# Anesthetic induced cardioprotection: from bench to bedside and retour

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# Chapter 2: Ischemic preconditioning phosphorylates mitogen-activated kinases and heat schock protein 27 in the diabetic heart

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## Abstract

Diabetes mellitus blocks protection by ischemic preconditioning (IPC), but the mechanism is not known. We investigated the effect of ischemic preconditioning on mitogen-activated protein kinases (extracellular signal-regulated kinases 1 and 2, c-Jun N-terminal kinases, p38 mitogen activated kinase) and heat shock protein 27 phosphorylation in diabetic and nondiabetic rat hearts in vivo. Two groups of anaesthetized nondiabetic and diabetic rats underwent a preconditioning protocol (3 cycles of 3 min coronary artery occlusion and 5 min of reperfusion). Two further groups served as untreated controls. Hearts were excised for protein measurements by Western blot. Four additional groups underwent 25 min of coronary occlusion followed by 2 h of reperfusion to induce myocardial infarction. In these animals, infarct size was measured. IPC reduced infarct size in the nondiabetic rats but not in the diabetic animals. In diabetic rats, IPC induced phosphorylation of the mitogenactivated protein kinases and of heat shock protein 27. We conclude that protection by IPC is blocked by diabetes mellitus in the rat heart in vivo without aff ecting phosphorylation of mitogen-activated protein kinases or heat shock protein 27. Therefore, the blockade mechanism of diabetes mellitus is downstream of mitogenactivated kinases and heat shock protein 27.

## Introduction

Coronary artery disease is one of the major causes of death in the Western world [1] . Diabetes mellitus increases the risk of coronary heart disease by a factor of 2– 4 [2, 3]. Nearly 50 % of deaths in diabetic patients are due to the consequences of coronary artery disease [4] . In-hospital and longterm mortality after myocardial infarction are also significantly higher in diabetic patients compared with nondiabetics [5, 6] . One possible explanation for the poor prognosis of diabetic patients with myocardial infarction is the blockade of endogenous protective mechanisms. The strongest endogenous mechanism against the consequences of ischemia is known as ischemic preconditioning (IPC). This concept was first described by Murry et al., who showed that short periods of ischemia protect the heart against the consequences of a consecutive longer ischemia [7]. One distinguishes two phases of protection: the early phase develops within minutes from the initial ischemic insult and lasts 2 to 3 h; the late phase of IPC becomes apparent 12 to 24 h later and lasts 3 to 4 d [8]. In the laboratory animal, diabetes mellitus blocks protection by early [9] and late IPC [10] . There is also evidence for a clinically relevant blockade of IPC in diabetic patients [11] . However, little is known about the mechanism by which diabetes mellitus blocks signal transduction of IPC. Activation of the phosphatidylinositol 3-kinase (PI3 K)-Akt is known to be an important step in the cellular signaling pathway of IPC [12, 13], and phosphorylation of PI3K-Akt is blocked in a model of type 2 diabetes mellitus [14]. Each subfamily of the mitogen-activated protein kinases (MAPK) – the 42 / 44-kDa extracellular signal-regulated kinases (ERK), the 45 / 54-kDa c-Jun N-terminal kinase (JNK), and the 38-kDa p38 MAPK – has been suggested to play a central role in the cardioprotection achieved by early IPC [15 – 17]. The heat shock protein 27 (HSP27) is a downstream target of the p38 MAPK [15, 18]; its involvement in cardioprotection by IPC also has been discussed [16].

In the present study, we investigated whether the phosphorylation of MAPK, HSP27, and (PI3 K)-Akt is blocked by diabetes mellitus. We aimed to investigate the blockade level at which ischemic preconditioning is abrogated in diabetes mellitus. Detecting the blockade mechanism is required before restoration of protection in diabetic patients can be made possible.

## Material and Methods

The present investigation conforms to the Guide for the Care and use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No.85-23, revised 1996) and was approved by the local Animal Care Committee.

## Induction of diabetes mellitus

We used a model of streptozotocin-induced type 1 diabetes. This model is well established in rats and other animals [19 – 21]. Eight weeks before the experiments, an intraperitoneal injection of 60 mg $kg^{-1}$ streptozotocin induced a diabetic state in the animals of the diabetic groups.

