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European Journal of Pharmacology 499 (2004) 163–169

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Intravenous atenolol and esmolol maintain the protective effect of ischemic preconditioning in vivo

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Received 16 February 2004; received in revised form 10 May 2004; accepted 20 July 2004
Available online 27 August 2004

Abstract

Catecholamines bind to α- and β-adrenoreceptors and are capable of preconditioning ischemic myocardium. Our purpose was to investigate the effect of acute either short or prolonged i.v. administration of β-adrenoreceptor antagonists on ischemic preconditioning in vivo. Fifty-five anesthetized rabbits were divided into 10 groups (n=5–7 per group) and were subjected to 30-min regional ischemia of the heart after ligation of a prominent left coronary artery and 3-h reperfusion after releasing the snare. Ischemic preconditioning was obtained by three cycles of 5-min ischemia separated by 10-min reperfusion. β-Adrenoreceptor blockade was obtained by the long acting β-adrenoreceptor antagonist atenolol or by the short acting esmolol, which were given as a short 5-min infusion or as a prolonged 45-min infusion, starting respectively 20 min before and ending 15 min before the beginning of sustained ischemia, or starting 45 min before and ending immediately before the beginning of sustained ischemia. Atenolol was given at a rate of 0.2 mg min^{-1} during 5 min or at a rate of 0.088 mg min^{-1} as a 45-min infusion. Esmolol was given as an initial dose of 500 μg kg^{-1} within 1 min, followed by a 4-min infusion at a rate of 50 μg kg^{-1} min^{-1} or as an initial dose of 3.4 mg within 1 min, followed by a 44-min infusion at a rate of 0.15 mg min^{-1}. Blood pressure and heart rate were continuously monitored. The infarcted and risk areas were delineated with the aid of tetrazolium chloride staining and fluorescent Zn–Cd particles. Infarct size was expressed in percent of the area at risk. All the animals without preconditioning developed an infarct size ranging between 36.3±2.4% and 49.6±7.6% (P=NS) and all the preconditioning groups developed an infarct size ranging between 14.9±1.2% and 21.0±2.2% (P=NS). All the preconditioning groups, independently of the use of β-adrenoreceptor antagonists, had a smaller infarct size than the control group, which developed an infarct size of 47.3±2.5% (P<0.01). Intravenous atenolol and esmolol, independent of timing and mode of administration, does not seem to interfere with protection afforded by ischemic preconditioning in vivo.

Keywords: β-Adrenoreceptor antagonists; Preconditioning; Infarction

1. Introduction

Multicenter trials have shown the effectiveness of administration of β-adrenoreceptor antagonists as life-saving agents for patients with coronary heart disease (Yusuf et al., 1988). In addition, blockade of β-adrenoreceptors during the first hours of an evolving myocardial infarction limits the infarct size and contributes to a better clinical outcome (International Collaborative Study Group, 1984, The MIAMI Trial Research Group, 1985). The treatment with β-adrenoreceptor antagonists started in these studies after the total occlusion of the responsible coronary artery. However, the blockade of β-adrenoreceptors before prolonged ischemia does not limit the infarct size in open chest anesthetized rabbits (Casati et al., 1997) but limits infarct size when given immediately before reperfusion, the β-adrenoreceptor antagonists acting as free radical scavengers (Feuerstein et al., 1998; Gao et al., 2000).

