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ORIGINAL CONTRIBUTION

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# Myocardial protection evoked by hyperoxic exposure involves signaling through nitric oxide and mitogen activated protein kinases

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**Abstract** *Background* Hyperoxic exposure in vivo (> 95 % oxygen) attenuates ischemia-reperfusion injury, but the signaling mechanisms of this cardioprotection are not fully determined. We studied a possible role of nitric oxide (NO) and mitogen activated protein kinases (MAPK) in hyperoxic protection. *Methods* Mice (n = 7–9 in each group) were kept in normoxic or hyperoxic environments for 15 min prior to harvesting the heart and Langendorff perfusion with global ischemia (45 min) and reperfusion (60 min). Endpoints were cardiac function and infarct size. Additional hearts were collected to evaluate MAPK phosphorylation (immunoblot). The nitric oxide synthase inhibitor L-NAME, the ERK1/2 inhibitor PD98059 and the p38 MAPK inhibitor FR167653 were injected intraperitoneally before hyperoxia or normoxia. *Results* Hyperoxia improved postischemic functional recovery and reduced infarct size ($p < 0.05$). Hyperoxic exposure caused cardiac phosphorylation of the MAPK family members p38 and ERK1/2, but not JNK. L-NAME, PD98059 and FR167653 all reduced the protection afforded by hyperoxic exposure, but did not influence performance or infarction in hearts of normoxic mice. The hyperoxia-induced phosphorylation of ERK1/2 and p38 was reduced by L-NAME and both MAPK inhibitors. *Conclusion* Nitric oxide triggers hyperoxic protection, and ERK1/2 and p38 MAPK are involved in signaling of protection against ischemia-reperfusion injury.

**Key words** contractile function – infarction – ischemia – MAP kinase – nitric oxide

## Introduction

Ischemic preconditioning is a powerful tool to protect organs against experimental ischemia-reperfusion injury. However, the clinical application of ischemic preconditioning has been hampered by the complexity of the underlying signaling pathways of protection. Despite two decades of preconditioning research, we are still far from identifying a suitable therapeutic approach of evoking the response. Preconditioning signaling involves trigger substances acting upon G-protein coupled membrane receptors, signal transduction cascades of phosphorylation reactions of protein kinases, with induction of cell survival programs, preservation of mitochondrial function and membrane integrity, and reduction of apoptosis and necrosis as end results [31]. We have established a clinically acceptable way of evoking a preconditioning-like effect by exposing animals to a hyperoxic environment with oxygen concentration over 95 % [25–27]. Similar to ischemic preconditioning hyperoxia has an immediate and a delayed phase of protection [25, 26]. Although the primary appeal of using hyperoxic exposure to evoke myocardial protection is the direct clinical applicability of the method, it is still necessary to understand the underlying molecular mechanisms of protection. Dissection of similarities between hyperoxic, ischemic, or other models of precondi-

tioning may provide new opportunities for developing cytoprotective agents.

Ischemic preconditioning may be triggered by non-receptor mediated substances such as oxygen free radicals [3] or nitric oxide. The role of endogenous nitric oxide (NO) for ischemic preconditioning is controversial, as it has been shown to have no effect [16] or to trigger protection [11, 12]. One group found that during preconditioning, the NO concentration rapidly increased, and this resulted in lower NO concentrations during prolonged ischemia [6]. The latter may have been connected to improved functional recovery caused by preconditioning [6]. Ischemic and hyperoxic preconditioning both lead to activation of the transcription factor nuclear factor kappa B (NFκB), and inhibition of NFκB activation during preconditioning abolishes protection [13, 27]. The upstream signaling to NFκB in ischemic preconditioning may involve protein kinase C [35], tyrosine kinases [13], and the mitogen activated protein kinases (MAPK). MAPKs are a highly conserved superfamily involved in intracellular signaling from membrane to nuclei and other intracellular targets [14, 34]. They are involved in a large variety of cellular regulatory processes such as growth, differentiation, development, cell cycle, survival, and death, and are activated by a plethora of stimuli [5, 21]. The major MAPK found in cardiac tissue are the extracellular signal-regulated kinases (ERK1/2), the stress-activated/c-Jun NH2 terminal kinases (SAPK/JNK), and p38 MAPK. Of these, especially ERK1/2 as well as p38 MAPK are suggested to be involved in protective signaling of ischemic preconditioning, although their roles in this are controversial [20]. NO activates cGMP and protein kinase C, which downstream can also involve other protein kinases [15]. Furthermore, exogenous NO donors activate MAPKs, especially p38 and ERK 1/2 [4, 11, 19]. We hypothesize that NO and the MAPK pathway are part of the signaling mechanisms inducing myocardial protection by hyperoxic exposure.