## Surgical preparation

The surgical preparation and the infarct-size measurement by triphenyltetrazolium chloride staining have been described in detail previously [22]. In brief, male Wistar rats were anesthetized by intraperitoneal S( + )-ketamine injection (150 mg $kg^{-1}$ ). After tracheal intubation, the lungs were ventilated with oxygen-enriched air and a positive end-expiratory pressure of 2 –3 cm $H_2O$. Respiratory rate was adjusted to maintain partial pressure of carbon dioxide within physiologic limits. Body temperature was maintained at 38 ° C by the use of a heating pad. The right jugular vein was cannulated for saline and drug infusion, and the left carotid artery was cannulated for the measurement of aortic pressure. Anesthesia was maintained by continuous α-chloralose infusion. A lateral left-sided thoracotomy followed by pericardiotomy was performed and a ligature (5-0 prolene) was passed below the main branch of the left coronary artery. The ends of the suture were threaded through a propylene tube to form a snare, and the coronary artery was occluded by tightening the snare. After surgical preparation, the rats were allowed to recover for 30 min before starting the experimental protocol. Successful coronary artery occlusion was verified by epicardial cyanosis.

## Experimental protocol

The study protocol is shown in Fig. 1 . For infarct-size measurement, 40 rats were randomly assigned to one of four groups, and all animals were subjected to 25 min of left coronary artery occlusion followed by 120 min of reperfusion. The control group (n = 10) and a group of diabetic animals (DM, n = 8) were not further treated.

