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Myocardial preconditioning and remote renal preconditioning

Identifying a protective factor using proteomic methods?

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Abstract It is still unknown whether remote ischemic preconditioning is mediated by a humoral or a neurogenic mechanism from the preconditioning to the preconditioned tissue. The purpose of the following study was to identify a possible humoral trigger of ischemic myocardial preconditioning and remote renal preconditioning. Open chest rats were subjected to a coronary artery occlusion period of 45 min followed by 2 h of reperfusion (Control animals; n = 6). The coronary preconditioned group (IPC, n = 6) was subjected to a preceding preconditioning period of 5 min coronary artery occlusion followed by 5 min of reperfusion, repeated three times. The renal preconditioned group (IPR, n = 6) was subjected to a preceding renal artery occlusion period of 10 min followed by 20 min of reperfusion. Area at risk (AAR) and infarcted area (IA) were determined at the end of each protocol. Blood samples were taken at the end of the preconditioning protocols from parallel experiments for proteomic analysis using two-dimensional gel electrophoresis (2-DE), matrix assisted laser desorption and ionization – time of flight – mass spectrometry (MALDI-TOF-MS), and liquid chromatography – electrospray ionization – tandem mass spectrometry (nanoLC-ESI-MS/MS). IA/AAR was 87.8 ± 10.7% in the control group. IPC and IPR significantly reduced IA/AAR (58.2 ± 9.3% and 56.9 ± 9.0%, $p < 0.001$). Proteomic analyses detected four protein spots which were either up- (n = 3) or down-regulated in the preconditioned groups vs. the control group. The three up-regulated protein spots were identified as albumin fragments, whereas the down-regulated spot was identified as liver regeneration-related protein (LRRG03). Interestingly, albumin modification by brief ischemia has been recently shown and evaluated for the clinical diagnosis of sublethal myocardial ischemia. However, no differentially abundant proteins which possess a known signaling function could be found. Hence, though there is a differential protein expression in blood following IPC and IPR, our data are not in favor of a humoral mediator of remote preconditioning with a molecular weight of more than 8 kDa. Our results rather suggest either a neurogenic pathway or a mediator smaller than 8 kDa.

Key words preconditioning – ischemia – myocardial infarction – proteomics – signal transduction

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Introduction

The phenomenon called "preconditioning with ischemia" was first described by Murry et al. [23] in 1986. In this pioneering paper, the authors referred to ischemic preconditioning (IP) as a myocardial adaptation to ischemic stress induced by repetitive brief periods of coronary artery occlusion followed by reperfusion.

During the last two decades, the understanding of the mechanisms underlying the pathogenesis of ischemia-reperfusion injury and its prevention by IP has been significantly enhanced by extensive investigations. Preconditioning with ischemia has been observed in several species and even in humans [13, 14, 33]. The preconditioning phenomenon of reducing ischemic injury is established for the heart as well as for a number of other organs including liver, skeletal muscle, and brain [26]. Numerous mediators, triggers and signaling pathways such as adenosine [20], bradykinin [22], opioid receptors [1, 4], protein kinase C [29], tyrosine kinase, mitogen-activated protein kinase (MAPK) [31], and ATP-dependent potassium channels [10, 30] have been controversially discussed (see reviews in [8, 13, 14]).

In 1993, Przyklenk et al. [27] showed that brief circumflex coronary occlusions in canine myocardium induce protection of remote myocardium subtended by the left anterior descending coronary artery. This phenomenon was termed "remote preconditioning" or "preconditioning at a distance", and has subsequently been shown to be not unique to the heart: Liauw et al. [19] demonstrated that ischemia of one skeletal muscle could protect the other one from ischemia-reperfusion injury. Furthermore, remote preconditioning is not limited to one specific organ system: McClanahan et al. [21] described that brief ischemia of the kidney can protect the myocardium against infarction and this result has been confirmed by other authors [25, 32]. Meanwhile, Gho et al. and Birnbaum et al. [3, 9] observed that brief mesenteric artery occlusion, and femoral artery occlusion respectively, confer protection against coronary artery occlusion (see reviews in [11, 28]). Though remote preconditioning is well established in rats and dogs and has also been described in pigs [17], recent observations could not demonstrate remote myocardial preconditining in a rabbit model [24].