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doi:10.1016/j.ejphar.2004.07.093

Ischemic preconditioning has been well recognised as an endogenous mechanism which protects hearts in all species studied, including men (Yellon et al., 1993; Ottani et al., 1995; Kloner et al., 1998). In brief, short cycles of ischemia prior to a prolonged ischemic insult render hearts more tolerant against a potentially lethal injury and reduce infarct size. In fact, other than early restoration of coronary blood flow, ischemic preconditioning is the strongest form of in vivo protection against myocardial ischemic injury (Kloner et al., 1998). Several ligands like adenosine (Liu et al., 1991), bradykinin (Goto et al., 1995), opioids (Schultz et al., 1997) and others may initiate the mechanism of preconditioning. Among the various ligands involved in preconditioning, catecholamines have been capable of initiating the cascade of protective reactions by binding to α- or β-adrenoreceptors (Tsuchida et al., 1994; Nasa et al., 1997; Lochner et al., 1999). It is of interest that the intracoronary infusion of norepinephrine limits the infarct size in anesthetized pigs in a dose related response (De Zeeuw et al., 2001). Blockade of α-adrenoreceptors abolishes the protective effect of ischemic preconditioning in man using the ST elevation changes as a surrogate end point (Tomai et al., 1997). Toombs et al., (1993) reported that protection from preconditioning was blunted in reserpine-treated rabbits because of the depletion of norepinephrine stores, while the effect of reserpine is not entirely conclusive in control and preconditioning dogs (Vander Heide et al., 1995). However, the role of β-adrenoreceptor blockade in ischemic preconditioning is not yet fully elucidated. Thus, in the present study, we sought to determine whether a short acting and a long acting β-adrenoreceptor antagonist, given i.v. for a short or a longer period of time, but always before sustained ischemia, negates the protective effect of preconditioning because of adrenoreceptor blockade. We induced preconditioning with a small number of brief ischemic periods in anesthetized rabbits in vivo.

2. Materials and methods

2.1. Materials

Commercially available atenolol (Tenormin, Astra Zeneca in 5 mg/10 ml vial) and esmolol (Brevibloc, Baxter Hellas in 100 mg/10 ml vial) were administered according to the protocol. New Zealand White male rabbits weighing between 2.7 and 3.4 kg were used in this study. All animals received proper care in compliance with the Principles of Laboratory Animal Care, formulated by the National Society for Medical Research, and the Guide for the Care and Use of Laboratory Animals, prepared by the Academy of Sciences and published by the National Institutes of Health (Institute of Laboratory Animal Resources Commission on Life Sciences, 1996).

2.2. Methods

2.2.1. Surgical preparation

All animals were anesthetized by slowly injecting 30 mg kg^{-1} of sodium thiopentone (Pentothal, Abbot) into an ear vein, intubated through a midline tracheal incision and mechanically ventilated with a positive pressure respirator for small animals (MD Industries, Mobile, AL, USA) at a rate adjusted to keep blood gases within the normal range. Two polyethylene catheters were inserted in the left jugular vein for fluids, drug administration or additional infusion of anaesthesia and in the carotid artery for continuous blood pressure monitoring via a transducer attached to a multichannel recorder (Nihon-Koden, Model 6000, Japan). A bipolar chest lead was used for continuous electrocardiographic recording. The chest was opened via a left thoracotomy in the fourth intercostal space and after pericardiotomy the beating heart was exposed. A 3–0 silk thread was passed through the myocardium around a prominent branch of the left coronary artery. Ischemia was induced by pulling the ends of the suture through a small segment of a soft tube which was firmly attached against the artery with a clamp. The successful induction of ischemia was verified by ST segment elevation on the electrocardiogram and by visual inspection (cyanosis) of the heart. Reperfusion was achieved by releasing the clamp and was verified by refilling of the artery.

2.2.2. Medication

We used two cardio-selective β-adrenoreceptor antagonists, one with a long and one with a short half-life. The doses of the drugs were properly adjusted according to body weight and our previous experience.

- Atenolol, with an elimination half-life of 6–9 h, given as a 5-min infusion at a rate of 0.2 mg min^{-1}, for acute intravenous administration, and as a 45-min infusion at a rate of 0.088 mg min^{-1}, for prolonged intravenous administration.
- Esmolol, with an elimination half-life of 9 min, given as an initial dose of 500 μg kg^{-1} within 1 min and followed by a 4-min infusion at a rate of 50 μg kg^{-1} min^{-1} for acute intravenous administration, and as an initial dose of 3.4 mg within 1 min, followed by a 44-min infusion at a rate of 0.15 mg min^{1} for prolonged intravenous administration.