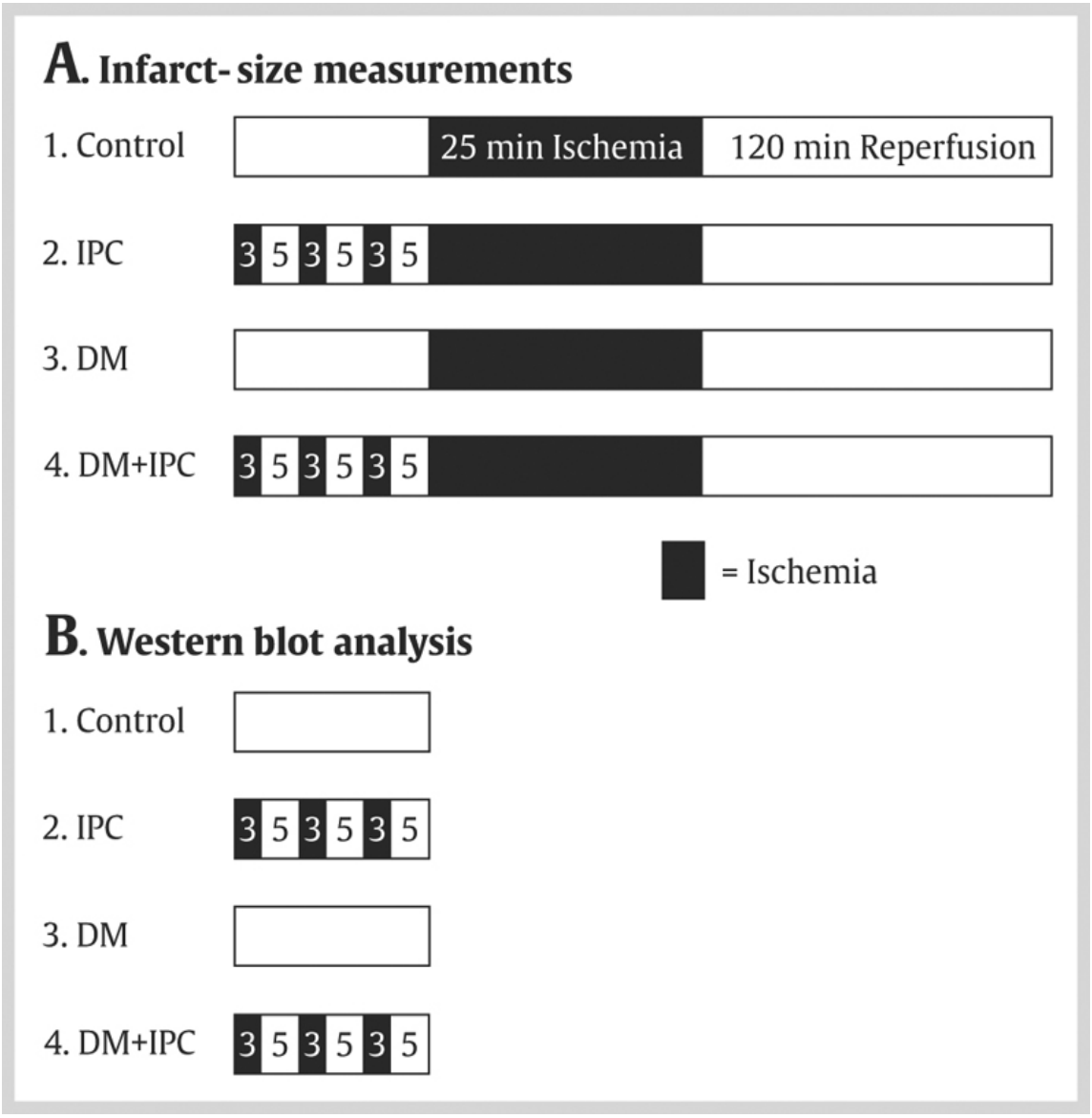


**Fig. 1** Experimental protocols. Panel *A*: Infarct-size measurements (top). All animals underwent 25 min of coronary occlusion followed by 120 min of reperfusion in order to induce myocardial infarction. The control group and a group of diabetic animals (DM) were not further treated. Ischemic preconditioning (IPC) was induced by three 3-min cycles of coronary occlusion followed by 5 min of reperfusion in a group of nondiabetic (IPC) and in a group of diabetic (DM+IPC) animals. At the end of each experiment, hearts were excised to measure infarct size. Panel *B*: Western blot analysis (bottom). A group of nondiabetic (IPC) and a group of diabetic (DM+IPC) rats underwent the preconditioning protocol of three 3-min cycles of coronary occlusion followed by 5 min of reperfusion. A group of nondiabetic (control) and a group of diabetic (DM) animals were not treated.

In a group of nondiabetic (IPC, n = 12) and one of diabetic animals (DM + IPC, n = 10), ischemic preconditioning was induced by 3 cycles of coronary artery occlusion for 3 min, each followed by 5 min of reperfusion before the infarct-inducing ischemia. To investigate MAPK, HSP27, and (PI3K)-Akt involvement, four groups of animals (control, IPC, DM, DM + IPC, each group n = 12) underwent the preconditioning protocol without index ischemia. Then the snare was

occluded while 1.5 ml of Evans blue was injected intravenously in order to identify the area at risk. The hearts were excised rapidly and the area at risk was cut out, frozen in liquid nitrogen, and stored at − 80 ° C for later Western blotting.

## Hemodynamics

The aortic pressure signal was digitized using an analogue-to digital converter (PowerLab / 8SP, ADInstruments Pty Ltd., Castle Hill, Australia) at a sampling rate of 500 Hz and continuously recorded on a personal computer using Chart for Windows v 5.0 (ADInstruments Pty Ltd.).

## Infarct-size measurement

For infarct-size measurement, hearts were excised after the 2 h reperfusion period. The size of the area at risk was then identified by Evans blue staining of the nonischemic area, and the infarct size within the area at risk was stained by triphenyltetrazolium chloride as described in detail previously [22]. The area at risk and the infarcted area were determined by planimetry using SigmaScan Pro 5 computer software (SPSS Science Software, Chicago, IL, USA).

