Nitric oxide donors protect murine myocardium against infarction via modulation of mitochondrial permeability transition

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Wang, Guangwu, David A. Liem, Thomas M. Vondriska, Henry M. Honda, Paavo Korge, Dawn M. Pantaleon, Xin Qiao, Yibin Wang, James N. Weiss, and Peipei Ping. Nitric oxide donors protect murine myocardium against infarction via modulation of mitochondrial permeability transition. *Am J Physiol Heart Circ Physiol* 288: H1290–H1295, 2005. First published November 4, 2004; doi:10.1152/ajpheart.00796.2004.—Mitochondrial permeability transition (MPT) pores have recently been implicated as a potential mediator of myocardial ischemic injury. Nitric oxide (NO) donors induce a powerful late phase of cardioprotection against ischemia-reperfusion injury; however, the cellular mechanisms involved are poorly understood. The role of MPT pores as a target of cardioprotective signaling pathways activated by NO has never been explored in detail. Thus mice were administered the NO donor diethylenetriamine (DETA)/NO (4 doses of 0.1 mg/kg iv each) 24 h before 30 min of coronary artery occlusion followed by 24 h of reperfusion. Infarct size was significantly reduced in DETA/NO-treated mice ($30 \pm 2\%$ of risk region in treated mice vs. $50 \pm 2\%$ in control mice; $P < 0.05$), which demonstrates powerful cardioprotection. To examine the role of MPT pores, mice were administered atractyloside (Atr; 25 mg/kg iv), which induces adenine nucleotide translocase-dependent MPT, 20 min before ischemia. Atr blocked the infarct-sparing effects of DETA/NO (infarct size, 58 ± 1 vs. $30 \pm 2\%$ of risk region in DETA/NO; $P < 0.05$), whereas Atr alone had no effect. Mitochondria isolated from DETA/NO-treated mice exhibited increased resistance to Ca^{2+}-induced swelling by 20 μmol/l $CaCl_2$ or by the higher concentration of 200 μmol/l, which suggests that cardioprotection involves decreased propensity for MPT. Preincubation of mitochondria from control hearts with 30 nmol/l of the pore inhibitor cyclosporin A prevented swelling by 200 μmol/l $CaCl_2$, thereby confirming that Ca^{2+} induces mitochondrial swelling via MPT. In accordance with the effects on infarct size, administration of Atr to the mice significantly abrogated DETA/NO-induced protection against Ca^{2+}-induced mitochondrial swelling. These phenotypic alterations were associated with an increase in the antiapoptotic protein Bcl-2, which suggests that the underlying mechanisms may involve inhibition of cell death by Bcl-2. These data suggest that a critical process during NO donor-induced cardioprotection is to prevent MPT pore opening potentially via targeting of the adenine nucleotide translocator.

myocardial infarct size; apoptosis; preconditioning; adenine nucleotide translocase; atractyloside

A CENTRAL ROLE OF MITOCHONDRIA to influence cell fate during stress has become increasingly apparent (9, 10, 13, 27, 28). In this regard, mitochondrial permeability transition (MPT), which is the opening of nonselective pores (MPT pores) that span the mitochondrial matrix to the cytosol, has been shown to be a deleterious correlate of ischemia-reperfusion injury (9). Although the precise molecular architecture of MPT pores is unknown, the core components of these pores are thought to include the adenine nucleotide translocator (ANT) in the inner mitochondrial membrane, voltage-dependent anion channels (VDACs) on the outer membrane, cyclophilin D (CyP-D) located in the mitochondrial matrix, and hexokinase II tethered to VDACs in the cytosol (9). Opening of MPT pores leads to a sudden and (partially) reversible increase in permeability of the inner membrane, loss of mitochondrial membrane potential, swelling of the mitochondrial matrix, and ultimately, rupture of the outer mitochondrial membrane and cell death. Recent reports have suggested that preventing MPT may be protective in the setting of ischemia-reperfusion injury (10, 13). In addition, modulation of MPT pore opening has been observed in cardioprotection by both classic ischemic preconditioning of the heart (14) and delayed ischemic preconditioning (22). In the latter report by Rajesh et al. (22), modulation of MPT occurred via the antiapoptotic protein Bcl-2.

