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Annexin A5 Scintigraphy of Forearm as a Novel In Vivo Model of Skeletal Muscle Preconditioning in Humans

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Background—Nonlethal ischemia and reperfusion reduce ischemia-reperfusion–induced cell death, a phenomenon called ischemic preconditioning. In animal models, this potent endogenous protection is mimicked in vivo by administration of adenosine. In humans, exploitation of ischemic preconditioning is hindered by the lack of an appropriate in vivo model to study this phenomenon. To solve this problem, we aimed to set up an easy-to-use human in vivo model to study ischemic or pharmacological preconditioning.

Methods and Results—Healthy male volunteers performed unilateral ischemic handgrip. At reperfusion, we intravenously injected technetium-99m–labeled Annexin A5, a presumed marker of ischemic injury, and we imaged both forearms and hands simultaneously with a gamma camera. Region of interest analysis (counts per pixel) and subsequent calculation of the percentage difference in radioactivity between experimental and control hands (thenar muscle; mean±SE) revealed significant uptake to the ischemically exercised tissue ($26\pm3\%$ at 4 hours after reperfusion; $P<0.05$). This selective localization of Annexin A5 was reduced by ischemic preconditioning (10 minutes of ischemia plus reperfusion before ischemic exercise) or by infusion of adenosine into the brachial artery to $6\pm1\%$ and $10\pm3\%$, respectively ($P<0.05$ versus ischemic exercise alone), resembling observations in animal models with infarct size as an end point. Appropriate control experiments supported our conclusion.

Conclusions—Annexin A5 scintigraphy can be applied to test pharmacological or physiological interventions for their ability to prevent ischemia-reperfusion injury. (*Circulation*. 2005;111:173-178.)

Key Words: adenosine ■ exercise ■ ischemia ■ scintigraphy

Ischemic preconditioning is defined as increased tolerance against ischemia-reperfusion injury resulting from a previous short exposure to ischemia.¹ Since the original description of this phenomenon by Murry et al¹ in 1986 in the canine heart, the underlying mechanism of action has partially been revealed.² Ischemia-induced release of adenosine activates adenosine receptors (A₁ and A₃) on cardiomyocytes, followed by activation of protein kinase C, ultimately resulting in opening of mitochondrial ATP-sensitive potassium channels.

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Influx of potassium and depolarization of the mitochondrial inner membrane delay cell death during subsequent periods of ischemia, probably by inducing a small increase in mitochondrial volume.³ Ischemic preconditioning reduces the release of mitochondrial cytochrome C and prevents apoptosis.^{4–6} Whether this explains the full protective action of ischemic preconditioning is not known. Apart from the heart, other organs such as liver, brain, and skeletal muscle are protected by ischemic preconditioning in various species such as rat, pig, dog, and rabbit.^{7–9} These findings suggest a

universal endogenous protective phenomenon against the deleterious sequelae of ischemia.

The mechanism underlying ischemic preconditioning suggested the possibility of pharmacological modulation of cellular tolerance to ischemia-reperfusion injury. In animal models, various drugs that are currently used in clinical practice to prevent or treat acute ischemic events interfere with ischemic preconditioning or modulate ischemia-reperfusion injury. For example, adenosine and opiates mimic ischemic preconditioning (pharmacological preconditioning),^{10,11} whereas ATP-sensitive potassium channel blockers and adenosine receptor antagonists inhibit ischemic preconditioning.¹² Insulin and statins reduce ischemia-reperfusion injury by a mechanism that is, at least partially, independent from the signal transduction pathway of ischemic preconditioning.^{13,14} Knowledge of the effect of (cardiovascular) drugs on ischemia-reperfusion injury will increase rational pharmacotherapy in patients who are at risk for ischemic events. Unfortunately, translation of these observations from animals to humans is hindered by the lack of a specific model to study ischemia-reperfusion injury in conscious humans in vivo.¹⁵