## Western blot analysis

Because of the small size of the area-at-risk specimens, two pieces from two different animals of the same group were always processed together. The frozen tissue was pulverized and dissolved in lysis buff er containing Tris base, EGTA, NaF, and $Na_3VO_4$ (as phosphatase inhibitors), a freshly added protease inhibitor mix (aprotinin, leupeptin, and pepstatin), okadaic acid, and dithiothreitol. The solution was vigorously homogenized on ice (Homogenisator, IKA, Staufen, Germany) and then centrifuged at 1 000 × g, 4 ° C, for 10 min to obtain the cytosolic fraction of the tissue. The supernatant, containing the cytosolic fraction, was centrifuged again at 16.000 × g, 4 ° C, for 15 min to clean up the fraction. After protein determination by the Lowry method [23], equal amounts of protein were mixed with loading buffer containing Tris-HCl, glycerol, and bromphenol blue. Samples were vortexed and boiled at 95 ° C before being subjected to sodium dodecylsulfate polyacrylamide gel electrophoresis. The proteins were separated by electrophoresis and then transferred to a polyvinylidene difluoride membrane by tank blotting. Unspecific binding of the antibody was blocked by incubation with 5 % fat dry milk powder solution in Tris-buffered saline containing Tween for 2 h. Subsequently, the membrane was

incubated overnight at 4 ° C with respective first antibody (phosphor specific or total in the case of p38 MAPK [Cell Signaling, Danvers, MA, USA], ERK 1 and ERK 2 [Cell Signaling, Danvers, MA, USA], and JKN-1 and JNK-2/3 [Cell Signal, Danvers, MA, USA], HSP27 [Affinity BioReagents, Golden, CO, USA], (PI3K)-Akt [Cell Signaling, Danvers, MA, USA], and anti-α-tubulin [Sigma, Saint Louis, MO, USA]) at indicated concentrations. After washing in fresh, cold, Tween-containing Tris-buffered saline, the blot was subjected to the appropriate horseradish peroxidase – conjugated secondary antibody for 2 h at room temperature. Immunoreactive bands were visualized by chemiluminescence detected on autoradiography film (Hyperfilm ECL; Amersham, Freiburg, Germany) using the enhanced chemiluminescence system Santa Cruz. The blots were quantified by SigmaScan Pro 5 computer software (SPSS Science Software, Chicago, IL, USA). The results were calculated as the ratio of phosphorylated protein to α-tubulin and to total protein. Values are presented as absolute values of average light intensity.