It was proposed that remote preconditioning is initiated by a humoral mechanism [9, 21], since a reperfusion period was obligate between ischemia of the peripheral organ and coronary artery occlusion. In addition, Dickson et al. [6] could show that the effluent collected during preconditioning of isolated perfused rabbit hearts can protect virgin isolated hearts from infarction. A very recent paper demonstrates that even acutely transplanted, i.e., denervated hearts can be preconditioned by remote ischemia [16]. On the other hand, ganglion blockade by hexamethonium was shown to abolish the effects of remote preconditioning, supporting a neurogenic pathway of protection [9].

Our research is based on the observation that remote preconditioning is as powerful as "classical" preconditioning in terms of protecting the heart, implying that both share a common pathway of infarct prevention. We hypothesized that a so far unidentified humoral trigger takes part in this pathway, which is carried by blood from the preconditioning to the preconditioned tissue. Therefore, the present study was designed to answer two fundamental questions: (1) Can we reproduce the effects of "classical" preconditioning and remote preconditioning by renal artery occlusion in our experimental protocols? and (2) Can we detect a possible humoral trigger in both groups, using two-dimensional gel electrophoresis (2-DE), matrix assisted laser desorption and ionization – time of flight – mass spectrometry (MALDI-TOF-MS), and liquid chromatography – electrospray ionization – tandem mass spectrometry (nanoLC-ESI-MS/MS)?

Materials and methods

General surgical preparation

Male Wistar rats, weighing 250–350 g, were used in all experiments. Rats were anesthetized by intraperitoneal injection of thiopental (120 mg/kg). A tracheotomy was performed and the trachea was intubated with a cannula connected to a rodent ventilator (model 683, Harvard Apparatus, Holliston, MA, USA). Rats were ventilated with room air supplemented with oxygen at 70 to 72 breaths per minute and a volume of 3 ml. Body temperature was maintained at 37 ± 1 °C using a heating pad.

The right carotid artery was cannulated with a heparinized catheter to measure blood pressure (model VF-1, Crystal Biotech, Hopkinton, MA, USA). A polyethylene catheter was placed in the right jugular vein for saline delivery (6 ml 0.9 % NaCl/4 h, Perfusor® Secura, B. Braun Melsungen AG, Melsungen, Germany). A left thoracotomy was performed followed by a pericardiotomy. A ligature (5-0 prolene) was passed below the left anterior descending coronary artery. The ends of the suture were threaded through a plastic button followed by a propylene tube to form a snare. Coronary artery occlusion was performed by pulling the ends of the suture taut and clamping the snare onto the epicardial surface with a hemostat. Epicardial cyanosis verified coronary artery occlusion.

Reperfusion of the heart was initiated by unclamping the hemostat and loosening the snare, and was confirmed by an epicardial hyperemic response. Additionally, in all three groups a laparatomy was performed and the right renal artery was dissected free. Renal artery occlusion in

the IPR group was performed as mentioned above for the left anterior descending coronary artery.

After the surgical preparation, animals were allowed to stabilize for 10–15 min before the different protocols were started.

Study groups and experimental protocols

The experiments were divided into two separate series. Series 1 (n = 18) was aimed at verifying the effects of ischemic preconditioning in our protocols using IA/AAR (infarcted area/area at risk). In series 2 (n = 18), blood samples were asservated after the preconditioning phase for the determination of a possible protective factor in the preconditioned groups vs. the control group.

Series 1

Rats were randomly assigned to one of the three following groups: control group (Ctrl, n = 6), coronary preconditioned group (IPC, n = 6), and renal preconditioned group (IPR, n = 6). All groups were subjected to a long period of sustained myocardial ischemia (45 min), followed by 2 h reperfusion. The control group underwent 30 min of free renal and myocardial perfusion before the sustained myocardial ischemia was started. In the IPC group, this long ischemia was preceded by a myocardial preconditioning protocol, consisting of 5 min coronary artery occlusion followed by 5 min of reperfusion repeated three times (in these animals, the renal artery was sham-operated, but no renal ischemia was performed). In the IPR group, sustained myocardial ischemia was preceded by a renal preconditioning protocol, consisting of 10 min renal artery occlusion followed by 20 min of reperfusion (Fig. 1). At the end of each protocol, the hearts were excised for the determination of the area at risk and the infarcted area.