## Methods

The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996), and was approved by the Ethics Committee for Animal Research at the University of Oslo, Norway.

### Pilot study

A pilot study consisting of n = 2 animals per group and time-point was conducted to explore the possible phosphorylation of MAPK by hyperoxic exposure. In the pilot study hearts were isolated at the end of 30 min of hyperoxia, at the end of a 20 min stabilization period in the Langendorff system, and at the end of 30 min of reperfusion after global ischemia in both normoxic and hyperoxic groups. After evaluating the results, it was decided to go forward with the rest of the study and block p38 and ERK1/2 as well as NO. The latter study was performed after moving to the laboratory, and a 15 min period of hyperoxic exposure was more efficient for cardioprotection than 30 min in the new environment.

### Isolated heart perfusion

Male C57BL/6 mice (22–25 g) purchased from B&K (Sollentuna, Sweden) were anesthetized by intraperitoneal injections of pentobarbital (50 mg/kg) with concomitant heparin (500 IU) injection. The hearts were excised through a median sternotomy, the aorta cannulated, and retrogradely perfused with gassed (5 % $CO_2$, 95 % $O_2$) Krebs-Henseleit buffer at a constant pressure of 55 mmHg as previously described [26]. The temperature of the heart was maintained at 37 °C through water-jacketed heating. Global ischemia was achieved by clamping the inflow tubing.

Left ventricular systolic (LVSP) and end-diastolic (LVEDP) pressures were recorded via a balloon inserted into the left ventricle, while left ventricular developed pressure (LVDP) was calculated (LVDP = LVSP-LVEDP). LVEDP was set to 4–7 mmHg at the end of the stabilization period. Coronary flow (CF) was continuously measured by a flowmeter (Transonic Systems Inc.). All recordings of LVSP, LVEDP, LVDP, and heart rate (HR) were registered in a computer system (PCLAB, AstraZeneca AB, Mölndal, Sweden).

### Experimental protocol

Awake mice were kept in a hyperoxic (> 95 % $O_2$) or normoxic environment for 15 min as previously described by us [26]. Immediately after induction of anesthesia, hearts were excised and Langendorff perfused. After 20 minutes of stabilization the hearts were subjected to 45 minutes of global ischemia, followed by 60 min of reperfusion. The study was performed in two different series of experiments with separate control groups.

The following groups were studied in the L-NAME series:

1. Normoxic controls (n = 9)
2. Hyperoxic exposure (n = 8)
3. Hyperoxic exposure 5 min after intraperitoneal injection of the unspecific nitric oxide synthase (NOS) inhibitor L-NAME (Sigma-Aldrich) 15 mg/kg (n = 8)
4. Normoxic time matching to group 3 after injection of L-NAME (n = 8)

The following groups were studied in the MAPK series:
1. Normoxic controls (n = 9)
2. Hyperoxic exposure (n = 8)
3. Hyperoxic exposure 5 min after intraperitoneal injection of the MEK1 (directly upstream to ERK1/2) inhibitor PD98059 (Sigma-Aldrich) 1 mg/kg dissolved in 5 % dimethyl sulfoxide (DMSO) (n = 9).
4. Normoxic time matching to group 3 after injection of PD98059 (n = 9).
5. Hyperoxic exposure 5 min after intraperitoneal injection of the specific p38 MAPK inhibitor FR167653 (a kind gift from Fujisawa Pharmaceutical Ltd.) 2 mg/kg (n = 7).
6. Normoxic time matching to group 5 after injection of FR167653 (n = 9)

The drug concentrations were chosen according the literature and after performing pilot studies [24, 29, 33].

### Determination of infarct size

At the completion of reperfusion, 1 mm thick sections of the hearts were incubated in 1 % triphenyl tetrazolium chloride (TTC, Sigma, St. Louis, MO) at 37 °C for 20 min, and fixed in 4 % paraformaldehyde. Digital images were obtained with a WILD M650 microscope and a Nikon Coolpix 5400 digital camera. Computerized planimetry of infarct size was carried out in Adobe Photoshop 7.0. Infarct areas from sections of one heart were averaged into one value for statistical analyses.