## Statistical analysis

Data are expressed as mean ± standard deviation. Statistical analysis was performed using Student's t-test to detect group differences followed by Bonferroni's correction for multiple comparisons. P-values smaller than 0.05 were considered statistically significant.

## Results

### Hemodynamics

Table 1 shows heart rate and mean aortic pressure of the experimental groups. In all groups, heart rate was stable during the experiments but was lower in the diabetic animals. Aortic pressure did not differ between the groups under baseline conditions (123 ± 22 mmHg), and there were no differences among the control, the IPC, and the DM groups. IPC in the diabetic animals led to a drop in mean aortic pressure, which stayed reduced until the end of the experiments. While mean aortic pressure in the control, IPC, and DM groups was 94 ± 17, 95 ± 18, and 73 ± 34 mmHg, respectively, after 120 min of reperfusion, it was only 59 ± 21 in the DM + IPC group ($p = 0.033$ vs. IPC).

**Table 1** Mean aortic pressure and heart rate during the experimental course in all groups.

| | Control | IPC | DM | DM + IPC |
|---|---|---|---|---|
| **mean aortic pressure (mmHg)** | | | | |
| baseline | 132 ± 17 | 125 ± 15 | 120 ± 29 | 113 ± 25 |
| preconditioning protocol | | | | |
| first IPC | 133 ± 13 | 126 ± 14 | 123 ± 28 | 91 ± 26 |
| first reperfusion | 131 ± 14 | 127 ± 12 | 118 ± 29 | 97 ± 34 |
| second IPC | 129 ± 15 | 124 ± 14 | 121 ± 33 | 94 ± 28 |
| second reperfusion | 126 ± 17 | 118 ± 10 | 119 ± 34 | 89 ± 32 |
| third IPC | 127 ± 17 | 115 ± 12 | 117 ± 33 | 79 ± 31 |
| third reperfusion | 125 ± 20 | 116 ± 13 | 110 ± 38 | 84 ± 34 |
| ischemia | | | | |
| 15 min | 127 ± 20 | 122 ± 13 | 109 ± 33 | 78 ± 29† |
| 24 min | 122 ± 15 | 114 ± 14 | 102 ± 34 | 69 ± 25† |
| reperfusion | | | | |
| 15 min | 115 ± 15 | 108 ± 17 | 93 ± 38 | 70 ± 21†† |
| 30 min | 109 ± 16 | 104 ± 18 | 99 ± 34 | 62 ± 22†† |
| 60 min | 99 ± 25 | 101 ± 20 | 85 ± 38 | 56 ± 19† |
| 120 min | 94 ± 17 | 95 ± 18 | 73 ± 34 | 59 ± 21† |
| **heart rate (beats per minute)** | | | | |
| baseline | 406 ± 22 | 397 ± 48 | 344 ± 61 | 318 ± 54 |
| preconditioning protocol | | | | |
| first IPC | 390 ± 25 | 402 ± 41 | 352 ± 62 | 335 ± 41 |
| first reperfusion | 387 ± 28 | 398 ± 37 | 329 ± 46 | 327 ± 49 |
| second IPC | 385 ± 30 | 417 ± 34 | 336 ± 52 | 329 ± 49†† |
| second reperfusion | 383 ± 33 | 394 ± 37 | 335 ± 54 | 321 ± 52 |
| third IPC | 383 ± 33 | 412 ± 32 | 336 ± 56 | 319 ± 54†† |
| third reperfusion | 390 ± 42 | 397 ± 33 | 339 ± 57 | 311 ± 54† |
| ischemia | | | | |
| 15 min | 424 ± 27 | 423 ± 32 | 344 ± 47* | 318 ± 44†† |
| 24 min | 413 ± 24 | 416 ± 31 | 341 ± 49 | 309 ± 52†† |
| reperfusion | | | | |
| 15 min | 384 ± 27 | 393 ± 35 | 323 ± 43 | 320 ± 40† |
| 30 min | 378 ± 31 | 385 ± 34 | 320 ± 33 | 310 ± 51† |
| 60 min | 376 ± 27 | 378 ± 34 | 320 ± 20* | 297 ± 50† |
| 120 min | 378 ± 26 | 373 ± 28 | 304 ± 39* | 309 ± 43† |

IPC = ischemic preconditioning; DM = diabetes mellitus

*$p < 0.05$ vs. control; †$p < 0.05$, ††$p < 0.01$, vs. IPC

## Infarct size

The diabetic animals had a reduced body weight compared with the nondiabetic animals ($p < 0.001$). Heart dry weight and area-at-risk weight was reduced by 93 mg and 47 mg in diabetic rats, respectively ($p < 0.001$). Table 2 shows animal weights and heart dry weights of the groups. Fig. 2 shows the infarct size in percentage of the area at risk. IPC reduced infarct size in the nondiabetic rats by 44 % compared with controls ($p = 0.01$). While diabetes mellitus itself had no effect on infarct size ($p = 1.0$ DM vs. control), it completely blocked the protective effect of IPC ($p < 0.001$ DM + IPC vs. IPC; $p = 1.0$ DM + IPC vs. DM).