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Recently, Annexin A5 scintigraphy has been developed to detect early signs of cell death in humans *in vivo*.^{16,17} Annexin A5 is an endogenous protein that binds with high affinity to negatively charged phosphatidylserine.¹⁸ Because of an active translocase, phosphatidylserine is located almost exclusively on the inner leaflet of the lipid bilayer of the normal cell membrane. Early in the process of apoptosis, the asymmetric distribution of phosphatidylserine is lost, and phosphatidylserine is exposed on the outer surface of the cell, thus providing binding sites for extracellular Annexin A5.¹⁹ In mice, 90 minutes of hindlimb ischemia followed by a 3-hour period of reperfusion induced apoptosis of vascular cells and skeletal muscle fibers, which was reduced by a caspase inhibitor.²⁰ To the best of our knowledge, apoptosis has not been studied after shorter bouts of ischemia combined with exercise. After mild ischemia, reversible phosphatidylserine externalization has recently been reported without subsequent development of cell death.²¹ Therefore, we hypothesize that a relatively short period of exercise during local blockade of the circulation induces externalization of phosphatidylserine and binding of Annexin A5, which can be used to detect early and reversible ischemia-reperfusion injury.

The aim of this study was to validate Annexin A5 scintigraphy as a tool to detect ischemia-reperfusion injury in humans *in vivo*. We reasoned that a relevant marker of ischemia-reperfusion injury should also be able to detect the protective effect of ischemic and pharmacological preconditioning with adenosine. We selected forearm skeletal muscle for 3 reasons. First, after intravenous administration of Annexin A5, the nonischemic forearm could serve as an internal control to subtract nonspecific background activity that results from circulating radiotracer. Second, a validated model to detect ischemia-reperfusion injury of forearm skeletal muscle would allow efficient pharmacological screening by infusion into the brachial artery of drugs that could potentially modulate tolerance to ischemia-reperfusion injury. Finally, forearm ischemic load is well defined by occlusion of the forearm circulation without confounding by collateral circulation.

This study addresses 3 main questions: Does ischemic isometric muscle exercise induce local targeting of radiolabeled Annexin A5? Can this targeting be prevented by a previous bout of ischemia and reperfusion (ischemic preconditioning)? And finally, does local infusion of adenosine protect against subsequent injury from ischemic exercise?

Methods

Subjects

After giving informed consent, 43 healthy male volunteers (age, 18 to 34 years) participated in 6 separate experiments. Subjects with cardiovascular disease, hypertension (systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg measured sphygmomanometrically in supine rest), or diabetes mellitus (fasting glucose >7.0 mmol/L or random glucose >11.0 mmol/L) were excluded. Volunteers were free of medications and were asked to abstain from caffeine-containing beverages for ≥ 24 hours before the start of the experiment. The protocol was approved by the Institutional Review Board of UMC Nijmegen.

General Procedures

Volunteers were studied in the sitting position after cannulation of an antecubital vein of the dominant arm for injection of radiolabeled

Annexin A5 (see below). Maximal voluntary contraction was determined in the nondominant arm with a handgrip dynamometer (Baseline Hydrolic Hand Dynamometer, Fabrication Enterprise Inc).

Ischemic Exercise

In 10 volunteers (ischemic exercise [Isch Ex]), the circulation of the nondominant forearm was occluded for 10 minutes with an upper arm cuff inflated to 200 mm Hg. Immediately after occlusion of the forearm circulation, ischemia was combined with isometric contractions of the finger flexors at 50% of maximum voluntary contraction. These contractions were performed rhythmically: 5-second contraction followed by 5-second relaxation until the volunteer was exhausted. The total duration of ischemia was 10 minutes independent from the duration of contractions. Radiolabeled Annexin A5 (0.1 mg protein, 500 MBq technetium [Tc]-99m) was administered intravenously immediately after the start of reperfusion. Forearms were imaged at 0, 1, 2, and 4 hours after injection. For this purpose, flexor muscles of both forearms were positioned on the gamma camera (Siemens Orbiter camera equipped with low-energy, high-resolution collimators) in a pronated position and scanned simultaneously until $\geq 100\,000$ counts were detected. After imaging of the flexor muscles, the palmar sides of both hands were placed on the gamma camera, and images of $\geq 50\,000$ counts were detected.