### Western blotting

Hearts were collected as described under the heading pilot studies. To study if the unspecific NOS inhibitor L-NAME, the specific p38 MAPK inhibitor FR167653, or the specific ERK1/2 inhibitor PD98059 influenced phosphorylation of MAPK after hyperoxic exposure, additional hearts were collected immediately after exposure to normoxia or hyperoxia (n = 6 hearts from each group). Frozen hearts were homogenized in Fast prep FP 120 (Thermo Savant), and proteins extracted with lysis buffer containing 1 mM $Na_3VO_4$, 1 % SDS and 1 % PMSF. Samples were centrifuged at 4 °C for 5 min (18000 g), and the supernatant was collected. Protein concentrations were determined with the BCA assay (Pierce, Rockford, IL, USA). Cell lysates (60 µg/lane) were separated on a 10 % denaturating polyacrylamide gel, transferred to nitrocellulose membranes, and ERK1/2, JNK and p38 phosphorylation was detected with phosphospecific rabbit anti-P-ERK1/2, anti P-JNK and anti P-p38 antibodies (1:1000, New England Biolabs, Beverly, MA, USA). Antibodies detecting the total proteins of p38, ERK1/2 and JNK (1:1000, Cell Signaling) were used to normalize for loading. Bands were visualized using a goat anti-rabbit secondary antibody coupled to horseradish peroxidase (1:1000, Pierce) and the Enhanced ChemiLuminescence kit (Amersham) according to the manufacturer's instructions.

Quantification of bands was performed by the following principle: Normoxic controls were used as reference for all immunoblots. First, normoxic density values corresponding to each gel of phosphorylated proteins were averaged separately on each film. The densitometric readings of all bands on each individual film were divided by this averaged normoxic signal. The same procedure was performed on densitometric readings of the corresponding total proteins. The generated relative numbers corresponding to the phosphorylated proteins were divided by the values of corresponding total proteins used as loading control. The resulting double-normalized values were used for statistical evaluation as individual entries coming from different gels, as artificial inter-film density differences due to exposure were removed and inter-film comparison of values became possible.

### Statistics

The data on graphs with heart function are shown as mean ± SEM to avoid overlap between groups. Optical density data and infarct size are shown as mean ± SD. Differences in the recovery of postischemic hemodynamic parameters were tested by using two-way analysis of variance (ANOVA) with repeated measures on one factor, taking treatment as an independent factor, and time as a dependent factor. The treatment-time interaction in ANOVA refers to the statistical test of whether mean profiles for one group are the same as for the other groups. In the case of significant interaction, simple effects, i. e., effects of one factor holding another factor fixed, were examined. Planned comparisons between the groups across factor time were then performed. The p-values were thereafter corrected according to the Bonferroni procedure. Comparisons of infarct size and optical density were performed by Mann-Whitney U test. $p < 0.05$ was considered significant.

## Results

### Cardiac MAPK expression after hyperoxic exposure

Mice were subjected to hyperoxia followed by heart isolation and Langendorff perfusion, and proteins were extracted serially and immunoblotted with phosphospecific antibodies against p38 MAPK, ERK1/2, and JNK. p38 and ERK1/2, and to a smaller degree JNK, were phosphorylated in hearts sampled immediately after 30

minutes of hyperoxic exposure (Fig. 1). When hearts were Langendorff-perfused for 20 minutes, most MAPKs were phosphorylated. The ERK1/2 phosphorylation was less marked in hearts from animals exposed to hyperoxia in vivo than in hearts from those exposed to normoxia. After 30 minutes of reperfusion ERK1/2 activation was still reduced in hearts of hyperoxic animals compared with normoxic (Fig. 1).

## Hemodynamic function

In order to investigate the functional role of MAPK phosphorylation and if nitric oxide was involved in the hyperoxic response, the unspecific NOS inhibitor L-NAME, the inhibitor of ERK1/2 through inhibiting MEK1 PD98059, and the p38 MAPK inhibitor FR167653 were given prior to hyperoxic exposure and compared with normoxia, hyperoxia, and normoxia plus the respective pharmacological agents.