Nitric oxide (NO) donors, which are clinically relevant drugs (15, 19), have been shown to induce a powerful "late phase" of cardioprotection in rabbits that manifests 24 h after administration of the drug and increases tolerance of the heart to ischemia-reperfusion insult (6, 7). However, the ability of NO donors to induce cardioprotection in live mice and the precise mechanisms by which NO donors engender protection against ischemia-reperfusion injury are unknown. Interestingly, numerous studies have reported that NO affects mitochondrial function. In noncardiac cells, NO has been shown (20) to attenuate apoptosis by inhibiting caspase activity concomitant with preventing mitochondrial membrane potential loss and the release of cytochrome *c*. It has been suggested (23) that in heart, mitochondria are a target of protective signaling by NO; however, this phenomenon has never been tested in vivo, and the associated signaling mechanisms remain unexplored. In this regard, the antiapoptotic protein Bcl-2 has been shown to inhibit MPT and cell death in some settings (28). It has been observed (8) that transgenic activation of Bcl-2 protects the heart against ischemic injury. However, the involvement of this protein in pharmacological preconditioning has never been examined.

The aim of the present study was to examine whether NO donors induce cardioprotection in mice and whether this phenomenon involves prevention of MPT. The data suggest that

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abrogation of ANT-dependent MPT is a critical protective task of NO and demonstrate for the first time functional regulation of MPT pores by NO in mouse heart. Furthermore, these effects appear to be associated with upregulation of Bcl-2.

MATERIALS AND METHODS

All experimental protocols conform to The National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (Publication No. 86-23).

Drugs. Diethylenetriamine (DETA)/NO (from Alexis) was dissolved in PBS (total volume infused, 200 μl). Atractyloside (Atr; Sigma) was dissolved in DMSO (Sigma) and added with PBS (total volume infused, 50 μl). Cyclosporin A (CsA; Sigma) was dissolved in PBS.

Myocardial ischemia-reperfusion surgery and infarct size analysis. Male ICR mice were subjected to myocardial ischemia-reperfusion as previously described (11, 21). Pentobarbital-anesthetized (50 mg/kg body wt ip) mice were intubated for positive-pressure ventilation with oxygen-enriched room air during the surgical procedure. After we performed a left thoracotomy between ribs three and four, the pericardium was opened, and a silk 8-0 suture was looped under the left anterior descending coronary artery 1–3 mm from the tip of the normally positioned left atrium. Ischemia was induced by ligation of the suture (a 1–2-mm section of polyethylene-10 tubing was placed between the suture and the artery to prevent damage to the vessel). Rectal temperature was continuously measured and maintained at 36.5–37.5°C. After a 30-min coronary artery occlusion, the suture was removed to allow coronary reperfusion followed by closure of the chest wall. After 24 h of coronary artery reperfusion, the heart was excised and postmortem perfusion was performed as previously described (11). The infarct region was determined by perfusion with a 1% solution of 2,3,5-triphenyltetrazolium chloride in phosphate buffer (pH 7.4, 37°C). To delineate the risk region, the coronary artery was tied at the site of the previous occlusion, and the aortic root was perfused with a 1% solution of Evans blue dye. Infarct size was measured by planimetry with NIH Image software and expressed as a percentage of the area at risk.

Treatment protocols for infarct size studies. Mice (8–12-wk old) were given four consecutive intravenous bolus doses, each separated by 25 min, of either DETA/NO (0.1 mg/kg each) or PBS (vehicle; Fig. 1). Ischemic injury was induced 24 h later by a 30-min occlusion of the left anterior descending coronary artery followed by 24 h of reperfusion. To study the role of MPT pores in the late phase of pharmacological preconditioning by DETA/NO, one group received Atr (25 mg/kg iv), which stabilizes ANT in the conformation compatible with pore opening, 20 min before ischemia. Atr was administered 20 min before ischemia in a group not treated with DETA/NO

to insure that this dose of Atr did not induce cell death and/or exacerbate basal ischemia-reperfusion injury.