Ischemic Preconditioning Followed by Ischemic Exercise

In a separate group of 8 volunteers, ischemic exercise was preceded by a 10-minute period of forearm ischemia and a 10-minute period of reperfusion without simultaneous exercise (IP+Isch Ex). Pilot studies (data not shown) indicated that this period of ischemia without concomitant exercise did not induce targeting of Annexin A5. Immediately after ischemic exercise, Annexin A5 was infused, and scintigraphic images were performed as described for the Isch Ex group.

Adenosine Followed by Ischemic Exercise

In a third group of 9 volunteers, the brachial artery was cannulated ≥ 30 minutes before drug infusion. In this group, adenosine ($50\ \mu\text{g} \cdot \text{min}^{-1} \cdot \text{dL}^{-1}$ forearm tissue; Adenocor, Sanofi-Synthelabo Inc) was infused into the brachial artery for 10 minutes beginning 20 minutes before the start of the ischemic exercise (ADO+Isch Ex). Immediately after ischemic exercise, Annexin A5 was infused, and scintigraphic images were performed as described for the Isch Ex group. In this group, the measurement at 2 hours after reperfusion was omitted.

Phentolamine Followed by Ischemic Exercise

In a separate group of 10 volunteers, phentolamine ($15\ \mu\text{g} \cdot \text{min}^{-1} \cdot \text{dL}^{-1}$ forearm tissue; Regitine, Novartis Pharma Inc) was infused instead of adenosine to study the effect of vasodilation per se on subsequent ischemia-reperfusion injury (PHENT+Isch Ex). In this group, the measurements at 0 and 2 hours after reperfusion were omitted for logistical reasons. Otherwise, the same protocol as in the ADO+Isch Ex group was performed.

Uptake of Tc-99m-Labeled Albumin After Ischemic Exercise

In an additional group of 3 volunteers, nonspecific mechanisms of targeting such as changes in capillary permeability or blood flow were explored. For this purpose, these individuals performed ischemic exercise as described above. Instead of Annexin A5, an equimolar dose of Tc-99m-labeled albumin was injected immediately on reperfusion. Subsequently, the forearms were scanned at 1 and 4 hours after reperfusion as described above.

Delayed Injection of Annexin A5 After Ischemic Exercise

Finally, the influence of timing of the Annexin A5 injection was studied in 3 volunteers. This group performed ischemic exercise as described above. In this group, Annexin A5 (0.1 mg protein, 500 MBq Tc-99m) was injected 1 hour after reperfusion instead of

immediately on reperfusion. Both forearms were scanned immediately and 3 hours after injection of Annexin A5 (1 and 4 hours after reperfusion) as described above.

Preparation of Radiopharmaceuticals

Radiolabeled Annexin A5 was freshly prepared before each experiment by adding Tc-99m Pertechnetate (1500 MBq) in the presence of stannous tricine to succinylhydrazinonicotinamide (HYNIC)-conjugated recombinant human Annexin A5 (NAS 2020, 0.275 mg per vial; Theseus Imaging Corp). Radiochemical purity as checked by instant thin layer chromatography was always >95%. The labeled Annexin A5 solution was filtered with a low-protein-binding 0.22- μ m filter (Millipore No. SLGV-0.25 BS, Millex GV). Finally, 500 MBq of the labeled Annexin A5 (0.1 mg) was further diluted with NaCl 0.9% and immediately administered to the volunteer.