In the L-NAME series, LVEDP increased during reperfusion of normoxic control hearts. When animals were exposed to 15 minutes of $>95\%$ $O_2$ before isolation, the postischemic increase of LVEDP was attenuated ($p = 0.02$). Administration of L-NAME before hyperoxic exposure abolished hyperoxia-induced protection ($p = 0.015$), but did not influence normoxic control hearts (Fig. 2). In the MAPK series, hyperoxia also attenuated the increase of LVEDP during reperfusion compared to normoxic control hearts ($p < 0.0001$). When PD98059 was given prior to hyperoxia, the beneficial effect on LVEDP was lost ($p < 0.0001$ compared with hyperoxia). PD98059 given before normoxia did not influence LVEDP compared to normoxia alone ($p = 0.014$ compared to hyperoxia) (Fig. 2). The p38 inhibitor FR167653 given before hyperoxia and normoxia caused LVEDP to be something in between hyperoxic and normoxic controls without causing any significant differences (Fig. 2).

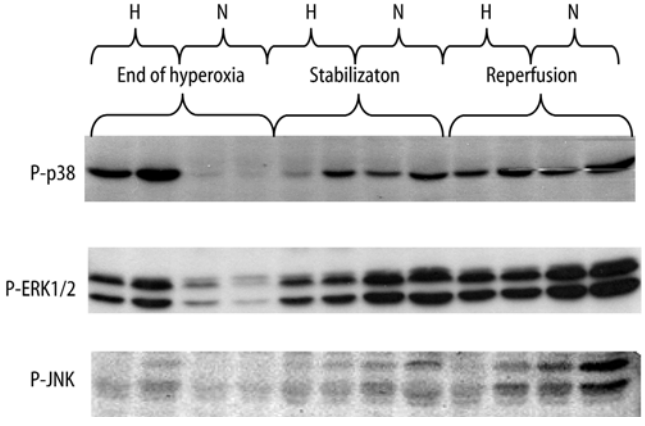


**Fig. 1** Immunoblots of protein extracts from mice hearts taken serially after in vivo exposure to a hyperoxic (H, > 95 % oxygen) or normoxic (N) environment for 30 min, thereafter heart isolation and perfusion in a Langendorff system for 20 min (stabilization) followed by 45 min induced global ischemia and 30 min reperfusion. Phosphospecific antibodies to the mitogen activated protein kinase family were used to detect p38, extracellular signal-regulated kinases (ERK1/2), and c-Jun NH2 terminal kinase (JNK)

In the L-NAME series, LVDP was not different between groups during reperfusion (Fig. 2). In the MAPK series, LVDP was depressed during reperfusion of normoxic controls. Hyperoxic exposure prior to heart isolation and induced global ischemia improved LVDP during reperfusion ($p < 0.0001$). PD98059 given before hyperoxia inhibited this improvement ($p = 0.024$), while PD98059 given before normoxic exposure did not influence LVDP ($p =$ n. s. compared to normoxia, $p = 0.007$ compared to hyperoxia). FR167653 given before hyperoxic exposure did not significantly reduce the preservation of LVDP induced by hyperoxia ($p = 0.06$). FR167653 did not significantly influence LVDP when given before normoxia ($p =$ n. s. compared to normoxia, $p = 0.06$ compared to hyperoxia)(Fig. 2).

Left ventricular systolic pressure, heart rate, and incidence of reperfusion arrhythmias were not different between groups in either series (results not shown).

## Coronary flow

Coronary flow gradually decreased during postischemic reperfusion after normoxic and also hyperoxic exposure (Fig. 2). The nitric oxide inhibitor L-NAME did not influence coronary flow in the L-NAME series neither when given before normoxia nor before hyperoxia. In the MAPK series the postischemic decrease of CF observed in normoxic hearts was attenuated by pretreatment with hyperoxia ($p < 0.0001$). When PD98059 was given prior to hyperoxia, the increase of CF was inhibited ($p = 0.03$). PD98059 given before normoxia did not influence CF compared to normoxia alone, and this group was different from the hyperoxic ($p = 0.0009$). The p38 MAPK inhibitor FR167653 did not influence the beneficial effect of hyperoxia on coronary flow, and did not influence CF when given before normoxia (Fig. 2).