Mitochondrial isolation. Mitochondria were isolated from adult mouse hearts by enzymatic digestion, homogenization, and differential centrifugation as described previously (3, 4, 17). Isolated mitochondria were resuspended in EGTA-free homogenization buffer (250 mM sucrose, 10 mM HEPES, pH 7.4 with Tris·HCl) to yield 3–5 mg/ml of mitochondrial protein. Mitochondria were kept on ice and used within 4 h.

Mitochondrial pore opening. Opening of MPT pores was determined by Ca^{2+}-induced swelling of isolated cardiac mitochondria (3). Opening of the pore causes mitochondrial swelling, which is measured spectrophotometrically as a reduction in absorbance at 520 nm (A_{520}). Isolated cardiac mitochondria were resuspended in swelling buffer [that contained (in mmol/l) 120 KCl, 10 Tris·HCl (pH 7.4), 20 MOPS, and 5 KH_2PO_4] to a final protein concentration of 0.25 mg/ml. Pore opening was induced by three different concentrations of $CaCl_2$ (200, 20, and 2 μmol/l) and was measured as A_{520}.

Western blotting. Myocardial proteins were resolved on 10% SDS-polyacrylamide gels and transferred onto nitrocellulose membranes as previously described (3, 4, 21, 26). Membranes were blocked with 5% nonfat milk and immunoblotted using anti-Bcl-2 antibodies (1:1,000 dilution), and signals were detected using the ECL detection system (Amersham). Bcl-2 and horseradish peroxidase-conjugated secondary antibodies were purchased from BD Pharmingen. Expression of Bcl-2 densitometry analysis was measured using Epson (3170 Photo) and Action Image software. Equal loading of proteins was confirmed by membrane staining with Ponceau S stain.

Statistical analysis. Infarct size was analyzed by one-way ANOVA followed by Student-Newman-Keuls test. Statistical significance was accepted when $P < 0.05$. Data are presented as means ± SE. Values for ΔA_{520}/min at 5 min were analyzed by Student's *t*-test and are presented as means ± SE.

RESULTS

NO affords a powerful cardioprotective effect in mice in vivo. Although the ability of NO donors to protect rabbit myocardium in vivo is well established, less is known regarding the ability of these compounds to reduce infarct size in murine heart. Thus mice were administered the NO donor DETA/NO 24 h before 30-min coronary artery occlusion and 24-h reperfusion. Compared with PBS-treated mice in the control group, infarct sizes were significantly reduced in the DETA/NO-treated mice (30 ± 2 vs. 50 ± 2% in control; $P < 0.05$), which demonstrates a potent NO donor-induced late phase of cardioprotection in mice (Fig. 2).
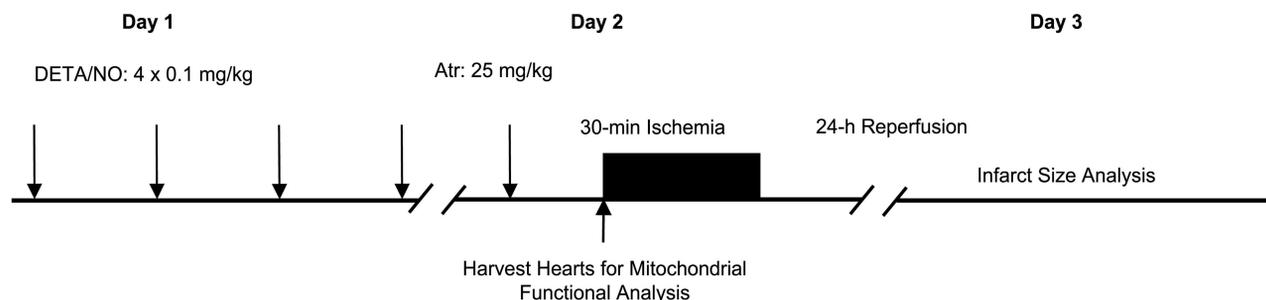