Radiolabeled albumin was prepared analogous to radiolabeled Annexin A5. Human serum albumin (200 mg/mL; Cealb, Sanquin CLB) was conjugated with HYNIC and stored in vials containing 0.6 mg HYNIC-conjugated albumin (approximately equimolar amount of protein compared with the Annexin A5 vials). Radiolabeled albumin was freshly prepared before each experiment by adding Tc-99m Pertechnetate (1500 MBq) in the presence of stannous tricine to a vial. Radiochemical purity as checked by ITLC was always >95%. The labeled albumin solution was filtered with a low-protein-binding 0.22- μ m filter (Millex GV). Finally, 500 MBq of the labeled albumin (0.2 mg) was further diluted with NaCl 0.9% and immediately administered to the volunteer.

Data Analysis

All the digitized gamma camera images were analyzed offline by the same investigator (W.O.) using Siemens ICON software. Two regions of interest were drawn in each forearm representing flexor muscles and thenar muscles. Special care was taken to avoid the major veins and arteries in the region of interest. Radioactivity was expressed as counts per pixel. To correct for background activity, the final result was expressed as the percentage difference between the experimental (nondominant) arm and control arm (targeting): $\text{targeting} = (\text{ROI}_{\text{experimental arm}} - \text{ROI}_{\text{control arm}}) \times 100\% / \text{ROI}_{\text{control arm}}$, where ROI represents region of interest (flexor or thenar muscle). The effect of ischemic exercise on targeting of Annexin A5 was statistically analyzed with a repeated-measures ANOVA with time as a within-subject factor and group as a between-subject factor. For this analysis, the first time point (immediately after reperfusion) was excluded because of the confounding effect of postischemic reactive hyperemia. Because this analysis revealed a significant interaction between time and group, results were also expressed as a change in targeting from 1 to 4 hours after reperfusion. For each time point, post hoc analysis was performed with 1-way ANOVA followed by Scheffé's test for post hoc comparisons (SPSS for Windows, release 10.0.7, SPSS Inc). Pilot studies have shown that forearm blood flow as measured by plethysmography increased from 5 ± 1 to 33 ± 3 mL \cdot min⁻¹ \cdot dL⁻¹ forearm (n=6, mean \pm SE) directly after ischemic exercise and reduced toward baseline levels within 1 hour.

Results

Groups were similar with respect to age, weight, height, blood pressure, random plasma glucose concentration, maximal voluntary handgrip force, and duration of the ischemic exercise. All volunteers quickly and uneventfully recovered from the ischemic exercise.

Directly after injection of radiolabeled Annexin A5, a large increase in activity was observed in all 3 groups in the thenar muscles of experimental compared with the control arm ($69 \pm 4\%$, $63 \pm 8\%$, and $78 \pm 13\%$ in Isch Ex, IP+Isch Ex, and ADO+Isch Ex, respectively; $P=0.50$ for between-group comparison, 1-way ANOVA). This initial increase almost completely disappeared at 1 hour after reperfusion in all

Course in Annexin A5 Targeting

	Time After Reperfusion, h			
	0	1	2	4
Thenar muscle				
Isch Ex (n=10)	69 \pm 4	19 \pm 1	23 \pm 2	26 \pm 3*
IP+Isch Ex (n=8)	63 \pm 8	9 \pm 3†	8 \pm 2‡	6 \pm 1‡
ADO+Isch Ex (n=9)	78 \pm 13	15 \pm 2	...	10 \pm 3‡
PHENT+Isch Ex (n=10)	...	21 \pm 7	...	25 \pm 7*§
Flexor muscle				
Isch Ex (n=10)	63 \pm 6	5 \pm 1	7 \pm 1	9 \pm 2*
IP+Isch Ex (n=8)	63 \pm 5	4 \pm 1	3 \pm 1	2 \pm 2
ADO+Isch Ex (n=9)	86 \pm 9	8 \pm 3	...	6 \pm 3
PHENT+Isch Ex (n=10)	...	7 \pm 2	...	8 \pm 2

Values shown are percent difference between experimental and control sides (mean \pm SE). Overall ANOVA for repeated measurements showed a significant interaction between group and time ($P=0.000$ and $P=0.006$ for thenar and flexor muscle, respectively).