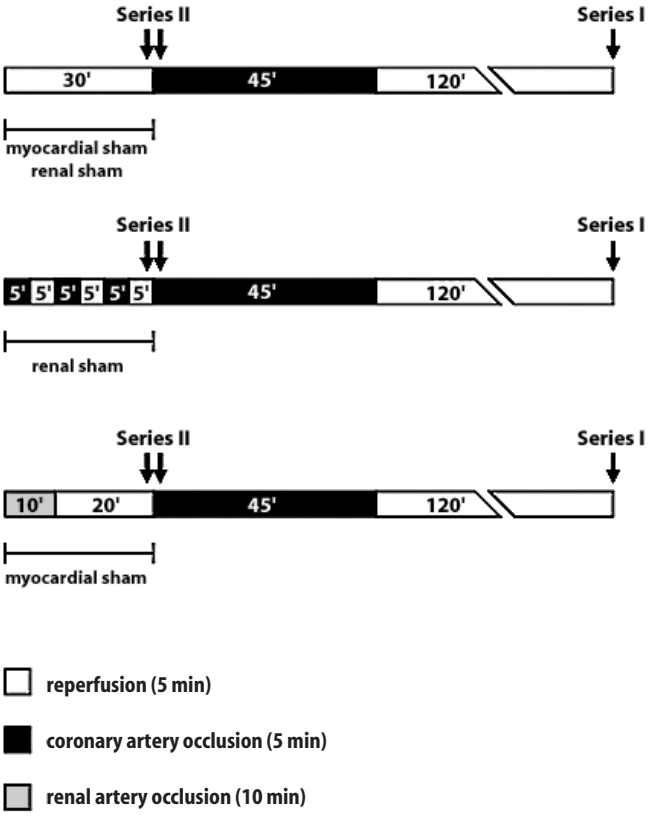

Fig. 1 Experimental protocol. All animals were subjected to a coronary artery occlusion period of 45 min followed by 2 h of reperfusion. The control group underwent 30 min of myocardial and renal sham anesthesia before coronary artery occlusion was started. IPC animals were subjected to a preceding preconditioning period of 5 min coronary artery occlusion followed by 5 min of reperfusion, repeated three times. Simultaneously, these animals underwent an abdominal sham period of 30 min. The IPR group was subjected to 10 min of renal artery occlusion followed by 20 min of reperfusion. At the same time, myocardial sham anesthesia was performed. Black arrows: time points when experiments from series 1 and 2 were terminated

Series 2

All three groups (all n = 6) were treated according to the same protocols as series 1, but the experiments were stopped before the 45 min coronary artery occlusion episode. Three milliliter blood samples were taken from the aorta of each animal for the following analysis using proteomic methods.

Determination of infarct size

Upon completion of series 1, the left coronary artery was reoccluded, and the area at risk was determined by negative staining with 3% lissamine green administered via the jugular vein. The normal area was stained green, while the AAR remained uncolored. The heart was excised and the left ventricle was dissected from the remaining tissue, and subsequently cut into six transverse sections. The slices were weighted (Sartorius Handy, Sartorius AG, Göttingen, Germany) and photographs were taken from the apical and basal side. For the delineation of the infarcted area, the slices were frozen at –80 °C for 5 min and subsequently incubated for 20 min with 1.0% 2,3,5-triphenyltetrazolium chloride (TTC) at 37 °C [36]. Tissues were stored overnight in 8% formaldehyde solution and photographed on the following day as mentioned above.

The photographs were digitized and AAR and IA were determined using Adobe Photoshop and NIH Image. The area at risk was expressed as a percentage of the left ventricle (AAR/LV). The infarcted area was represented as a percentage of the left ventricle (IA/LV) and as a percentage of the area at risk (IA/AAR).

Sample preparation for 2-DE

The blood samples taken from series 2 were centrifuged at 8.000 g (Biofuge 13, Heraeus Instruments, Newtown, CT, USA) at room temperature for 15 min. Twenty µl sample buffer (9 M Urea, 70 mM DTT and 2% carrier ampholytes pH 2-11) were added to 100 µl serum. After 30 min of gently stirring at room temperature, the samples were centrifuged at 150.000 g (Beckmann TL-100, Palo Alto, CA, USA) at 20 °C for 30 min. The supernatants were stored in aliquots at –80 °C. The total protein concentrations were determined via the Bradford protein assay, using bovine serum albumin (BSA) as a standard.

Two-dimensional gel electrophoresis

Without further purification or fractionation, the samples were subjected to 2-DE analysis according to Klose and Kobalz [15].