**Table 2** Body weight, heart dry weights (whole heart, area at risk, infarcted area), area at risk in percentage of the whole heart, and blood glucose concentration before the experiments.

| | Control | IPC | DM | DM + IPC |
|---|---|---|---|---|
| body weight (g) | 394 ± 15 | 409 ± 19 | 249 ± 30** | 223 ± 37‡ |
| heart dry weight (mg) | 182 ± 18 | 213 ± 29 | 117 ± 11** | 108 ± 17‡ |
| area at risk (mg) | 78 ± 30 | 97 ± 36 | 39 ± 16* | 49 ± 15†† |
| area at risk/whole heart (%) | 42 ± 15 | 45 ± 14 | 36 ± 14 | 46 ± 13 |
| blood glucose concentration (mg $dl^{-1}$) | 5.7 ± 0.4 | 6.9 ± 1.3 | 31.3 ± 7.7 | 33.4 ± 5.0‡ |

IPC = ischemic preconditioning; DM = diabetes mellitus

*$p < 0.05$, **$p < 0.0001$ vs. control; ††$p < 0.01$, ‡$p < 0.0001$ vs. IPC

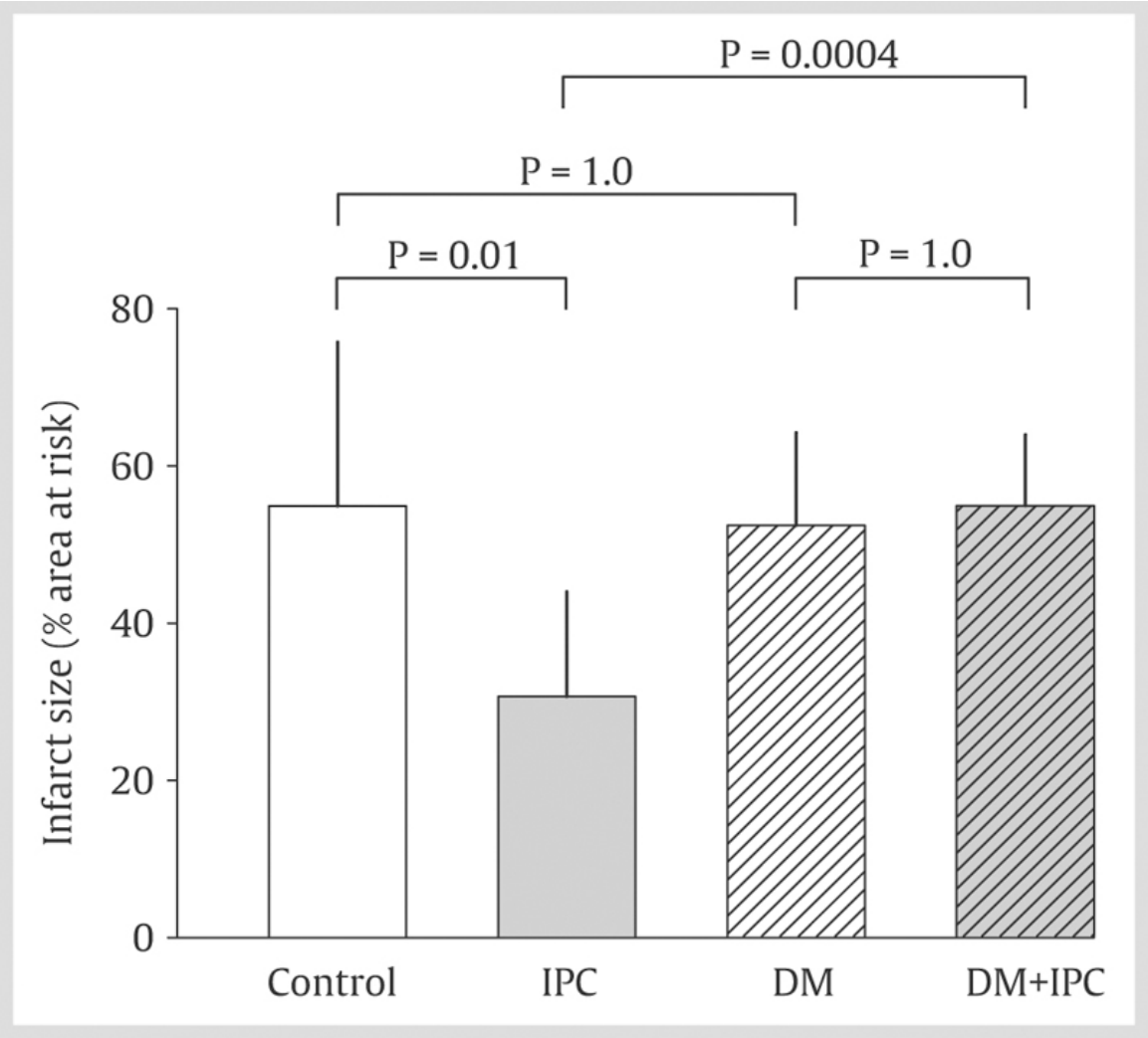


**Fig. 2** Infarct size in percentage of the area at risk. Ischemic preconditioning (IPC) reduced infarct size significantly compared with controls. While diabetes mellitus (DM) had no effect on myocardial damage, DM completely blocked the protection provided by IPC (DM+IPC).

## Western blot

Fig. 3 shows the Western blot results of the phosphorylated MAPKs p38 MAPK, JNK-1, JNK-2 / 3, ERK-1, and ERK-2 and of phosphorylated HSP27. In diabetic rats, IPC led to phosphorylation of all MAPKs and of the downstream target HSP27. In nondiabetic rats, IPC also resulted in an increase in the phosphorylated form of the MAPKs and of HSP27 but reached statistical signifi cance in p38 MAPK, ERK-1, ERK-2, and JNK-2/3 only. Phosphorylated (PI3 K)-Akt showed no significant differences in either diabetic (IPC, 0.56 ± 0.57 vs. DM + IPC, 0.39 ± 0.30; p = 0.52) or nondiabetic animals (control, 1.41 ± 0.99 vs. IPC, 1.23 ± 1.06; p = 1.0).