* $P<0.01$ for effect of time (ANOVA for repeated measurements; only t=1 and t=4 included in this analysis).

† $P<0.05$, ‡ $P<0.01$ vs Isch Ex (1-way ANOVA, followed by Scheffé's post hoc test; PHENT+Isch Ex was excluded from this analysis).

§ $P<0.005$ vs ADO+Isch Ex for interaction between time and group (repeated-measures ANOVA).

groups and is interpreted as the result of postocclusive hyperemia (the Table).

At the end of the subsequent 3 hours, the annexin labeling in the ischemic hand of the Isch Ex group was $26 \pm 3\%$ greater than in the control hand. In contrast, after ischemic preconditioning (IP+Isch Ex) and after previous infusion of adenosine (ADO+Isch Ex), this difference in activity further decreased to $6 \pm 1\%$ and $10 \pm 3\%$, respectively (the Table and Figure 1). Consequently, the increase over time of Annexin A5 targeting from 1 to 4 hours after reperfusion was significantly blunted in the IP+Isch Ex and ADO+Isch Ex groups compared with the Isch Ex group (Figure 2).

To assess the effect of vasodilation per se, adenosine was replaced by phentolamine (PHENT) in an extra series of experiments. In this PHENT+Isch Ex group, Annexin A5 targeting increased over time, resulting in a difference between the 2 hands of $25 \pm 7\%$, a number closely resembling the results of the Isch Ex group. As a consequence, Annexin A5 targeting in the PHENT+Isch Ex group was significantly higher compared with the IP+Isch Ex and ADO-Isch Ex group (the Table and Figure 2).

In contrast to Annexin A5, Tc-99m-albumin was not retained in the experimental hand after ischemic exercise. The difference in activity between the hands was $2 \pm 0.5\%$ (range, 1% to 3%) and $0 \pm 1\%$ (-3% to 1%) at 1 and 4 hours of reperfusion, respectively (n=3) and significantly differed from the Isch Ex group, in which Tc-99m-Annexin A5 was used instead of albumin (mean \pm SE; $P<0.01$ for effect of group; $P<0.05$ for interaction between group and time, repeated-measures ANOVA).

In a final control experiment, Tc-99m-Annexin A5 was not injected directly after reperfusion but 1 hour later. With this approach, Annexin A5 targeting to the thenar muscle was