The first dimension, isoelectric focusing (IEF), was performed in vertical rod gels (0.15 cm diameter), containing 9 M Urea, 4% acrylamide, 0.3% piperazine diacrylamide, 5% glycerol, 0.06% TEMED, and 2% carrier ampholyte (pH 2-11). For analytical runs, 100 µg, and, for preparative runs, up to 500 µg of the protein samples were applied to the anodic side of the gel and focused at 8820 Vh. After focusing, the gels were incubated in equilibration buffer, containing 125 mM Tris-phosphate (pH 6.8), 40% glycerol, 65 mM dithiothreitol (DDT), and 3% sodium dodecyl sulfate (SDS) for 10 min, and subsequently frozen at –80 °C.

For the second dimension, large-format SDS-PAGE gels (sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels, 23 × 30 cm) were prepared, containing 375 mM Tris-HCL-Buffer (pH 8.8), 15% acrylamide, 0.2% bisacrylamide, 0.1% SDS and 0.03% TEMED, as described by Laemmli [18]. After thawing, the equilibrated IEF gels, replacing the stacking gels of the Laemmli system, were immediately applied to the SDS-PAGE gels. Electrophoresis was performed using a two-step increase of current, starting with 15 min at 65 mA, followed by a run of 6 h at 140 mA, until the front reached the end of the gel.

After 2-DE separation, analytical gels were stained with silver nitrate (0.1%), according to Jungblut and Seifert [12], whereas preparative gels were stained with 0.066% Coomassie brilliant blue G-250 (CBB G-250) as described by Doherty et al. [7].

Image analysis and quantification

Silver-stained gels used for comparison analysis were digitized at a resolution of 150 dpi using a computer-assisted densitometer (Mirage II, Umax, Willich, Germany). Two-dimensional image analysis was performed using the ProteomeWeaver software (Definiens AG, Munich, Germany). The identifiable spots were outlined, quantified and matched on the gels. Quantification of proteins was represented as intensity of the spots.

Identification of 2-DE separated proteins

For analysis, the protein spots were excised of the CBB G-250 stained gels. After in-gel tryptic digestion, the peptides were extracted using ZipTip columns (Millipore, Bedford, MA, USA). Samples were mixed with an equal volume of α-cyano-4-hydroxycinnamic acid matrix. Because identification by MALDI-MS (Bruker Reflex III mass spectrometer, Bruker Daltonik GmbH, Bremen, Germany) was not successful, we used nanoLC-ESI-MS/MS (LCQ™ Deca XP mass spectrometer, Thermo Finnigan, San Jose, CA, USA and NanoFlow LC system, series 1000, Agilent Technologies, Palo Alto, CA, USA). The peptide mass data were fed into the MASCOT search engine (http://www.matrixscience.com) and searched against the NCBI protein database.

Statistical analysis

IA/AAR values are expressed as mean ± SEM. As a first step, the two-tailed unpaired student t-test was used to

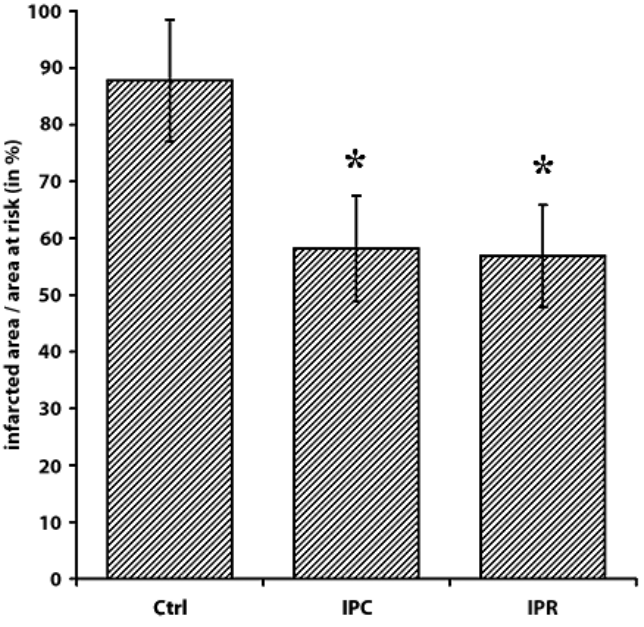

Fig. 2 Infarct size expressed as a percentage of the area at risk. All values are presented as mean ± SEM. IPC and IPR group show a significant reduction of IA/AAR vs. control (*$p < 0.001$)