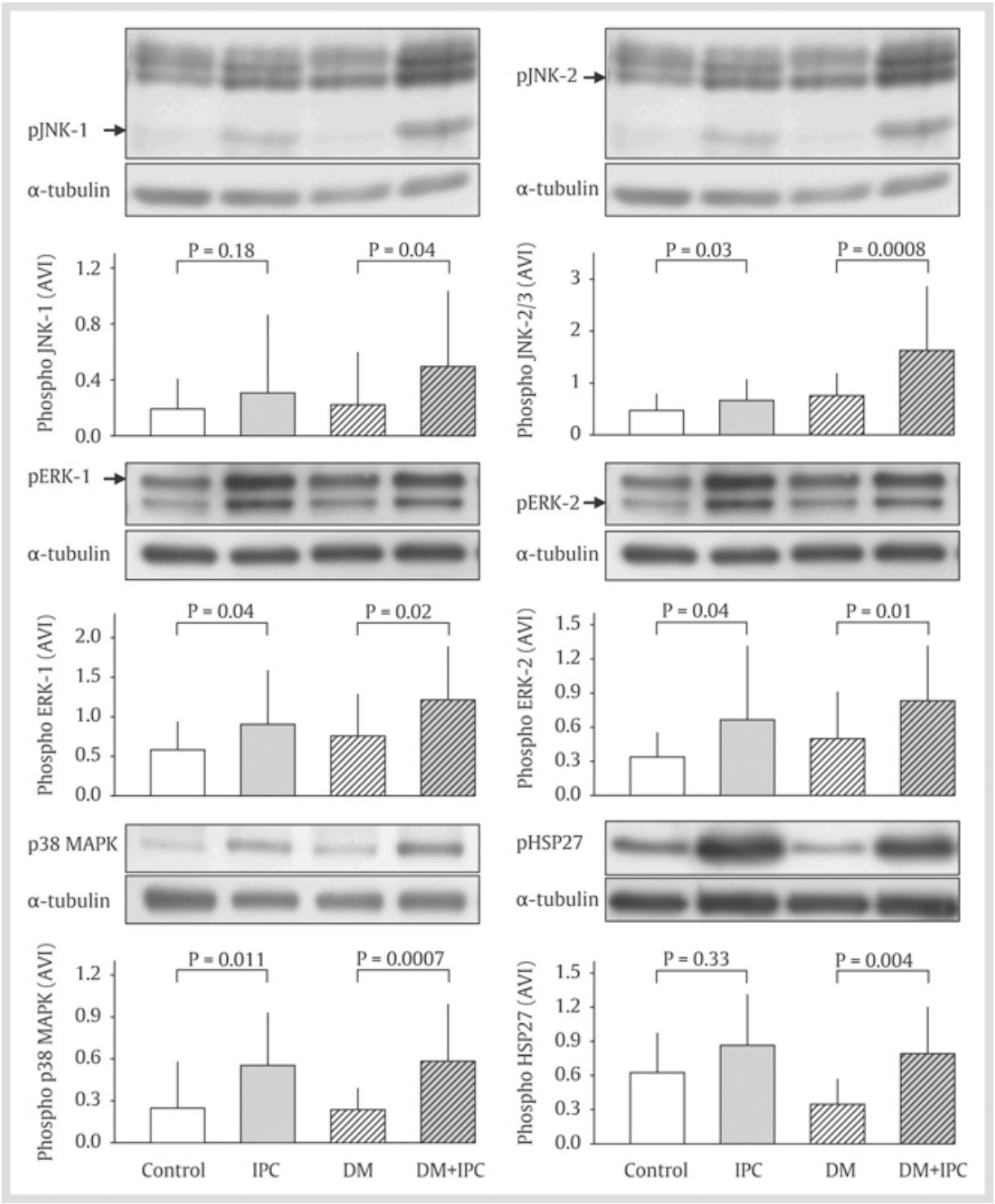


**Fig. 3** *Phosphorylation of the mitogen-activated protein kinase (MAPK) p38 MAPK, the extracellular regulated kinases (ERK)-1 and ERK-2, the c-Jun N-terminal kinases (JNK)-1 and JNK-2/3, and heat shock protein 27 (HSP27), which is a downstream target of the p38 MAPK. α-tubulin is shown as reference. In diabetic animals (DM) ischemic preconditioning (IPC) resulted in phosphorylation (activation) of all analyzed MAPKs and HSP27.*

## Discussion and Conclusions

The present study shows that diabetes mellitus abolishes the protection provided by early ischemic preconditioning in the rat heart in vivo without affecting the phosphorylation of any MAPK (p38 MAPK, ERK-1 and ERK-2, JNK-1 and JNK-2/3) or of HSP27. Therefore, the blockade mechanism in the diabetic animal is located downstream of MAPK and HSP27.

In contrast to patients with diabetes mellitus, nondiabetic patients with preinfarction angina show a better outcome than patients without such an ischemic event before acute myocardial infarction [11, 24, 25]. These findings suggest a clinically relevant blockade of ischemic preconditioning in diabetic patients. Gosh et al. failed to precondition human diabetic myocardium in vitro [26] in insulin-dependent and noninsulin-dependent diabetic patients. Therefore, it is likely that the blockade mechanism is independent of antidiabetic medication (sulfonylurea blocks cardiac $K_{ATP}$ channels), as suggested by Cleveland and colleagues [27] . In the laboratory animal, hyperglycemia as well as diabetes mellitus blocked cardioprotection by early IPC [9, 28, 29] independently of osmolarity and insulin levels [9]. In contrast, glucose deprivation can induce preconditioning by opening of mitochondrial ATP-sensitive potassium ($mK_{ATP}$) channels [30]. Opening of $mK_{ATP}$ channels plays a central role in the signal transduction cascade of IPC leading to activation of MAPK [31, 32]. Opening of $mK_{ATP}$ channels can be altered by hyperglycemia and diabetes mellitus [33]. Kersten and colleagues showed that hyperglycemia and diabetes mellitus blocked the preconditioning effect induced by the $mK_{ATP}$ channel opener diazoxide [34]. One may hypothesize that the blockade of $mK_{ATP}$ channel opening by hyperglycemia causes the lack of cardioprotection by IPC in diabetic animals and patients. However, the blockade of IPC by a diabetic state could also be shown under “normoglycemic” conditions [35], while being dependent on the duration of the diabetic state [36]. These findings suggest a mechanism of blockade independent of or at least additional to $K_{ATP}$ channel attenuation by hyperglycemia. Tsang and colleagues [14] investigated the (PI3K)-Akt (protein kinase B) pathway as an important upstream (trigger) part of the signal transduction of early IPC [12, 37]. They found a blockade of early IPC in a rat model of type 2 diabetes when using one 5 min cycle of ischemia, while 3 cycles of IPC were protec-