Fig. 1. Schematic overview of experimental protocols. On *day 1*, mice were treated either with four bolus injections of diethylenetriamine/nitric oxide (DETA/NO; 4 × 0.1 mg/kg iv), each separated by 25 min, or by the DETA/NO solvent PBS. On *day 2*, ischemia was induced in all groups by 30-min coronary artery occlusion. To examine the role of mitochondrial permeability transition (MPT) pores, one group that received DETA/NO on *day 1* was treated with atractyloside (Atr; 25 mg/kg) 20 min before ischemia on *day 2*. A control group was administered Atr alone 20 min before ischemia on *day 2* after treatment with DETA/NO vehicle PBS on *day 1*. For analysis of mitochondrial function, hearts were harvested on *day 2* in lieu of ischemia, and mitochondria were isolated as described (see MATERIALS AND METHODS).
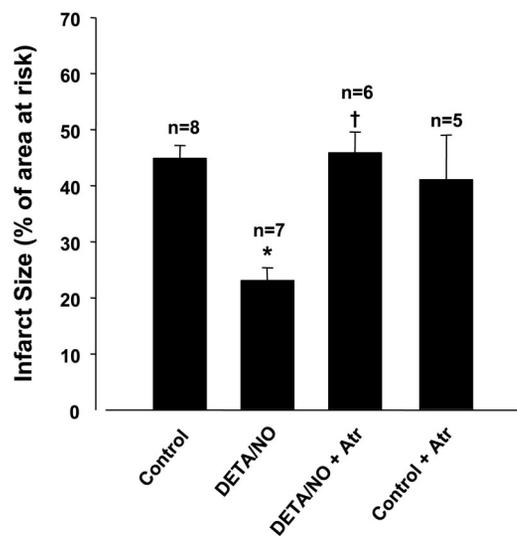
Fig. 2. Infarct sizes in all groups are expressed as a percentage of area at risk. Cardioprotection by DETA/NO (4×0.1 mg/kg) was abolished by treatment with Atr (25 mg/kg) 20 min before ischemia. Atr by itself did not affect infarct size. $*P < 0.05$ vs. PBS; $\dagger P < 0.05$ vs. DETA/NO.

Involvement of MPT pores in NO donor-induced cardioprotection. To examine whether MPT pores were involved in cardioprotection by NO, we administered the ANT ligand and MPT inducer Atr to mice before the ischemic insult on *day 2* (see Fig. 1). Atr blocked the infarct-sparing effects of the NO donor but had no effect in altering the susceptibility of the myocardium to infarction when administered alone (see Fig. 2). These findings indicate that opening of MPT pores blocks protection by NO donors.

Atr blocks NO donor-induced protection of mitochondria. To examine whether resilience of mitochondria to Ca^{2+}-induced swelling is a subcellular alteration associated with the infarct-sparing effects of NO, we examined mitochondrial swelling by 200 μmol/l $CaCl_2$ (measured by decrease in A_{520}; Fig. 3A) in mitochondria from normal or NO donor-treated mice. The dose of 200 μmol/l Ca^{2+} induced a clear decrease of A_{520} over 5 min. In mitochondria from DETA/NO-treated mice, the decrease of A_{520} over 5 min was abrogated at the same time point at which the heart was protected against ischemia-reperfusion injury (24 h after DETA/NO administration, on *day 2*) hereby demonstrating protection against Ca^{2+}-induced swelling. This mitochondrial protection against swelling was blocked by the same mechanism that blocked the infarct-sparing effects of DETA/NO, namely, administration of Atr to mice 20 min before swelling analyses. Also similar to the infarct-size data, administration of Atr alone did not exacerbate mitochondrial swelling (Fig. 3A). For each group, representative traces from three independent experiments are shown. These data support the concept that ANT-dependent MPT is blocked by NO donors in the mouse heart.

Effects of different Ca^{2+} concentrations on mitochondrial swelling. We additionally studied the effects of different Ca^{2+} concentrations (200, 20, and 2 μmol/l $CaCl_2$) on mitochondrial swelling in both the control and the DETA/NO-preconditioned groups. In all three concentrations, Ca^{2+}-induced mitochondrial swelling was inhibited in the DETA/NO-preconditioned mice compared with PBS treated animals (Fig. 3, *B, C,* and *D*).