Table 1 The ten protein spots chosen for identification

Super Spot-ID	$CTRL_A$[1]	IPC_A[2]	IPR_A[3]	t-test IPC vs. CTRL	t-test IPR vs. CTRL	IPC_A / $CTRL_A$	IPR_A / $CTRL_A$	Regulation
11	0.132	0.042	0.072	0.0021*	0.0097*	0.32	0.55	down
64	0.175	0.085	0.102	0.0149*	0.0162*	0.49	0.58	down
67	0.165	0.384	0.240	0.0032*	0.0127*	2.33	1.45	up
241	0.019	0.041	0.040	0.0183*	0.0341*	2.16	2.11	up
242	0.175	0.468	0.365	0.0019*	0.0054*	2.67	2.09	up
474	0.082	0.220	0.169	0.0025*	0.0045*	2.68	2.06	up
558	0.095	0.251	0.195	0.0032*	0.0091*	2.64	2.05	up
605	0.178	0.338	0.290	0.0220*	0.0287*	1.90	1.63	up
698	0.012	0.021	0.027	0.0076*	0.0043*	1.75	2.25	up
704	0.179	0.388	0.356	0.0088*	0.0154*	2.17	1.99	up

Averages in spot intensity of the two preconditioned groups differ from the control group in the same direction. Bold numbers indicate the spots which were significantly different expressed according to ANOVA.
*: $p < 0.05$ by unpaired t-test
[1]: Averages in spot intensity of the control group
[2]: Averages in spot intensity of the coronary preconditioned group
[3]: Averages in spot intensity of the renal preconditioned group

compare two different groups of animals in order to yield a high degree of discrimination. As a second step, ANOVA (FisherPLSD-test) was used to confirm these findings using appropriate statistical methods. A p-value smaller than 0.05 was considered to be statistically significant.

Drugs and materials

Thiopental was purchased from BYK Gulden, Constance, Germany. Lissamine green was obtained from Waldeck GmbH & CoKG, Münster, Germany. TTC and α-cyano-4-hydroxycinnamic were purchased from Sigma-Aldrich, Seelze, Germany. DTT was purchased from Biomol GmbH, Hamburg, Germany. Ampholyte (Servalyte, pH 2-11), bisacrylamide and acrylamide were purchased from Serva, Heidelberg, Germany. All other chemicals were purchased either from BioRad (Munich, Germany) or Merck (Darmstadt, Germany).

Results

Infarcted area and area at risk

Left ventricular mass and area at risk expressed as a percentage of the left ventricle (AAR/LV) were not significantly different in any of the three groups (Control: 30.3 ± 7.4, IPC 31.8 ± 7.5, IPR 36.2 ± 4.6; all in %). Figure 2 shows IA/AAR (in %) for each group. IA/AAR in the control animals averaged 87.8 ± 10.7%. IPC significantly reduced IA/AAR to 58.2 ± 9.3% ($p < 0.001$ vs. control group). Similarly, IPR resulted in a marked reduction of IA/AAR (56.9 ± 9.0%, $p < 0.001$) compared to the control group.

Fig. 3a A representative 2-DE gel of proteins extracted from a control animal. The protein spots were detected by silver nitrate staining. The ten identified up- or down-regulated protein spots are marked by big numbers

Proteomic analysis

To identify protein changes during the preconditioning phase in the IPC and the IPR group, the total protein extracted from blood samples of all three groups were separated on 2-DE gels and silver stained. In the first step, using the formally inappropriate albeit sensitively discriminating t-test, 48 protein spots were observed which differed between IPC, IPR and control group. To underline our hypothesis of IPC and IPR acting in the same manner, we have chosen those protein spots for identification which have shown: (i) a significant t-test (IPC vs. Ctrl and IPR vs. Ctrl) and (ii) an average in spot intensity, which is higher or lower in both groups vs. control. Using the t-test, eight protein spots were reproducibly detected to be more abundant in the IPC and IPR groups over control, but two protein spots were less abundant in the two preconditioned groups vs. controls (Table 1). However, of these 10 proteins spots, only four were found to still meet the criteria following ANOVA-testing. Of these four spots, three were up- und one was down-regulated. Representative 2-DE gels of each group show the positions of the ten up- or down-regulated protein spots (Fig. 3 a–c).

Using nanoLC-ESI-MS/MS and the MASCOT search engine, the up-regulated spots were identified as albumin (spots 242, 474, 704). Respectively, the down-regulated spot was identified as liver regeneration-related protein LRRG03 (spot 11) (Tables 2 and 3).