2.2.3. Study design

Fifty-five animals were divided into 10 groups and were all subjected to 30-min regional ischemia and 3-h reperfusion.

– Control group (n=5): the animals were subjected to ischemia and reperfusion without previous intervention.
– Ischemic preconditioning group (n=6): the rabbits were subjected to three cycles of 5 min ischemia

separated by 10-min reperfusion before the prolonged ischemia.
- Atenolol plus ischemia group (n=5): the animals received a 5-min atenolol infusion starting 20 min before the sustained ischemia.
- Atenolol plus preconditioning group (n=5): atenolol was infused as previously described for 5 min between the second and third cycle of preconditioning, starting 20 min before the sustained ischemia.
- Atenolol for a prolonged period plus ischemia group (n=5): the animals were subjected to prolonged ischemia and reperfusion, after receiving atenolol for 45 min.
- Atenolol for a prolonged period plus preconditioning group (n=5): the rabbits received a 45-min infusion, during the three cycles of 5-min ischemia separated by 10 min of reperfusion before the prolonged ischemia.
- Esmolol plus ischemia group (n=7): the rabbits received a 5-min esmolol infusion starting 20 min before the onset of sustained ischemia.
- Esmolol plus preconditioning group (n=7): esmolol was infused as previously described for 5 min between the second and third cycle of preconditioning, starting 20 min before the sustained ischemia.
- Esmolol for a prolonged period plus ischemia group (n=5): the animals were subjected to prolonged ischemia and reperfusion, after having received esmolol intravenously for 45 min.
- Esmolol for a prolonged period plus preconditioning group (n=5): the rabbits received a 45-min esmolol infusion, during the three cycles of 5-min ischemia separated by 10 min of reperfusion before the prolonged ischemia.

The protocol is shown in Fig. 1.

We used this protocol based on indications that there are differences in protection between one and three cycles of preconditioning (Sandhu et al., 1997). Thus, we tested the effect of a short-acting and a long-acting β-adrenoreceptor antagonist before all three preconditioning stimuli and before the last stimulus.

2.2.4. Hemodynamics

Heart rate and blood pressure were continuously monitored and measured at baseline (5 min before sustained ischemia), in the middle of sustained ischemia (15th minute) and at the end of the long reperfusion period (180th minute).

2.2.5. Risk area and infarct size

After 3 h of reperfusion, the hearts were harvested, mounted on a reperfusion apparatus and perfused (50 mm Hg) for 2 min retrogradely via the aorta with normal saline (15 ml min^{-1}, 20 °C). When all residual blood had been removed from the coronary arteries, the coronary ligature was retightened at the same site and 5 ml of Zn–Cd fluorescent particles (1–10 μm diameter, Duke Scientific, Palo Alto, CA, USA), suspended in saline, were infused over 5 min for the delineation of the normally perfused tissue from the risk zone. Hearts were then frozen at −20 °C and 24 h later, were sliced into 3-mm-thick sections from the apex to base. The slices were incubated in 1% triphenyl tetrazolium chloride (TTC) in