## Infarct size

Myocardial infarct size as a percentage of total heart volume is presented in Fig. 3. Hearts of normoxic control animals in the L-NAME series had an average infarct size of 51 %. This was reduced to 29 % when the hearts were exposed to hyperoxia prior to heart isolation and induced ischemia ($p = 0.013$). When L-NAME was given prior to hyperoxia, the infarct limiting effect of hyperoxia was lost ($p = 0.013$). Normoxic control animals with L-NAME treatment were not different from

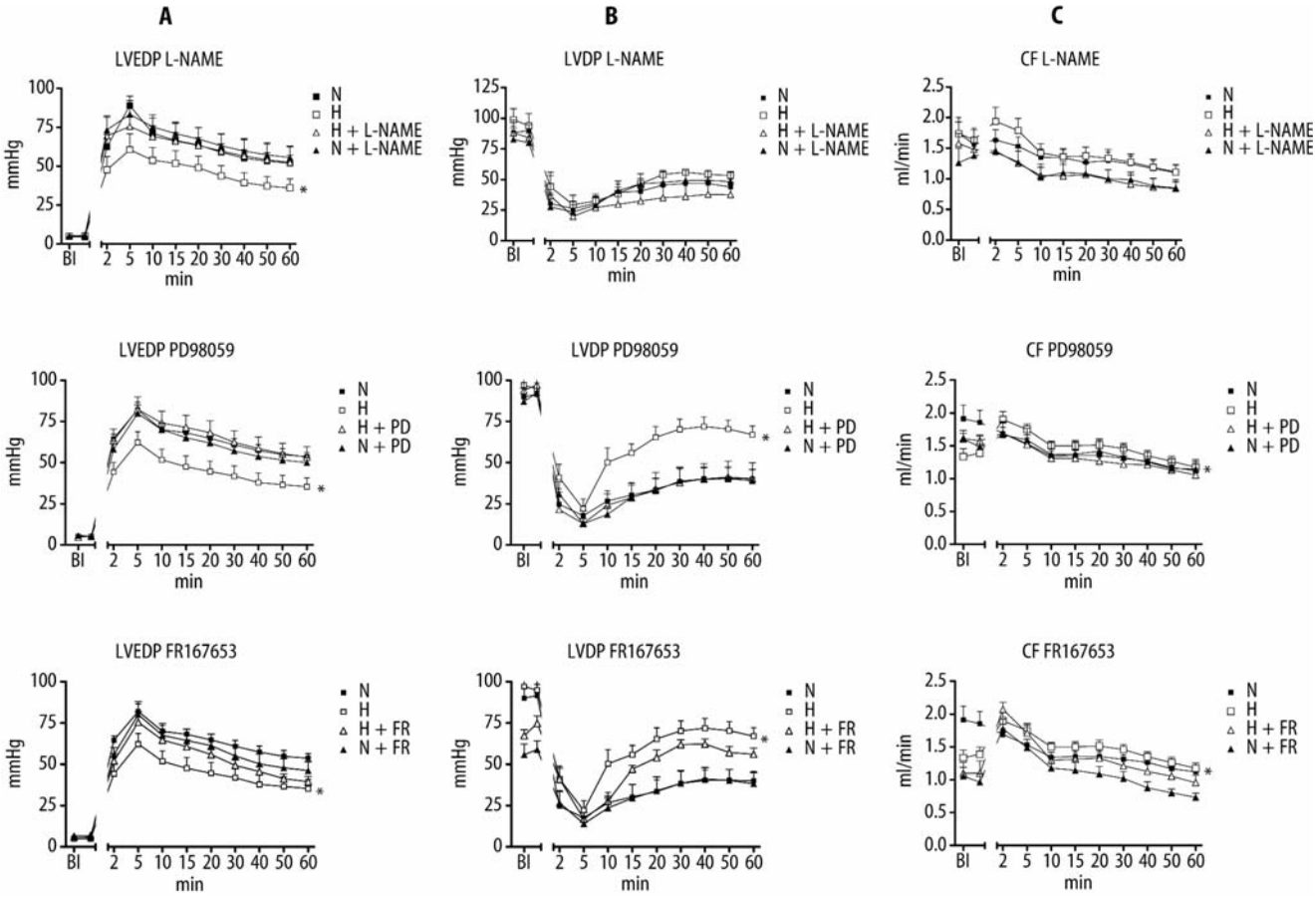


**Fig. 2** Left ventricular end-diastolic pressure (LVEDP), left ventricular developed pressure (LVDP) and coronary flow (CF) in Langendorff-perfused mouse hearts subjected to 45 min global ischemia and 60 min reperfusion. Mice were kept in normoxic (N) or hyperoxic ($O_2$ > 95 %) (H) environment 15 min prior to heart isolation and perfusion. The nitric oxide synthase inhibitor L-NAME, the MEK1 (immediately upstream to ERK1/2) inhibitor PD98059 (PD) or the p38 inhibitor FR167653 (FR) were given intraperitoneally 5 min before exposure to either hyperoxia or normoxia and Langendorff perfusion. Data are mean ± SEM of 7–9 hearts in each group. BI = before global ischemia, times denote minutes of reperfusion. * denotes $p < 0.05$

normoxic controls. In the MAPK series normoxic control animals had an infarct size of 52 %, and this was reduced to 29 % in hyperoxic animals. Both PD98059 ($p = 0.001$) and FR167653 ($p = 0.001$) inhibited the infarct-limiting effect of hyperoxia. Neither of the inhibitors influenced infarct size when given before normoxia (Fig. 3).