Discussion

First series

In the present study, we were able to reproduce the protective effect of classical and remote preconditioning regarding IA/AAR using our own experimental design.

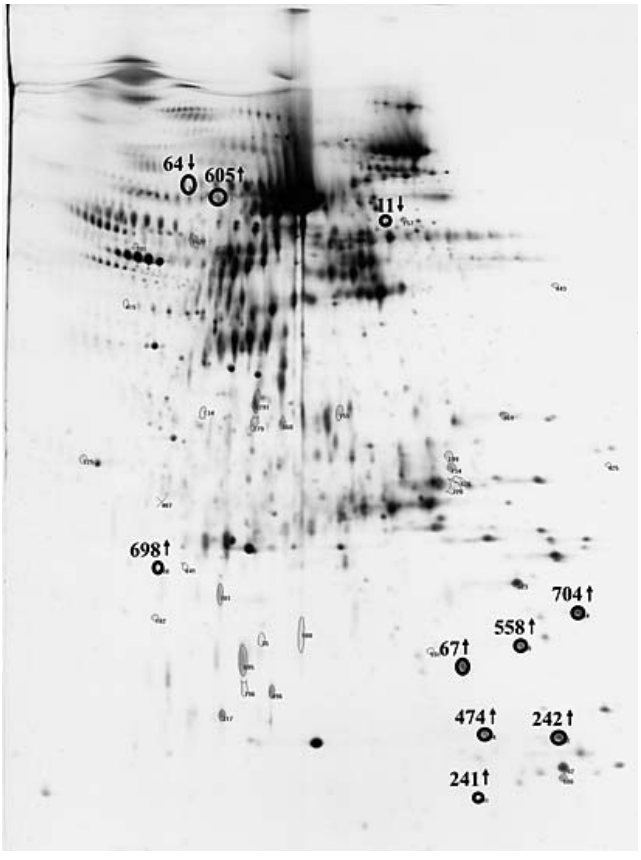

Fig. 3b A representative 2-DE gel of proteins extracted from an animal, which was subjected to the myocardial preconditioning protocol. The protein spots were detected by silver nitrate staining. The ten identified up- or down-regulated protein spots are marked by big numbers. ↓: down-regulation vs. control; ↑: up-regulation vs. control

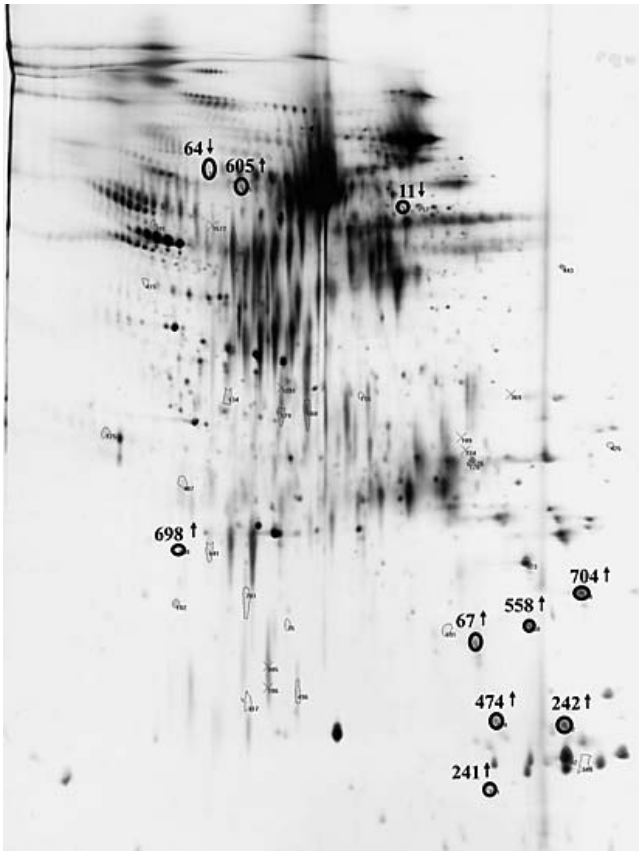

Fig. 3c A representative 2-DE gel of proteins extracted from an animal, which was subjected to the renal preconditioning protocol. The protein spots were detected by silver nitrate staining. The ten identified up- or down-regulated protein spots are marked by big numbers. ↓: down-regulation vs. control; ↑: up-regulation vs. control

Table 2 nanoLC-ESI-MS/MS analysis of the three up-regulated protein spots in the IPC and the IPR group immediately after the preconditioning phase