Confirming that Ca^{2+}-induced mitochondrial swelling is due to MPT, 5-min incubation with the pore inhibitor CsA before addition of 200 μmol/l $CaCl_2$ abolished the effect on dissipation of A_{520} in both groups in mitochondria from control and DETA/NO-treated mice. In both the control and the DETA/NO-preconditioned groups, the higher Ca^{2+} concentrations of 200 and 20 μmol/l showed an enhanced total change of A_{520} over 5 min of Ca^{2+}-induced mitochondrial swelling, whereas the low dose of 2 μmol/l did not exhibit a decrease in absorbance during 5 min of Ca^{2+}-induced swelling in both groups (Fig. 3*E*).

NO donors induce increased Bcl-2 expression. To further examine the molecular mechanism(s) involved in protection of cardiac mitochondria by NO, we studied the expression of Bcl-2 24 h after NO donor administration. Immunoblotting indicates a global increase in Bcl-2 expression in hearts 24 h after administration of NO (178 $\pm$ 11.3% of control Bcl-2 expression; $P < 0.05$). The experiments were repeated three times. Blots show 4 hearts from a total of 12 animals. Equal loading onto nitrocellulose membranes was confirmed by membrane staining with Ponceau S stain (Fig. 4). These findings suggest that Bcl-2 is a critical protective protein that is upregulated by NO donors in heart and may be involved in the MPT-blocking effects associated with the cardioprotective phenotype.

DISCUSSION

Although numerous investigations have recently emphasized the importance of mitochondria in the ischemic myocardium (3, 10, 13, 27), the relationship of this organelle's function to myocyte survival in this setting has only begun to be understood. There are several novel findings in this study that address this gap in our knowledge. First, we have demonstrated for the first time that in vivo administration of NO donors reduces the propensity for pathological MPT coincident with reducing infarct size. Moreover, this ability of NO to protect cardiac mitochondria against MPT was associated with ANT-dependent protection against cell death. The infarct-sparing effects of NO donors, which are well established in animal models such as rabbits, were confirmed in live mice, and this effect was blocked by the ANT ligand Atr. Last, these NO donor-induced beneficial organ and organelle endpoints were associated with increased Bcl-2 protein expression in cardiac cells.