## MAPK expression after NOS and MAPK inhibition

To study the effect of the nonselective NOS inhibitor L-NAME, the MEK1 inhibitor (directly upstream to ERK1/2) PD98059, or the p38 MAPK inhibitor FR167653 on p38 and ERK1/2 phosphorylation by hyperoxic pretreatment, hearts were collected for protein extraction and immunoblotting with phosphospecific antibodies, and the phosphorylated forms were related to the expression of the corresponding total protein. After hyperoxic exposure, phosphorylation of ERK1/2 increased ($p = 0.004$). This was inhibited by the MEK1 inhibitor PD98059 and p38 MAPK inhibitor FR167653 ($p = 0.002$ compared to hyperoxia, respectively). After hyperoxic exposure, p38 MAPK was phosphorylated compared to normoxia ($p = 0.016$). This increase was blocked by the p38 MAPK inhibitor FR167653 ($p = 0.016$) and the MEK1 inhibitor PD98059 ($p = 0.0004$) (Fig. 4). To investigate if NO was involved in signaling of the hyperoxia-induced phosphorylation of p38 MAPK and ERK1/2, the nonselective nitric oxide synthase (NOS) inhibitor L-NAME was used (Fig. 4). Hyperoxia induced cardiac phosphorylation of ERK1/2 compared to normoxia ($p = 0.008$), and this was blocked when animals were pretreated with L-NAME ($p = 0.001$). When normoxic animals were pretreated with L-NAME, there was no influence on the phosphorylation of ERK1/2 compared with normoxia alone ($p = 0.36$) (Fig. 4). Hyperoxia increased cardiac phosphorylation of p38 MAPK compared to normoxia ($p = 0.016$). When L-NAME was given to animals before they were exposed to hyperoxia, the

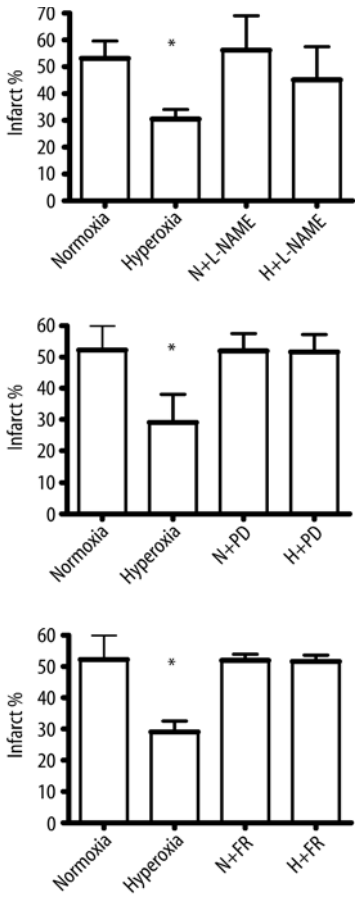


**Fig. 3** Infarct size at the end of 45 min global ischemia and 60 min reperfusion in hearts isolated immediately after 60 min normoxia (N) or hyperoxia ($O_2 > 95\%$, H). The nitric oxide synthase inhibitor L-NAME, the MEK1 (immediate upstream to ERK1/2) inhibitor PD98059 (PD) or the p38 inhibitor FR167653 (FR) were given intraperitoneally 5 min before exposure to either hyperoxia or normoxia and Langendorff perfusion. Data are mean ± SD of 7–9 experiments in each group. * denotes $p < 0.05$ hyperoxia versus all other groups

hyperoxia-induced phosphorylation of p38 MAPK was reduced ($p = 0.0004$). When L-NAME was given to animals that were kept in normoxia, the phosphorylation of p38 MAPK remained unchanged ($p = 0.83$). P-JNK was not increased by hyperoxia, and was not influenced by L-NAME or the MAPK inhibitors (data not shown).