Spot #	Identified protein	NCBI number	M_r exp*	M_r calc[1]	Matched sequences
242	albumin	19705431	1895.98	1895.95	509 – 524: RPCFSALTVDETYVPK+P
			2152.18	2152.01	528 – 545: AETFTFHSDICTLPDKEK+P
			1356.79	1356.64	570 – 581: TVMGDFAQFVDK
			1961.98	1961.93	585 – 602: AADKDNCFATEGPNLVAR+P
474	albumin	19705431	1895.98	1895.95	509 – 524: RPCFSALTVDETYVPK+P
			2152.18	2152.01	528 – 545: AETFTFHSDICTLPDKEK+P
			1961.98	1961.93	585 – 602: AADKDNCFATEGPNLVAR+P
704	albumin	19705431	1675.99	1675.86	470 – 483: LPCVEDYLSAILNR+P
			1896.99	1895.95	509 – 524: RPCFSALTVDETYVPK+P
			2152.18	2152.01	528 – 545: AETFTFHSDICTLPDKEK+P
			1356.79	1356.64	570 – 581: TVMGDFAQFVDK

*: Experimental m/z transformed to a relative molecular mass
[1]: Relative molecular mass calculated from the matched peptide sequence
P: Propionamide; Ox: Oxidation; Me: Me-ester

Table 3 nanoLC-ESI-MS/MS analysis of the down-regulated protein spot in the IPC and the IPR group immediately after the preconditioning phase

Spot #	Identified protein	NCBI number	M_r exp*	M_r calc[1]	Matched sequences
11	Liver-regene-ration-related-protein LRRG03	33187764	1682.98	1683.79	252 – 264: KPVDQYEDCYLAR+Me;P
			1628.98	1628.84	274 – 287: NGDGKEDLIWEILK
			1421.99	1422.75	298 – 310: SKDFQLFGSPLGK
			1205.99	1207.62	300 – 310: DFQLFGSPLGK
			1389.99	1391.76	332 – 343: LYLGHSYVTAIR
			1555.99	1557.73	347 – 361: EGVCPEGSIDSAPVK+P
			1345.99	1347.64	455 – 466: ASDSSINWNNLK
			1575.99	1577.74	575 – 587: QEDFQLLCPDGTK+Me;P
			1315.99	1316.56	633 – 642: DCTGNFCLFR+2P

*: Experimental m/z transformed to a relative molecular mass
[1]: Relative molecular mass calculated from the matched peptide sequence
P: Propionamide; Me: Me-ester

Moreover, we could show – for the first time to our knowledge – that preconditioning via renal artery occlusion using a rat model under normothermia is highly protective against myocardial infarction.

Three previous studies back up our results by use of a rabbit model. McClanahan et al. [21] were the first to describe a remote preconditioning protocol by renal artery occlusion. Infarct size expressed as a percentage of the ischemic risk zone was 43 ± 3% in control animals and 11 ± 2% in the transient renal ischemia group. Pell et al. [25] found corresponding results, reporting that the infarct-to-risk ratio was limited from 32.7 ± 4.0% (control group) to 17.8 ± 3.0% in the transient renal ischemia group. In addition, Takaoka et al. [32] demonstrated that IA/AAR was limited to 19.6 ± 1.3% in the renal preconditioned group (vs. 42.8 ± 3.5% in the control group).

In contrast to those findings, Gho et al. [9] reported that preconditioning via renal artery occlusion failed to limit infarct size (72 ± 5% vs. 68 ± 2% in the control group) in a rat model under normothermia, but was successful under hypothermia (46 ± 6%).

In conclusion, all these results are in concordance with our findings that remote preconditioning via renal artery occlusion is as effective and powerful as classical preconditioning via coronary artery occlusion. Both protective phenomena may be mediated by similar mechanisms [16, 17, 32].

Second series

Regarding our assumption of a humoral factor in correspondence to previous studies [6, 9, 21], Konstantinow

and coworkers [16] and Weinbrenner et al. [34] recently again underlined the humoral hypothesis, reporting that a reperfusion period has to be necessarily interspaced between infrarenal occlusion of the aorta and the coronary artery occlusion. Furthermore, they demonstrated that hexamethonium, a ganglion blocker, could not block the protection achieved by remote preconditioning. However, Gho et al. [9] found controversial data, since pretreatment with hexamethonium abolishes the protection of the heart, supporting a neurogenic way.