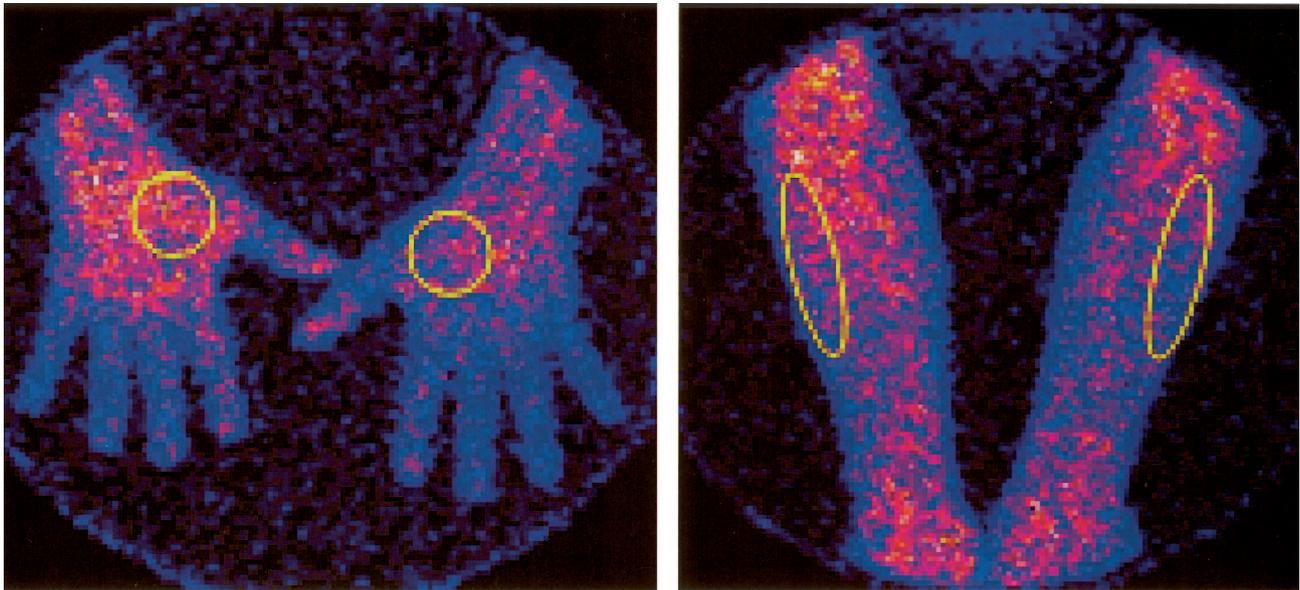


Figure 1. Typical image of thenar (left) and flexor (right) muscle at 4 hours of reperfusion. Regions of interest are marked. In this individual, percentage difference in radioactivity (counts per pixel) between experimental and control sides was 37% and 10% for thenar and flexor muscle, respectively. In this particular experiment, ischemic exercise was performed without previous preconditioning. Exercise is required during ischemia for targeting of annexin A5. In addition, distribution of radioactivity is in accordance with uptake in muscle instead of bone or skin.

only $7 \pm 2\%$ (range, 5% to 11%) at 4 hours after reperfusion ($n=3$), which was significantly different from the $26 \pm 3\%$ (see the Table, Isch Ex group) when Annexin A5 was injected immediately on reperfusion ($P < 0.01$ for effect of group, repeated-measures ANOVA).

In general, targeting of radiolabeled Annexin A5 showed a similar pattern for the flexor muscle, although less pronounced than for the thenar muscle (the Table and Figure 2). Results did not relevantly change when the statistical analysis was refined with workload as a covariate.

Discussion

This study reveals 3 novel findings: (1) Ischemic handgrip increases uptake of Annexin A5 in the hand and forearm; (2) this targeting of Annexin A5 is prevented by a previous bout of 10 minutes of ischemia and 10 minutes of reperfusion (ischemic preconditioning); and (3) intra-arterial infusion of adenosine provided protection against ischemia-reperfusion injury (pharmacological preconditioning). These observations were most pronounced in the thenar muscle of the hand but also occurred in the flexor muscle and resemble the effect of ischemic preconditioning and intra-arterial infusion of adenosine on infarct size in skeletal muscle of pigs after a more prolonged ischemic insult.^{9,10} Thus, our observations are in accordance with our hypothesis that Annexin A5 scintigraphy validly detects ischemia-reperfusion injury, ischemic preconditioning, and pharmacological modulation of ischemia-reperfusion injury.

We did not observe retention of radiolabeled albumin in the experimental arm, indicating that the observed targeting of Annexin A5 is not caused by nonspecific targeting such as changes in vascular permeability or reactive hyperemia. Delay of the Annexin A5 injection by 1 hour did not result in uptake of Annexin A5. Finally, in a pilot study in 6 healthy volunteers, the AV difference in plasma creatine kinase

activity across the experimental forearm did not change in response to this ischemic exercise, excluding relevant skeletal muscle necrosis (data not shown). Taken together, these observations are fully compatible with a short-lasting availability of Annexin A5 binding sites after this relatively mild ischemic stimulus and may provide an argument against the occurrence of apoptosis in this model. This finding contrasts with the long-lasting availability of Annexin A5 binding sites after myocardial infarction or apoptosis as observed by others.^{16,17,19,22} Our observation is in agreement, however, with reversible availability of externalized phosphatidylserines in response to mild hypoxia or ischemia as has been reported by others.^{21,23,24}

