cycles/min; MAP, mean aortic pressure; CF, coronary blood flow; MVO_2, myocardial oxygen consumption; AVO_2, coronary arteriovenous difference in oxygen content; AVlactate, coronary arteriovenous difference of lactate. *$P<0.05$, at least, vs. baseline.

Table 2
Hemodynamic variables

		Baseline	Ischemia		Reperfusion		
			30 min	60 min	60 min	120 min	180 min
HR (c/min)	NP	135±5	139±12	140±13	136±8	134±11	132±11
	EP	141±17	132±17	130±7	136±8	120±15	139±17
	LP	126±12	122±7	124±10	126±9	126±5	123±15
	EP+5HD	142±8	136±12	145±14	150±12	153±8	144±14
	NP+5HD	139±5	134±7	137±7	127±9	125±12	129±13
SAP (mmHg)	NP	112±17	113±13	124±15	125±14	122±16	128±14
	EP	115±18	117±3	115±5	118±5	126±3	117±13
	LP	114±9	113±5	120±2	121±4	111±6	114±7
	EP+5HD	119±7	111±6	107±8	115±3	115±13	112±5
	NP+5HD	112±5	108±8	115±10	127±10	119±10	117±11
DAP (mmHg)	NP	85±9	82±11	89±9	86±9	82±8	86±7
	EP	78±15	87±9	83±8	88±5	85±8	93±8
	LP	81±9	86±5	89±1	92±2	89±5	86±5
	EP+5HD	82±4	81±4	76±4	80±4	79±11	80±5
	NP+5HD	80±8	78±7	88±8	88±8	91±9	83±7

HR, heart rate; SAP, systolic aortic pressure; DAP, diastolic aortic pressure; 5HD, 5 hydroxydecanoate; NP, non-preconditioned dogs; EP, early preconditioned dogs; LP, late preconditioned dogs; EP+5HD, early preconditioned dogs plus 5HD; NP+5HD, non-preconditioned dogs plus 5HD.

Table 3
Transfer of blood to the dog circulation (ml)

	Ischemia	Reperfusion	Total
G1 (NP)	116±8	62±9	178±7
G2 (EP)	102±11	50±11	153±18
G3 (LP)	102±13	63±12	165±19
G4 (EP+5HD)	101±15	68±8	169±10
G5 (NP+5HD)	114±11	58±10	172±10

G, group; NP, non-preconditioned dogs; EP, early preconditioned dogs; LP, late preconditioned dogs; EP+5HD, early preconditioned dogs plus 5HD; NP+5HD, non-preconditioned dogs plus 5HD.

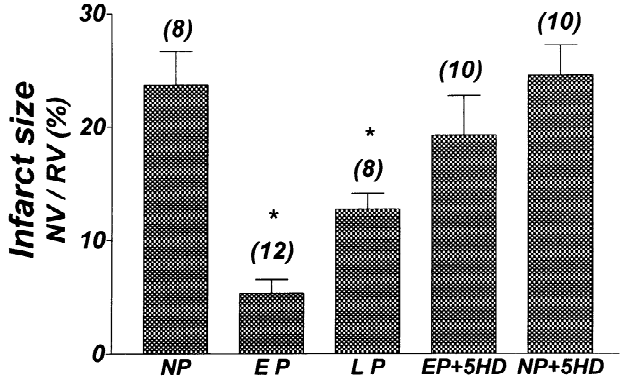

Fig. 2. Infarct size expressed by the necrotic volume (NV) as a percentage of the risk volume (RV) of the left ventricular wall. NP, non-preconditioned dogs; EP, early preconditioned dogs; LP, late preconditioned dogs; EP+5HD, early preconditioned dogs plus 5 HD; NP+5HD, non-preconditioned dogs plus 5HD. Figures in parentheses represent number of dogs. *$P<0.05$ vs. NP.

for groups 1 to 5, respectively) nor in left ventricular wall volumes (80.7±9.1 cm^3, 74.3±6.9 cm^3, 75.7±6.2 cm^3, 82.4±7.4 cm^3 and 79.3±8.7 cm^3 for groups 1 to 5, respectively).

4. Discussion

Our results show that exercise induces early and late preconditioning of myocardial infarct size in the dog and that the early effect is mediated by mitochondrial ATP sensitive potassium channels. These protective effects cannot be explained by changes in hemodynamic variables during ischemia and reperfusion because these variables were not significantly different between the groups. These effects cannot be explained either by changes of these variables by effect of the preconditioning exercise because the hemodynamic variables were allowed to return to basal values before occluding the coronary artery. Furthermore, the protective effect of exercise cannot be attributed to differences in risk region sizes or to changes in its collateral flow. Finally, the preconditioning effect of exercise was not due to ischemia because: (a) arterial blood levels of lactate were normal at rest and the myocardial extraction of lactate increased during exercise, (b) the venous coronary O_2 content did not decrease by more than 10%, a variation less than that usually observed in healthy dogs during exercise [10–12], and (c) during exercise no ischemic electrocardiographic alterations were observed.

The early preconditioning effect due to exercise in conscious dogs was much larger (75% reduction in infarct

size) than that observed due to tachycardia (50% reduction) in anesthetized dogs [4], although the size of the risk zone (about 45%) and the increase in myocardial oxygen consumption (about 60%) were similar in both studies. These results suggest that during exercise stimuli other than myocardial O_2 consumption, activate the metabolic pathway through which preconditioning is produced. Several metabolites that are known to be released during exercise like α adrenergic agonists [13,14], bradykinin [15,16], opioids [17,18], nitric oxide [3] and reactive oxygen species [19,20] induce preconditioning. Whatever the initial stimuli may be, the metabolic pathway during preconditioning by exercise is probably similar to that described during preconditioning by tachycardia [4–6], that is an increase in interstitial adenosine content and, at least in early preconditioning, activation of mitochondrial ATP-dependent potassium channels.

Our results agree with those of Yamishata et al. [7] who recently showed that exercise provides a biphasic cardioprotection in rats. The authors showed that both phases of the protection are mediated by manganese superoxide dismutase activation probably by increasing the production of oxygen species, TNFα and IL-1β in the myocardium during exercise. Besides, the late protection seems to be mediated also through protein kinase C [8]. The authors, however, did not study the participation of mitochondrial ATP sensitive potassium channels.

We observed that the protective effect of late preconditioning was smaller than the early one. However this finding may depend on the time selected to assess the late effect. Thus, Yamashita et al. [7] reported a late preconditioning effect on infarct size less or equal to the early effect according to the time selected for the assessment of the late effect. This is probably due to the evolution in time of the synthesis of mediators for this protection [7].

The induction of myocardial preconditioning by exercise may be related to the well known fact that the incidence of acute myocardial infarction is less in individuals who practice moderate or intense physical activity regularly [21,22]. Although this is probably due to the maintenance of a proper endothelial function and a decrease in risk factors for atheromatosis, it is reasonable to think that the myocardium is permanently preconditioned against ischemia according to the magnitude of its metabolic activity and other stimuli on the heart (as mentioned above) due to physical activity. This is even more important considering that exercise produces late preconditioning and that several of the mediators that increase during exercise induce late preconditioning. Thus, recent studies [18] in rats have shown that opioids not only induce early preconditioning but also preconditioning 24 h after its administration, a finding that also has been found for nitric oxide [3].

In summary, our results show that physical exercise induces early and late myocardial preconditioning of infarct size in dogs. The early effect is mediated by mitochondrial ATP sensitive potassium channels.

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