With the increased appreciation that mitochondria, in addition to energy production, also play a central role in regulating apoptotic and necrotic cell death (9, 10, 27, 28), the purported MPT pores, which are multiprotein complexes in mitochondria that can induce pathological nonselective permeablization of the inner membrane, have become a target of investigation in heart. The precise components of MPT pores are unknown, but commonly accepted components include ANT in the inner mitochondrial membrane, VDACs on the outer membrane, hexokinase, and CyP-D, which is a peptidyl prolyl *cis-trans* isomerase located in the matrix (9). Under normal aerobic conditions, electron transport generates a large electrochemical gradient across the inner mitochondrial membrane that is used to synthesize ATP via oxidative phosphorylation. Maintenance of membrane potential requires that the inner membrane re-
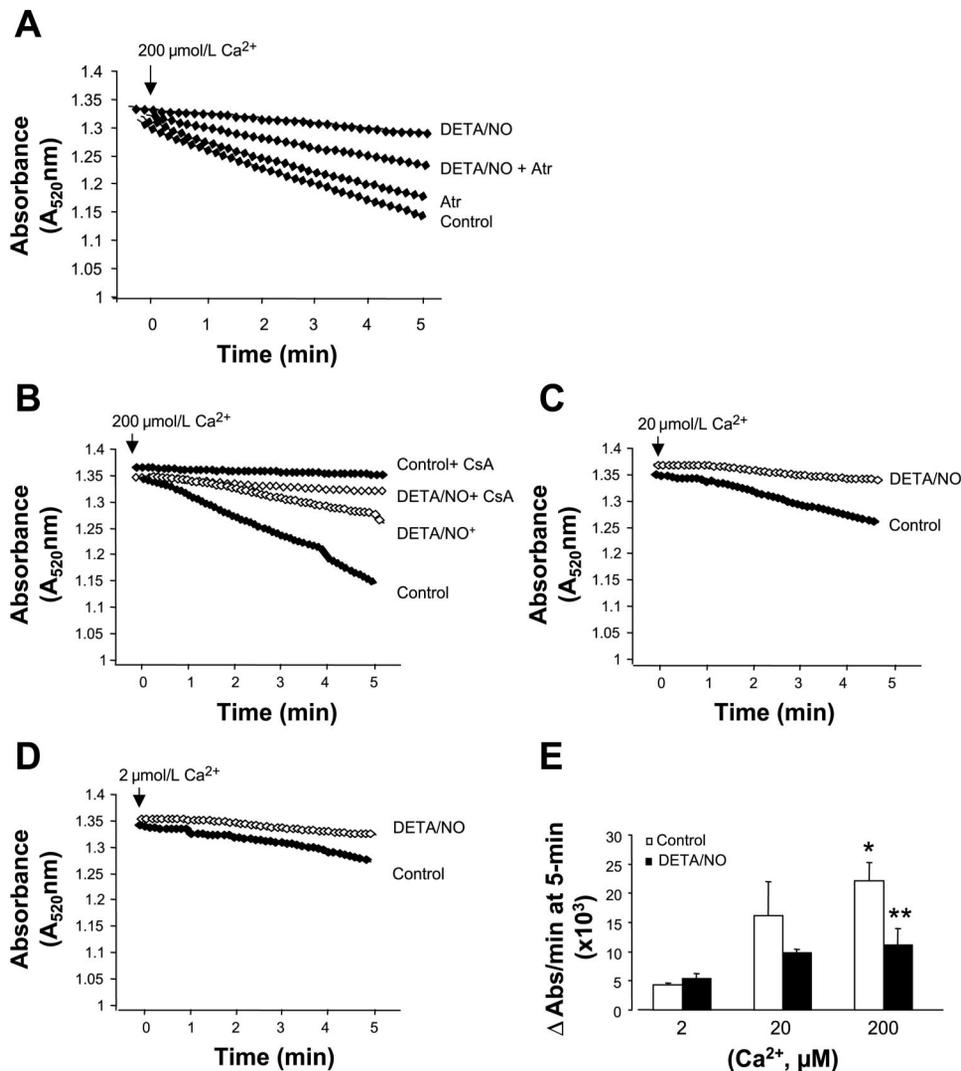
Fig. 3. NO donors protect against MPT. Ca^{2+}-induced swelling, which is an index for MPT, was measured as a decrease in absorbance at a 520-nm wavelength (A_{520}) in isolated mitochondria 24 h after administration of DETA/NO (4×0.1 mg/kg), Atr (25 mg/kg), or PBS vehicle. Representative traces from three independent experiments are shown for cardiac mitochondria isolated from vehicle control, DETA/NO, DETA/NO + Atr, and Atr-treated mice (*A*). Treatment of mice with DETA/NO protected against mitochondrial swelling at the same time point at which it reduced infarct size (24 h after treatment). Adenine nucleotide translocator (ANT) ligand and the MPT-inducing molecule Atr blocked this effect. Effects of 200 (*B*), 20 (*C*), and 2 μmol/l (*D*) Ca^{2+} on A_{520} in mitochondria from both control group and DETA/NO-preconditioned group are shown. Pore inhibitor cyclosporin A (30 nmol/l) abolished the effects on A_{520} in both groups, thereby confirming that Ca^{2+}-induced mitochondrial swelling is due to MPT. A total change in A_{520} over 5 min of Ca^{2+}-induced mitochondrial swelling was indicated for all three Ca^{2+} concentrations (*E*). *$P < 0.05$ vs. 2 μmol/l Ca^{2+}; **$P < 0.05$ vs. control.

main impermeable to ions and that ANT maintain its function as a translocase to exchange ADP for ATP. During pathological insults such as ischemia-reperfusion injury, ANT can be converted from a nucleotide transporter into a nonselective pore that contributes to MPT. Pore opening leads to an influx of solutes and water that causes mitochondrial matrix swelling. Eventually this increased volume can rupture the outer membrane and cause release of inner membrane space contents such as proapoptotic factors.