Alternatively, one might argue that targeting of Annexin A5 in our experiments is driven mainly by reactive hyperemia. Although this concept provides an explanation for the lack of uptake of Annexin A5 when injected 1 hour after reperfusion, it does not explain why Annexin A5 targeting was prevented by ischemic preconditioning or adenosine infusion. Reactive hyperemia was similar for the 3 groups, as reflected by a similar distribution of Annexin A5 directly after reperfusion; nevertheless, significant differences in retention of Annexin A5 were observed 4 hours after reperfusion. Our interpretation that reactive hyperemia is not significantly involved in targeting of Annexin A5 after ischemic exercise is further supported by the lack of uptake of Tc-99m-albumin. The observed action of ischemic and pharmacological preconditioning on Annexin A5 targeting after ischemic exercise closely resembles the effect of these interventions on infarct size as demonstrated in various animals and organs, including skeletal muscle,^{9,10,25} and a clinical trial in the heart.²⁶ This further indicates that this technique detects relevant early signs of ischemic exercise and reperfusion-induced injury and can be used for future pharmacological research to evaluate agents intended to protect against ischemia-reperfusion injury in humans in vivo.

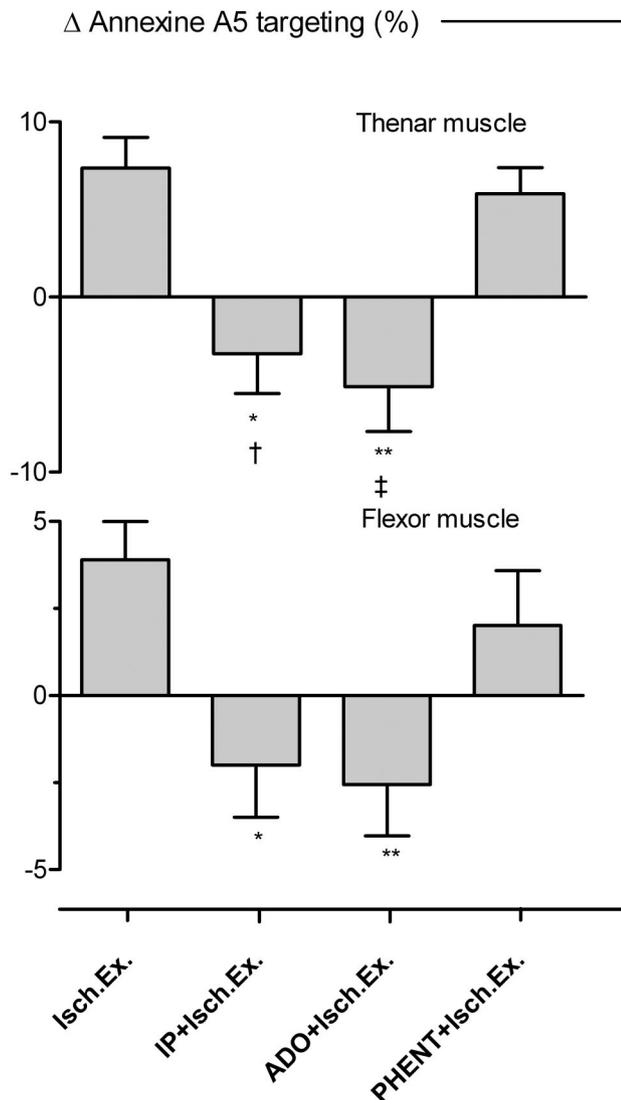


Figure 2. Change in percentage difference between experimental and control sides from 1 to 4 hours after reperfusion. * $P < 0.05$ vs Isch Ex; ** $P < 0.01$ vs Isch Ex; † $P < 0.05$ vs PHENT+Isch Ex; ‡ $P < 0.01$ vs PHENT+Isch Ex.