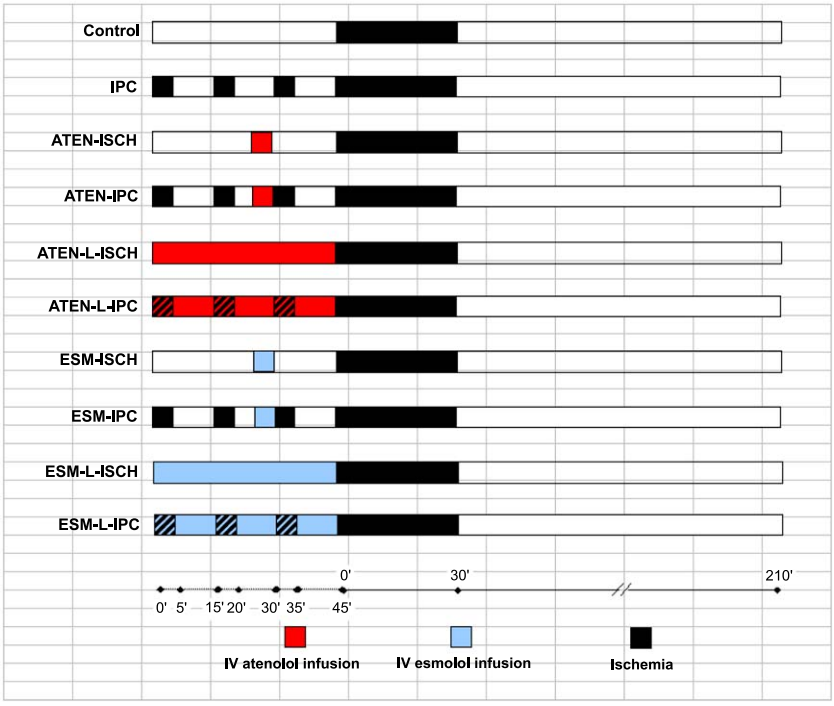

Fig. 1. Diagrammatic representation of the experimental protocol. ISCH: ischemia, IPC: ischemic preconditioning, ATEN: atenolol, ESMO: esmolol, L: prolonged.

Table 1
Mean values±S.E.M. expressed in cm^3 of the areas of infarct and risk in the various study groups

	N	Infarct size	Risk area
Control	5	0.69±0.07	1.46±0.14
IPC	6	0.23±0.05[a]	1.41±0.14
ATEN-ISCH	5	0.56±0.05	1.33±0.17
ATEN-IPC	5	0.18±0.04[a]	1.45±0.14
ATEN-L-ISCH	5	0.52±0.07	1.42±0.14
ATEN-L-IPC	5	0.28±0.04	1.33±0.12
ESMO-ISCH	7	0.74±0.16	1.47±0.11
ESMO-IPC	7	0.24±0.05[a]	1.38±0.12
ESMO-L-ISCH	5	0.56±0.18	1.51±0.13
ESMO-L-IPC	5	0.16±0.05[a]	1.38±0.13

ISCH: ischemia, IPC: ischemic preconditioning, ATEN: atenolol, ESMO: esmolol, L: prolonged.
[a] $P<0.05$ vs. control group.

isotonic phosphate buffer solution, pH 7.4 for 20 min at 37 °C. TTC reacts with dehydrogenate enzymes and nicotinamide adenine dinucleotide in viable tissue; the infarcted area was defined as the negative staining region. The heart slices were immersed in 10% formaldehyde solution for 24 h to delineate the infarcted areas more clearly. For examination, the slices were pressed between glass plates; to identify the borders between the risk zone and the normal area, slices were examined under ultraviolet light (wavelength 366 nm). The infarcted, the risk and the normal areas were traced onto an acetate sheet which had been placed over the top glass plate. The tracings were then photographically enlarged (Adobe Photoshop 6.0) and were quantified by planimetry with the aid of the Scion Image program interfaced with a computer. The areas of myocardial tissue at risk and of infarction were automatically transformed into volumes by multiplying the corresponding areas by thickness (3 mm). Infarct and risk area volumes were expressed in cm^3 and the percent of infarct to risk area calculated as previously described (Iliodromitis et al., 1997).

2.2.6. Statistical analysis

Values are expressed as mean±S.E.M. All tests were computed with the CSS-Statistical Software package. Differences in infarct to risk ratio in percent were assessed using one way analysis of variance (ANOVA) for each variable between groups. Hemodynamic parameters were evaluated using two-factor ANOVA (for group and time) with repeated measures across the second factor. Post-hoc analysis with the least significant difference test was used for the statistical comparisons. A calculated P value less than 0.05 was considered to be statistically significant.