tive. One IPC cycle resulted in a smaller degree of (PI3K)-Akt phosphorylation compared with 3 IPC cycles. Whether this reduction in phosphorylation caused the lack of protection remains unclear. Phosphorylation of (PI3K)-Akt after IPC was clearly shown in in vitro models [12, 13]. In our in vivo rat model, we could not show an increased phosphorylation of (PI3K)-Akt in either nondiabetic or diabetic animals. A difference between the in vivo and the in vitro situation could therefore be suggested. Whether this lack of (PI3K)-Akt phosphorylation is only an expression of bad luck or suggests this to be an in vitro phenomenon remains unclear. However, we also investigated the MAPK and HSP27 pathway, which is located downstream of (PI3K)-Akt phosphorylation and $mK_{ATP}$ opening [16, 38, 39]. Our data clearly show that all MAPKs (p38 MAPKK, ERK-1 and ERK-2, JNK-1 and JNK-2/3) and HSP27 are phosphorylated in the diabetic animals, despite the absence of cardioprotection. These findings indicate that the diabetes-induced blockade of the signal transduction cascade of early IPC is situated downstream from these proteins and, therefore, downstream of (PI3K)-Akt and the $mK_{ATP}$ channel. Does one finding exclude the other? Not necessarily. A blockade downstream of MAPK and HSP27 can be present parallel with reduced (PI3K)-Akt phosphorylation. Reduced phosphorylation does not necessarily mean blockade of the preconditioning signal. However, Tsang et al. [14] could show an effect of diabetes on protein phosphorylation in the signal transduction of preconditioning. Therefore, it seems worthy to continue looking for a blockade of protein phosphorylation activation.

In contrast to our findings, Hassouna and colleagues induced protection by pretreatment with the protein kinase C (upstream of MAPK [38]) activator PMA and the p38 MAPK activator anisomycin in atrial tissue of diabetic patients in vitro. Possible explanations for this discrepancy are the unspecificity of the activators used, the comparableness of the type of diabetes in the two studies (streptozotocin-induced type 1 diabetes vs. type 2 diabetes), species differences (rat vs. human), tissue differences (ventricular myocardium vs. atrial tissue), or model differences (in vivo vs. in vitro). They also found impaired depolarization and superoxide production in isolated mitochondria of diabetic patients, indicating but not proving a lack of $mK_{ATP}$ channel opening. Hassouna's findings suggest an upstream blockade resulting in the lack of $mK_{ATP}$ channel opening. Hassouna's study seems to contradict our results of an intact downstream phosphorylation of MAPKs and HSP27. Whether this is a result of study-

ing completely different models or of still unknown mechanisms cannot be answered. A dual role of $mK_{ATP}$ channel opening as trigger and mediator may also be an explanation [40].

Strniskova and colleagues investigated the phosphorylation of p38 and ERK after 5 and 30 min of regional ischemia in isolated rat heart in a model of streptozotocin-induced diabetes mellitus [41]. Although Strniskova's group also showed MAPK phosphorylation, these results are not comparable to our study because the experiments were performed only one week after induction of diabetes. However, blockade of cardioprotection by ischemic preconditioning is not observed before 5 weeks of existing diabetes mellitus [36, 42, 43]. Therefore, no conclusion about signal transduction of ischemic preconditioning in the diabetic state can be drawn from their study.

We used a rat model of type 1 diabetes (streptozotocin induced) that may not reflect conditions of the more clinically relevant type 2 diabetes in patients. However, this model is well established, and the duration of 8 weeks of diabetes before performing the experiments is long enough to have pathomorphologic changes typical for diabetes. Furthermore, after 8 weeks of streptozotocin-induced diabetes, preconditioning is blocked in vitro [35], indicating changes in the myocardium independent of the current blood glucose concentration.

In the current investigation, we also observed the expected increased phosphorylation of JNK-1 and HSP27, but this increase did not reach statistical significance using a conservative test such as Student ' s t -test with Bonferroni ' s correction. However, activation of these proteins by early IPC is well established [44 –46]. Whether diabetes mellitus amplifies the phosphorylating effect of IPC on these proteins cannot be concluded from this study. A shift of the time course of activation by diabetes mellitus could be a possible explanation, but this is only speculation. One could argue that despite phosphorylation, the investigated proteins may not work properly in the diabetic myocardium, causing the blockade of preconditioning. However, we showed a phosphorylation of HSP27 that is controlled by the p38 MAPK [15], indicating that at least this protein is working.

The involvement of HSP27 in the cardioprotection by early IPC can only be assumed. At the moment, the lack of a specific blocker or activator of HSP27 that

may be employed in vivo makes it impossible to prove a causal link between HSP27 phosphorylation and protection by IPC. However, the activation of HSP27 in the diabetic animals in our model supports the hypotheses that the upstream proteins of the signal transduction of IPC are not only phosphorylated but also have effects on downstream targets.

We showed that diabetes mellitus blocks protection provided by early IPC in the diabetic rat heart in vivo. The streptozotocininduced diabetes did not affect activation of p38 MAPK, ERK-1 and ERK-2, JNK-1 and JNK-2 / 3, or HSP27. These findings show that the blockade of IPC-induced cardioprotection by diabetes mellitus is situated downstream of the MAPK and the HSP27 in the signal transduction cascade of early IPC.

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