In this study, we used phentolamine as a control vasodilator for adenosine. In contrast to other vasodilators such as nitric oxide donors or calcium channel blockers, phentolamine does not have a known direct effect on cellular tolerance against ischemia-reperfusion injury. It should be emphasized, however, that the vasodilator action of phentolamine is limited given a certain baseline α -adrenergic tone. We did not measure the vasodilator response to adenosine and phentolamine in these experiments. It is well known from previous studies with these drugs that forearm blood flow measured with strain-gauge plethysmography increases by a factor of 3 to 5 in response to phentolamine^{27,28} and by a factor of 10 in response to adenosine.²⁹ Therefore, our observation with phentolamine indicates that an increase in flow before ischemic exercise to $\approx 10 \text{ mL} \cdot \text{min}^{-1} \cdot \text{dL}^{-1}$ forearm tissue does not affect Annexin A5 scans after ischemic exercise. However, our observation does not exclude that part of the protective action of adenosine could

involve vasodilation, eg, by increasing shear stress and subsequent release of nitric oxide before the ischemic exercise. Therefore, the mechanism of protection of exogenous adenosine may be different from ischemic preconditioning. However, this possibility also occurs in the animal studies exploring the protective action of adenosine in the heart or skeletal muscle with infarct size as an end point. The present study was not intended to elucidate the mechanism of adenosine-induced protection.

Models previously used to study ischemic preconditioning in humans have important pitfalls. Epidemiological studies have shown that preinfarct angina reduces infarct size.^{30–32} However, these studies may have been biased by differences in reperfusion time, which is significantly shorter in patients with preinfarct angina.³³ Repeated PTCA reduces lactate formation and ischemia-associated ECG changes. Like infarct size in animal models, these surrogates for ischemia-reperfusion injury are responsive to adenosine receptor antagonists and glibenclamide.^{34,35} However, possible recruitment of collateral circulation, which could be reduced by the pharmacological treatment, complicates interpretation of these observations. Changes in venous lactate do not necessarily reflect differences in ischemic injury but may result from differences in ischemic load. Observations in animals indicate that ECG is a poor surrogate end point to detect ischemia-reperfusion injury, especially when pharmacological interventions such as glibenclamide interfere with the electrophysiological properties of the sarcolemma.³⁶ Finally, repeated PTCA is a complicated procedure with inherent risk that makes this model difficult to use for pharmacological screening in humans in vivo. An elegant model of ischemic preconditioning is restricted to patients undergoing coronary artery bypass surgery.³⁷ This limitation hinders efficient screening of pharmacological or physiological interventions for their potential to interfere with ischemic preconditioning in conscious persons. In contrast to the limitations of these methods, ischemic forearm exercise followed by Annexin A5 scintigraphy is not biased by collateral circulation, detects membrane changes that directly result from ischemia-reperfusion injury, and can easily be applied to volunteers with minimal risk of serious complications.

In conclusion, Annexin A5 scintigraphy reliably detects ischemia-reperfusion injury and a protective effect of interventions known to reduce infarct size in skeletal muscle. Our observations support the use of this model as a possible screen for pharmacological interventions that aim at reducing ischemia-reperfusion injury in clinically relevant organs such as the heart or brain. For this purpose, however, further research, including elucidation of signal transduction pathways, is needed to validate extrapolation of findings in the forearm to other organs.

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Disclosure

At the time that these studies were performed, Dr Steinmetz was vice president and medical director of Theseus Imaging Corporation, a company that develops technetium-labeled Annexin A5 as a tool to visualize apoptosis in humans in vivo. Otherwise, none of the authors have any competing financial interest related to this study.

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