3. Results

3.1. Mortality and exclusions

Of the 61 animals entered into the study 6 animals—from different groups—died, because of pump failure, intractable ventricular fibrillation or technical reasons, and were excluded from the study. Therefore, 55 animals completed the study successfully.

3.2. Hemodynamic variables

Hemodynamic variables were not different at baseline. There was a trend for slower heart rate and mean blood pressure during ischemia in some groups which received atenolol or esmolol compared to the baseline. Although the changes in mean blood pressure reached statistical significance in some groups treated with β-adrenoreceptors antagonists, the relative change in blood pressure was not statistically different between all the groups. Moreover, we did not observe significant differences in blood pressure between all the groups at the time of ischemia and reperfusion.

3.3. Infarct size

Table 1 details the infarct size and the risk zone size for the various study groups. No significant differences were detected in risk areas between groups. In contrast, significant differences existed in the infarct zones.

Fig. 2 shows the infarct to risk ratio in percent. As shown, the control group, which was subjected to extended ischemia without beta blockade or preconditioning, developed infarcts, which represented 47.3±2.5% of the risk zone size. As shown in the preconditioning group (with three cycles of ischemia), the infarct size was significantly smaller in comparison to the control group (15.7±2.9%, $P<0.01$).

It is remarkable that the atenolol plus ischemia and esmolol plus ischemia groups, in which atenolol or esmolol were given intravenously for a 5-min period, shortly before the prolonged ischemia, developed infarcts which represented 43.2±4.1% and 47.7±6.4% of the risk zone, correspondingly (P=NS vs. control). We also did not find

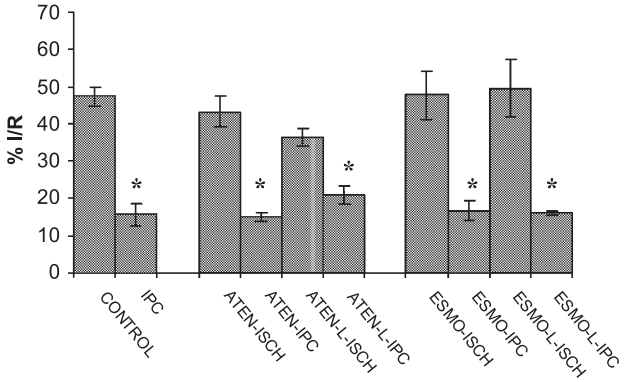

Fig. 2. The effect of β-adrenoreceptor blockade on infarct size, expressed as a percentage of risk zone size, after sustained ischemia–reperfusion and ischemic preconditioning. $P<0.01$ vs. control group. ISCH: ischemia, IPC: ischemic preconditioning, ATEN: atenolol, ESMO: esmolol, L: prolonged.

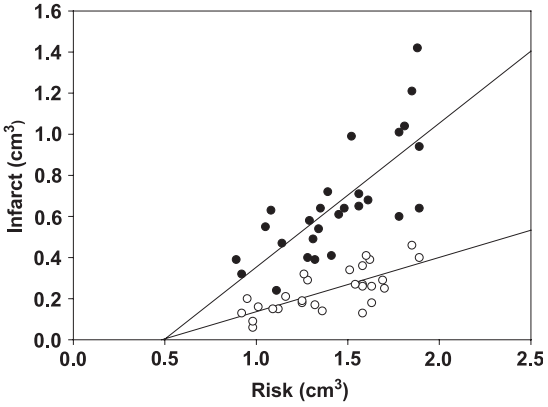

Fig. 3. Absolute infarct volume plotted against absolute risk zone volume in rabbit hearts. Closed symbols depict individual hearts from control and open symbols depict preconditioned hearts.

a significant reduction of infarct size in the atenolol for a prolonged period plus ischemia and esmolol for a prolonged period plus ischemia groups, which were treated with a prolonged infusion of atenolol, or esmolol, before sustained ischemia–reperfusion (36.3±2.4% and 49.6±7.6%, respectively, P=NS vs. control).