## Discussion

The main findings of the present study were that exposure to > 95 % oxygen for 15 min before heart isolation with induced global ischemia and reperfusion improved hemodynamic recovery and reduced infarct size. These beneficial effects were inhibited in the presence of L-NAME, PD98059, and FR167653, indicating that the protection afforded is signaling through NO, ERK1/2, and p38 MAPK. The latter was confirmed by the finding that the phosphorylation of p38 and ERK1/2 induced by hyperoxia was reduced by L-NAME as well as by the respective MAPK inhibitors.

The fact that hyperoxic exposure (> 95 % oxygen) adapts the myocardium to tolerate ischemia is in accordance with previous studies from our group in rats and mice with either normal or severely atherosclerotic arteries [9, 26, 27]. The present finding that L-NAME injection inhibited the hyperoxia-induced protection indicates that NO is an important substance for protective signaling. As there are no similar studies published on high doses of oxygen and myocardial protection, the following discussion is focused on possible similarities to ischemic preconditioning. The role of NO for evoking delayed protection after ischemic preconditioning is well established [7], while its role in classic ischemic preconditioning is more controversial. Some studies demonstrate that endogenous NO is not a trigger of ischemic preconditioning [16], while other studies indicate a trigger role of endogenous NO [11, 12]. Exogenous NO donors are shown to activate ERK1/2 and p38 through low-molecular-weight G proteins [11, 19]. We have previously found that mice deficient for the inducible NO synthase gene could not be protected by classic ischemic or hyperoxic preconditioning, and interpreted that NO acted as a trigger or early mediator of the protective response rather than as an organ effector, as the time frame for transcription and translation of the gene product was insufficient [30]. The present study supports this interpretation.

The downstream signaling to NO in ischemic preconditioning involves protein kinase C [18] and p38 MAPK [11], although the role of the latter for evoking ischemic preconditioning is a matter of debate [23]. During short episodes of ischemia and reperfusion p38 phosphorylation occurs [13]. Blocking of p38 phosphorylation abolishes the preconditioning effect in myocytes [2]. In the present study we found an increased phosphorylation of p38 after hyperoxic exposure similar to that previously found after ischemic preconditioning. When the p38 inhibitor FR167653 was administered before hyperoxic exposure, p38 phosphorylation was reduced, and the protective effects on infarct size reduction disappeared. p38 MAPK did not alter infarct size in normoxic animals as demonstrated by Tanno et al. [28]. This indicates that p38 MAPK is essential for signaling of protection after hyperoxic exposure, in accordance with others' findings after ischemic preconditioning in rats [10, 22]. However, FR167653 also abrogated the hyperoxia-induced phosphorylation of ERK1/2, confounding this interpretation somewhat. FR167653 is a new p38 MAPK inhibitor, which has previously been found to be specific in rat hearts [33]. Thus, the finding that it also reduced ERK1/2 phosphorylation may be a mouse-specific phenomenon.

