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Effects of nitric oxide donors on cardiac contractility in wild-type and myoglobin-deficient mice

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1 The effects of the nitric oxide (NO) donors S-nitroso-N-acetylpenicillamine (SNAP), sodium(*Z*)-1-(*N,N*-diethylamino)diazen-1-ium-1,2-diolate (DEA-NONOate), and (*Z*)-1-[*N*-(2-Aminoethyl)-*N*-(2-ammonioethyl)amino]diazen-1-ium-1,2-diolate (DETA-NONOate) on force of contraction (F_c) were studied in atrial and ventricular muscle strips obtained from wild-type (WT) and myoglobin-deficient ($myo^{-/-}$) mice.

2 SNAP slightly reduced F_c in preparations from WT mice at concentrations above 100 μM; this effect was more pronounced in $myo^{-/-}$ mice.

3 DEA-NONOate reduced F_c in preparations from $myo^{-/-}$ mice to a larger extent than those from WT mice.

4 DETA-NONOate reduced F_c in preparations from $myo^{-/-}$ but not from WT mice.

5 Pre-incubation with an inhibitor of the soluble guanylyl cyclase (1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one; 100 μM) prevented the effects of SNAP, DEA-NONOate and DETA-NONOate on F_c in $myo^{-/-}$ mice.

6 It is suggested that, in physiological conditions, myoglobin acts as intracellular scavenger preventing NO from reaching its intracellular receptors in cardiomyocytes, whereas, in myoglobin-deficient conditions, NO is able to reduce contractility *via* activation of the soluble guanylyl cyclase/cyclic GMP pathway.

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Abbreviations: DEA-NONOate, sodium(Z)-1-(N,N-diethylamino)diazen-1-ium-1,2-diolate; DETA-NONOate, (Z)-1-[N-(2-Aminoethyl)-N-(2-ammonioethyl)amino]diazen-1-ium-1,2diolate; DMSO, dimethylsulphoxide; eNOS, endothelial NO synthase; F_c, force of contraction; $myo^{-/-}$, myoglobin-deficient; NO, nitric oxide; NS, not statistically significant; ODQ, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one; sGC, soluble guanylyl cyclase; SNAP, S-nitroso-N-acetylpenicillamine; WT, wild-type

Introduction

It is well established that nitric oxide (NO) represents an important signal molecule involved in several physiological processes including regulation of vascular tone and platelet aggregation (Moncada *et al*., 1991). However, the effects of NO on myocardial contractility are still discussed controversially. For instance, NO or related substances have been reported either to reduce (Flesch *et al*., 1997; Smith *et al*., 1991), to increase (Kojda *et al*., 1996) or not to affect cardiac contractility (Nawrath *et al*., 1995; Weyrich *et al*., 1994). Several possibilities have been suggested to account for this discrepancy. Developmental changes in the expression of the NO/cGMP signalling pathway may induce different responses to NO at different ages of the preparations used (Ji *et al*., 1999; Vulcu *et al*., 2000). Alternatively, the myoglobin content in cardiac muscle, which is different among species (O'Brien *et al*., 1992) and even between cardiac muscle preparations of one species (Ishibashi *et al*., 1993) may account for the differences in NO actions on the heart since myoglobin acts as an NO scavenger (Flögel *et al*., 2001; Mittal *et al*., 1978). In the present study, the latter hypothesis was tested by investigating the effects of different NO donors on force of contraction (F_c) in cardiac muscle preparations from wild-type mice (WT) and myoglobin-deficient ($myo^{-/-}$) mice.

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Methods

$Myo^{-/-}$ mice were obtained from the Dept. of Cardiovascular Physiology, Düsseldorf, at which they were generated as described (Gödecke *et al*., 1999). Briefly, myoglobin-specific genomic clones were isolated from a genomic library of mouse strain 129Sv by using a 266-bp, PCR-amplified cDNA fragment of the murine myoglobin gene spanning exon 2. A targeting vector containing the neomycin resistance gene instead of exon 2 was used to transfect the embryonic stem cell line R1. Transfected stem cells were aggregated with murine embryos isolated from NMRI mice.

Mice of either sex were killed by cervical dislocation at the age of 4–12 weeks. The heart was quickly removed and immersed in warmed (37°C) and oxygenated buffer solution (containing in mM: NaCl 137, KCl 5.4, $CaCl_2$ 3.6, $MgCl_2$ 1, $NaHCO_3$ 12, NaH_2PO_4 0.42, glucose 5.6; aerated with 95%