Infarct size was small in the other four preconditioned groups and the addition of β-adrenoreceptor antagonists, independently of the mode of administration (prolonged or short), did not change the infarct size (atenolol plus preconditioning group: 14.9±1.2%, atenolol for a prolonged period plus preconditioning group: 21.0±2.2%, esmolol plus preconditioning group: 16.7±2.4%, esmolol for a prolonged period plus preconditioning group: 16.0±0.5%, P<0.01 vs. control).

Interestingly, the infarct size was smaller in all the preconditioning groups than the β-adrenoreceptor antagonists-treated groups, showing that preconditioning is a stronger form of protection than the acute, either short or prolonged, infusion of β-adrenoreceptor antagonists (Fig. 3).

4. Discussion

This study demonstrates that acute, either short or prolonged, β-adrenoreceptor blockade with a long or short acting β-adrenoreceptor antagonist does not adversely affect the classic ischemic preconditioning in vivo, which remains a powerful mode of protection against irreversible ischemic injury.

β-Adrenoreceptor antagonists have been routinely used in clinical practice in patients with coronary heart disease and the various preparations of these drugs appear to have equal efficacy (Prissant et al., 1994). Ischemic preconditioning seems to have clinical relevance and is the strongest mode of protection other than early reperfusion (Kloner et al., 1998). Among the various ligands involved in the mechanism of protection are adenosine (Liu et al., 1991; Tsuchida et al., 1992; Lim and Laskey, 1997), bradykinin (Goto et al., 1995), opioids (Schultz et al., 1997), catecholamines (Banerjee et al., 1993; Tsuchida et al., 1994; Lochner et al., 1999) and others (Wang et al., 1996; Tritto et al., 1997; Htun et al., 1998; Iliodromitis et al., 1998). However, there are differences in triggers between species; adenosine does not initiate the mechanism of preconditioning in rats (Liem et al., 2001; Li and Kloner, 1993) but has a significant role in many other species, including man (Walker et al., 1995). Furthermore, there are controversies over the "frequency-dependency" of preconditioning (Cohen et al., 1994; Iliodromitis et al., 1997; Lawson et al., 1993) and the intensity of the initial trigger (Liu et al., 1991; Sandhu et al., 1997; Schultz et al., 1998). Since various ligands of varying potency are capable of initiating the mechanism of preconditioning, they must often act synergistically in order to reach a certain threshold level which triggers the mechanism of protection (Goto et al., 1995). Regarding the intracellular mediators, it appears that the stimulation of one or more receptors results in subsequent phosphorylation or activation of many "in parallel" or "in series" mediators (Schulz et al., 2001). Catecholamines activate phospholipases C and D and induce translocation of protein kinase C from its inactive form in the cytoplasm to its active form on the cellular membrane (Talosi and Kranias, 1992; Cohen et al., 1996; Yabe et al., 1998). This event cascade appears to play a pivotal role in rabbit, as well as in human hearts, rendering them more tolerant against irreversible damage (Speechly-Dick et al., 1995; Hu et al., 1996).