To assess the effects of Ca^{2+} in a dose-dependent fashion, we assayed different Ca^{2+} concentrations, including 2, 20, and 200 μM. The effects of Ca^{2+} on MPT in isolated mitochondria have previously been studied by several groups using different Ca^{2+} concentrations. In the aforementioned study by Rajesh et al. (22), the Ca^{2+} concentration to induce MPT was 10-fold higher than in our present study. In contrast, Korge et al. (18) used lower Ca^{2+} concentrations (10–20 μM) to elicit MPT. Thus the Ca^{2+} concentration does have a significant effect on mitochondrial swelling, a phenomenon that is related to the K_m. This concentration-dependent effect of Ca^{2+} on MPT has also recently been investigated by Argoud et al. (2). In that study, the authors increased Ca^{2+} concentration starting from

20 μM. Isolated mitochondria from rabbit hearts after index ischemia showed MPT at a Ca^{2+} overload of 180 μM, whereas mitochondria that were subjected to ischemic preconditioning showed MPT at an extended Ca^{2+} overload of 300 μM. Nevertheless, in our study, the mouse hearts were harvested 24 h after DETA/NO treatment without index ischemia.

In the present study, we used the ANT inhibitor Atr to open the MPT pores. Atr is a ligand that binds and stabilizes ANT in the c conformation, which is compatible with pore opening (28). Intravenous administration of Atr before coronary artery occlusion completely attenuated the protective effects of the NO donor, which suggests that ANT-dependent pore formation prevents the beneficial effects of NO on mitochondria. Because Atr had no effect on infarct size in control (i.e., non-DETA/NO-treated) mice, the possibility that this dose of Atr itself induced cell death can be ruled out. Likewise, the ability of NO to exhibit mitochondrial-dependent protection of the heart is bolstered by the finding that isolated mitochondria from NO donor-treated mice are resistant to Ca^{2+}-induced mitochondrial swelling, whereas those from DETA/NO-treated mice that were also administered Atr are indistinguishable from control (i.e., unprotected) animals. These findings provide a functional
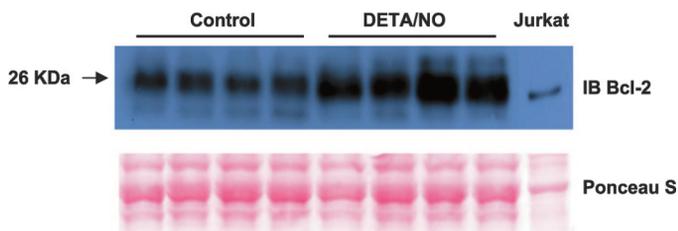
Fig. 4. NO donor-induced protection involves upregulation of the antiapoptotic protein Bcl-2. Immunoblotting for Bcl-2 in control or DETA/NO-treated hearts reveals a significant increase in Bcl-2 expression (178 $\pm$ 11.3% of control Bcl-2 expression; $P < 0.05$) 24 h after administration of DETA/NO, which suggests that upregulation of this antiapoptotic protein may contribute to protective signaling induced by NO donors. Expression of Bcl-2 densitometry analysis was done using Epson (3170 Photo) and Action Image software, and Ponceau S staining was performed. Experiments were repeated three times. Blots show 4 hearts from a total of 12 animals.

link between the actions of NO to reduce infarct size and to protect mitochondria against MPT-inducing stimuli.