The major finding of the study is that brief myocardial and renal ischemia alters the protein composition of blood. The sensitive proteomic methods used in our study were in fact suited to detect a unitary change in the abundance of four protein spots in the IPC and the IPR group compared to the control animals and to identify these differentially expressed proteins: several albumin fragments were found to be increased in myocardial as well as renal preconditioning, whereas the level in liver regeneration-related protein (LRRG03) was decreased. However, we detected no proteins or peptides which were already known for their signaling function (according to results from database searches). Although we cannot exclude *indirect* signaling effects of the observed proteomic alterations, i.e., modulation of protein binding and, thus, availability of an unknown mediator and/or its receptor, the results of our study support a neurogenic rather than a humoral mechanism of remote preconditioning. Nevertheless, as the methods used are only suitable to detect molecules with a molecular mass of more than 8 to 10 kDa, we cannot exclude a mediator with a lower molecular mass. As the proteomic analysis of tissue extracts was not the primary aim of our study, the interesting hypothesis of a humoral response in the heart following neurogenic transmission of the preconditioning signal remains to be elucidated.

Ischemia modified albumins

We unexpectedly found a significant increase in several albumin-fragments, which was highly consistent between the groups. Though this was at first a surprising finding, there is evidence that albumin metabolism may be of importance in acute ischemia: recently, ischemia-modified albumin (IMA) was shown to be a new marker for myocardial ischemia. The importance of IMA in the clinical setting was reported by Bar-Or [2], comparing IMA with established markers of myocardial ischemia, including CK-MB, myoglobin, and cTn-I, in 41 patients undergoing percutaneous transluminal coronary angioplasty (PTCA). IMA showed a significant increase in the albumin-cobalt binding (ACB) assay directly after intervention and returned to baseline within 6 h. CK-MB, myoglobin, and cTn-I were not altered immediately after PTCA, but were significantly elevated above baseline after 6 and 24 h. Comparable results were achieved by Christenson et al. [5], enrolling 256 acute coronary syndrome patients at four medical centers: ACB assay of the first blood sample, immediately taken after presentation at the medical center, had high negative predictive value (96 %) and sensitivity (83 %) for predicting troponin-negative or -positive results 6–24 h later. Christenson et al. concluded that the ACB test could play an important role to distinguish between high-risk and low-risk patients. These observations demonstrate that albumin is structurally altered following ischemia. Like IMA, the consistent occurrence of albumin fragments in the IPC group immediately after ischemia might point into the same direction. As albumin fragments were also seen in the IPR group, this phenomenon is apparently not restricted to the heart.

In conclusion, we detected a significant increase of albumin fragments in the preconditioned groups compared to the control animals. As it was shown for IMA, albumin modification appears to be a useful tool for the early diagnosis of myocardial and – as firstly shown – renal ischemia. Nevertheless, as signaling functions are not known for the peptides found to be differentially abundant in preconditioned and control animals, our study argues against the hypothesis of a protein or peptide as protective mediator and strengthens a neurogenic mechanism to underlay remote preconditioning. However, a mediator with a molecular weight less than 8 to 10 kDa cannot be excluded.

Critique of methods

In our study, blood samples were drawn directly prior to the sustained ischemic episode. As protein synthesis, posttranslational protein processing, and protein exocytosis are time-consuming processes, the latest possible time-point before index ischemia should be the most promising time-point to detect these proteins in the blood. As a limitation, this approach might overlook very rapidly available peptides or proteins, i.e., quickly released, labile or unstable compounds from the myocardial and/or renal cell in response to brief preconditioning ischemia. However, using proteomic methods, neither such proteins nor their possible degradation products could be detected. The same holds true for possible degradation artefacts during sample processing, inasmuch as these problems must have constantly occurred in all three groups.

Our research is based on the observation that both remote renal and classical myocardial preconditioning represent highly conserved phenomena that share a common pathway of infarct prevention. As it has been shown that an analog intracellular pathway of protection is activated in myocardium and kidney, i.e., involvement of mitochondrial ATP-dependent potassion channels, as

recently again underlined by Kristiansen et al. [17], we hypothesized that these analog intracellular mechanisms are activated by the same humoral trigger(s). However, our approach does not rule out the possibility that different mediators may contribute to the two phenomena, which might explain the observation that – in some models – the initiation of ischemic myocardial preconditioning and remote renal preconditioning (and vice versa) has not been equally successful [9, 17, 21, 24, 25, 32].

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