Stimulation of α-adrenoreceptors, in rabbit hearts (Tsuchida et al., 1994) or human atrial trabeculae (Cleveland et al., 1997) and activation of β-adrenoreceptors by i.v. isoproterenol in isolated perfused rat hearts (Yabe et al., 1998; Lochner et al., 1999) mimic ischemic preconditioning. Thus, one could assume that α- or β-adrenoreceptor antagonists would adversely affect the process of ischemic preconditioning. Indeed, phentolamine, an α-adrenoreceptor antagonist, negates preconditioning during coronary angioplasty in humans (Tomai et al., 1997). However, little is known regarding the interference of β-adrenoreceptor blockade in the mechanism of ischemic preconditioning and conflicting reports exist as to whether β-adrenoreceptor blockade prevents ischemic preconditioning. Thornton et al. (1993) have shown that, although catecholamines can induce adenosine receptor-mediated protection in rabbits, this benefit is not abolished by the β-adrenoreceptor antagonist propranolol. Cohen et al. (1996) reported that propranolol prevents ischemic preconditioning in isolated rabbit hearts not by blocking the adrenoreceptors but, interestingly, by blocking the diacylglycerol production initiated by the stimulation of adenosine receptors. In contrast, Yabe et al. (1998), who demonstrated that cardioprotection induced by β-adrenoreceptor agonist preconditioning, like ischemic-preconditioning, is mediated by protein kinase C activation in isolated rat hearts, found that

timolol, another β-adrenoreceptor antagonist, did not prevent the protection afforded by ischemic preconditioning in the same experimental model. Thus, they claimed that preconditioning with short ischemia and preconditioning with β-adrenoreceptor agonists may be exerted through distinct mechanisms, although these may share certain intracellular signals. Interestingly, Nasa et al. (1997), who suggest that a brief period of β-adrenoreceptor stimulation exerts the preconditioning-like protective effect against post-ischemic contractile dysfunction in perfused rat hearts, found that pre-treatment with the β-adrenoreceptor antagonist timolol abolished only the α-adrenergic-induced and not the β-adrenergic-induced preconditioning. The reason for these divergent results is unclear, but may be the variety in the second messenger systems, which are activated under different experimental conditions (Saurin et al., 2000; Garcia-Dorado et al., 2002; Iliodromitis et al., 2003). However, the mode of administration of the β-adrenoreceptor antagonist is also important in evaluating its role in preconditioning; Lochner et al. (1999) assessed in an isolated perfused rat heart model, the β-adrenoreceptor characteristics at different times during the ischemia–reperfusion protocol with or without preconditioning and concluded that ischemia-induced activation of the β-adrenergic signaling pathway during preconditioning should also be considered a trigger in eliciting the mechanism of protection.

In the present study, we examined whether acute, short or prolonged, intravenous β-adrenoreceptor blockade by atenolol (a long half-life β-adrenoreceptor antagonist), or by esmolol (a short half-life β-adrenoreceptor antagonist) may adversely affect the protection conferred by ischemic preconditioning. Our data are in agreement with most of the above studies since we demonstrated, for the first time in vivo, that β-adrenoreceptor blockade does not seem to interfere with the mechanism of classic ischemic preconditioning. Notably, we found that both β-adrenoreceptor antagonists, used either before all three short bouts of ischemia or before the last, do not abolish the protection afforded by ischemic preconditioning in vivo. Although we do not have data on markers for the mediators of intracellular signaling, our findings may be of importance in clinical practice. We suggest that patients taking β-adrenoreceptor antagonists and who suffer transient episodes of non-lethal short ischemic insults prior to myocardial infarction may still benefit by preconditioning. It is well recognized that, at the time of the short ischemic insults, a number of ligands—other than catecholamines—trigger the protective mechanism when a certain concentration is exceeded (Goto et al., 1995) and overcome a possible blockade of β-adrenoreceptors. However, the intravenous treatment with a β-adrenoreceptor antagonist with a long or short elimination half-life, given shortly before or during a more prolonged period, but without preconditioning, does not reduce the infarct size when the heart is exposed to a sustained period of ischemia.

In conclusion, intravenous atenolol and esmolol, independent of timing of administration, maintain the protective effect of ischemic preconditioning in vivo. Neither short nor prolonged β-adrenoreceptor blockade with a long or a short acting β-adrenoreceptor antagonist adversely affects protection conferred by ischemic preconditioning.

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