ERK1/2 is upregulated in a protein kinase C-depen-

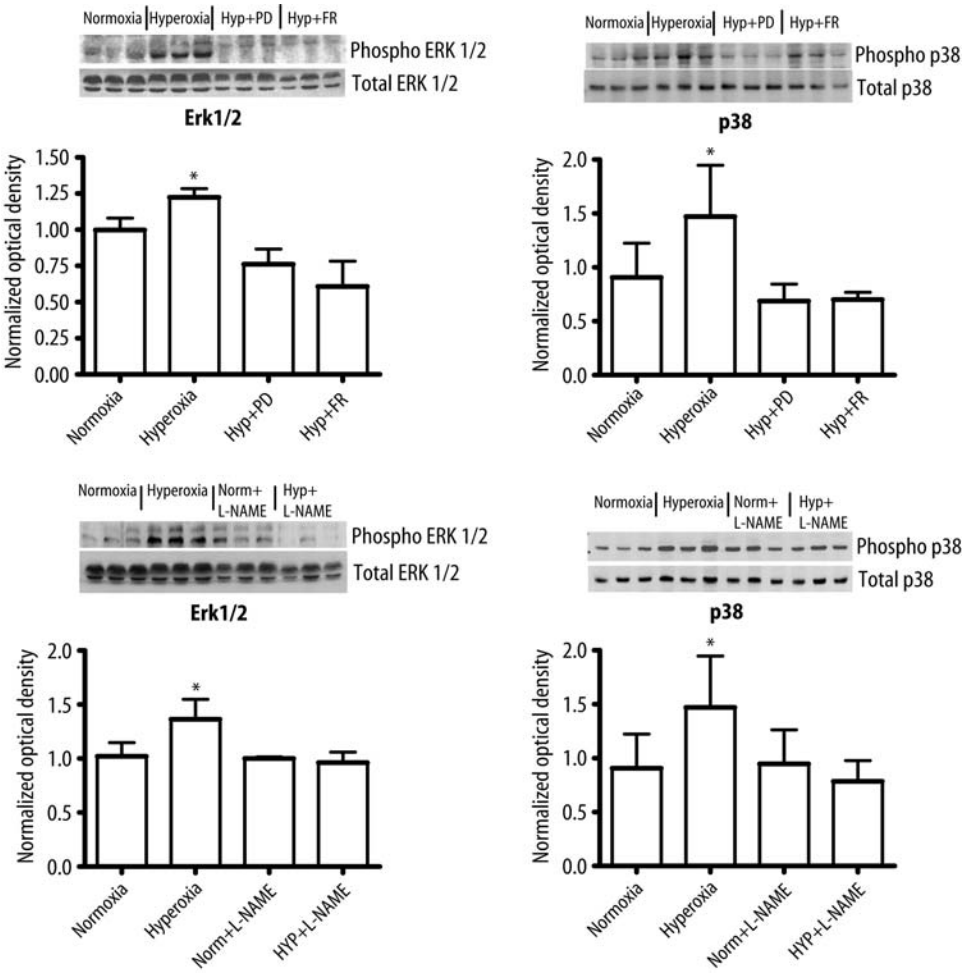


**Fig. 4** Immunoblots of cardiac protein extracts from mice treated with normoxia (N) or hyperoxia for 15 min ($O_2 > 95\%$, H), or treated with intraperitoneal injection nitric oxide synthase inhibitor L-NAME, the MEK1 (immediately upstream to ERK1/2) inhibitor PD98059 (PD) or the p38 inhibitor FR167653 (FR). Hearts were sampled immediately after exposure to normoxia or hyperoxia. The nitrocellulose membranes show representative immunoblots with three samples from each group, where band densities of phosphospecific MAPK were related to the total expression of the corresponding MAPK. The bar graphs show mean ± SD of densitometric readings of all together 6 samples in each group, where the phosphospecific protein is related to total protein as described in M&M. * denotes $p < 0.05$ compared with the normoxia group

dent pathway during ischemic preconditioning [17]. In accordance with findings for ischemic preconditioning, ERK1/2 was phosphorylated after exposure to hyperoxia. This was reduced by administration of PD98059 before exposure. Inhibition of MEK1 inhibited the beneficial effects of hyperoxia on function and infarct size, indicating that ERK1/2 was important for the protection afforded. This was also shown in rabbits during ischemic preconditioning [17]. However, because PD98059 also reduced phosphorylation of p38 MAPK the respective roles of p38 and ERK1/2 for hyperoxic protection remain unresolved.

L-NAME administration prior to hyperoxia completely blocked the hyperoxia-induced phosphorylation of p-ERK1/2 and p38 MAPK, suggesting that NO is upstream to ERK1/2 during hyperoxic exposure. A NO-mediated increase of cGMP concentrations could be a possible explanation for this finding [15]. In the present study no convincing activation of JNK took place after hyperoxic exposure. In general, the activation of JNK seems to follow the activation pattern of p38, although the role of JNK in ischemic preconditioning is even more controversial than for the other MAPK family members [1, 34]. Downstream to the MAPK signaling cascade is the transcription factor NFκB, which plays a crucial role in evoking protection in ischemic and also hyperoxic preconditioning [13, 27]. We have previously proposed that one of the beneficial effects of NFκB activation during preconditioning is upregulation of its own inhibitory factor IκBα, which then reduces the activation of NFκB during ischemia and reperfusion, and thereby reduces the inflammatory reaction [32]. In rat hearts subjected to hyperoxic preconditioning, an increase of IκBα was found during sustained ischemia and early reperfusion [27]. After ischemic preconditioning in rats [27], expression of some NFκB regulated genes were reduced later during reperfusion [8].

In conclusion, hyperoxia protects hearts against ischemia-reperfusion injury. NO is important for evoking this protection. The intracellular signaling of protection involves ERK1/2 and p38 MAPK. However, further elucidation of their relative contribution is needed.

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