It is important to note that the dynamics of the mitochondrial proteome and therefore mitochondrial function are likely organ specific. Briefly, Kokoszka et al. (16) have shown that mitochondria from liver of systemic ANT-1-knockout mice combined with ANT-2-conditional knockout mice were used to genetically inactivate ANT. Although the authors claim that both ANT-1 and ANT-2 were not detectable, the liver mitochondria retained their MPT pore activity with abnormal Ca^{2+} sensitivity. Accordingly, a conclusion was made that ANT may not be an essential component in MPT pores of mitochondria. As recently discussed by Halestrap (12), these results are in complete contradiction with a substantial number of previous studies. It is surprising that mice without hepatic ANT are able to survive the subsequent loss of these essential metabolic pathways. It might be possible that another member of the mitochondrial carrier family takes over the role of ANT when it is knocked out. Alternatively, the liver might switch to using much more glucose to generate ATP by glycolysis in the cytosol. A final possibility is that the mitochondria in this study (16) were not totally devoid of ANT activity. Several groups have observed that heart mitochondria express large amounts of ANT-1 isoform and little ANT-2, whereas livers (and other organs) express low amounts of ANT-1 and large amounts of ANT-2 (25). Moreover, liver is more glucose producing than glucose utilizing, which is in complete contrast with the heart. Furthermore, the reported liver mitochondria exhibit unique functional characteristics distinct from those of cardiac mitochondria with respect to membrane permeability to ions. It was recently observed in liver mitochondria that ANT can either function as or modulate mitochondrial ATP-sensitive K^+ (K_{ATP}) channels (1). Nevertheless, the role of these three functions of ANT are not yet fully established in either heart or liver. These considerations taken with the fact that two forms of cardioprotection [induced by PKC-ε transgenesis (3) or NO donors (see Fig. 2)] are blocked by Atr support a potential role for this molecule in MPTs in heart. It is also important to note the agreement of the organ (infarct size) and organelle (mitochondrial swelling) endpoints with regard to MPT inhibition in the present study.

Neither the susceptibility of the myocardium to infarction nor that of the mitochondria to undergo MPT was affected by administration of Atr alone. However, Atr was effective to block the protective effects of DETA/NO in both of these respects. These findings support the idea that ANT-dependent manipulation of MPT with Atr is an effective approach for unraveling the role of MPT in cardioprotection without simultaneously affecting the basal susceptibility of the heart to injury. Furthermore, it should be noted that although Atr was administered before ischemia in the present study, the possibility cannot be excluded that this compound (or its effects on ANT) are still present during the reperfusion period. Indeed, previous studies have implicated involvement of MPT pores at reperfusion (13), and thus our findings complement rather than supplant these previous investigations. Furthermore, consistent with previous studies (13, 22), Atr by itself did not enhance infarct size in ischemia-reperfusion. One possible scenario to explain this observation is that after ischemia-reperfusion, MPT pores are already fully open, and thus MPT cannot be further enhanced by Atr. A second possible scenario is that ischemia-reperfusion injury renders MPT pores less sensitive to Atr. However, the precise molecular mechanism to clarify this remains elusive and requires additional characterization. Future studies are required to unequivocally discern the temporal involvement of MPT pores in ischemia-reperfusion injury.

Previous studies documented a late phase of NO donor-induced cardioprotection in conscious rabbits (6) and in isolated mouse heart (5), and the present study extends these findings to the in vivo setting. Administration of NO to rabbits is known to induce transcription of cardioprotective genes including inducible NO synthase (4) and to activate a signaling module that contains PKC-ε and Src tyrosine kinase (16). However, targeting of the mitochondria in this setting remained uncertain. It has been suggested that NO might induce the opening of mitochondrial K_{ATP} channels (24); however, the mechanism for such action remains unknown. In addition, a connection between NO and mitochondria-mediated apoptosis including suppression of caspase activity and/or cytochrome *c* release (20) has been suggested. In the present study, the doses of NO sufficient to reduce myocardial infarct size were associated with beneficial effects on mitochondria. These effects include upregulation of the antiapoptotic protein Bcl-2 and ultimately decreased propensity for mitochondrial swelling. Previous investigations have implicated Bcl-2 in cell survival (for review, see Ref. 28), and cardiac transgenesis of Bcl-2 is sufficient to protect against apoptotic cell death (8). However, to our knowledge, the present findings are the first to indicate that pharmacological cardioprotection by NO involves activation of Bcl-2. Our previous studies have demonstrated localization of the protective kinase PKC-ε to mitochondria (3) and have shown that PKC-ε transgenesis is associated with improved mitochondrial function and cardioprotection. Because of the established role of PKC-ε signaling in the setting of NO donor-induced cardioprotection, it is reasonable to hypothesize that this kinase may also play a role in mediating the improved mitochondrial function that we observed in the present investigation. Future studies will unravel the specific manner in which this kinase regulates MPT pores in response to NO.

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