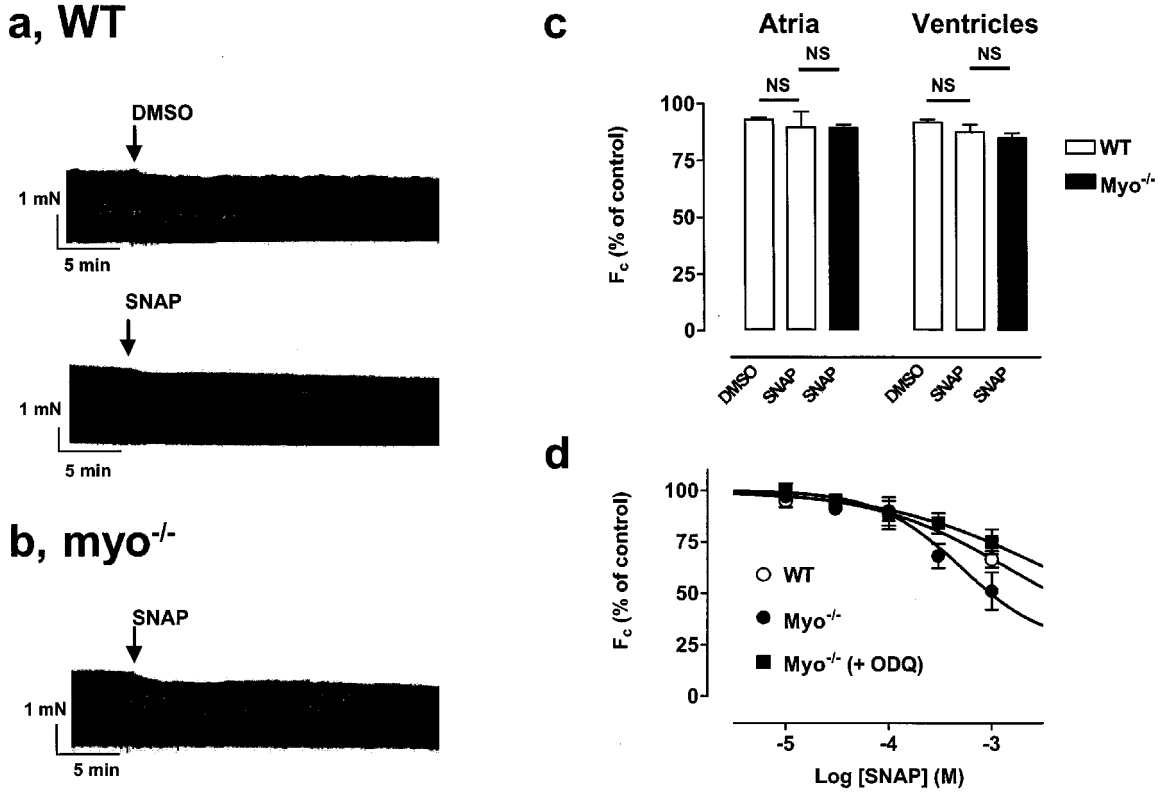

Figure 1 Effects of SNAP on F_C in cardiac muscle from WT and myo$^{-/-}$ mice. (a, b) Original recordings of F_C from atrial preparations of WT (a) and myo$^{-/-}$ mice (b). Arrows indicate the times at which SNAP (100 μM) or the solvent DMSO (0.5%, v v^{-1}) was added. (c) Bars represent means$\pm$s.e.mean ($n=6-19$). Data were obtained 5 min after the addition of SNAP (100 μM) or DMSO. NS indicates the absence of a statistically significant difference. (d) Concentration-response curves of SNAP in atria from WT mice and from myo$^{-/-}$ mice in the absence and presence of ODQ (100 μM). Symbols represent means$\pm$s.e.mean ($n=3-4$).

$O_2+5\%$ CO_2; pH 7.4). The left and right atria were supplied at either end with silk ligatures as well as ventricular muscle strips as described (Wegener & Nawrath, 1997).

The cardiac preparations were mounted vertically in organ baths (5 ml) containing oxygenated buffer solution at 36 ± 1°C. One end was fixed to a hook of a muscle holder while the other end was connected to an inductive force-displacement transducer whose output was fed to a carrier frequency preamplifier (TA2000, Gould, www.gouldis.de). The preparations were stretched to the apex of the preload active tension curve, mounted next to two platinum electrodes built in a muscle holder, and electrically stimulated by square-wave voltage pulses at 3 Hz (1 ms duration, voltage 20% above threshold; Grass S4, www.astro-med.com). Drugs were applied as single dose or cumulatively to achieve the concentrations indicated.

S-nitroso-N-acetylpenicillamine (SNAP), sodium(*Z*)-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (DEA-NONOate), (*Z*)-1-[*N*-(2-Aminoethyl)-*N*-(2-ammonioethyl)amino]-diazen-1-ium-1,2-diolate (DETA-NONOate), and 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ) were purchased from Alexis (www.alexis-corp.com). Stock solutions of SNAP and ODQ were prepared in dimethylsulphoxide (DMSO) and further diluted to the concentrations indicated. The DMSO content in the test solutions did not exceed 0.5% v v^{-1}. All other chemicals used were as pure as commercially available and purchased from Sigma (www.sigma-aldrich.com).

Data are presented as original recordings or expressed as means$\pm$s.e.means. The magnitude of F_c was measured as the difference between resting and peak tension. Statistical analysis was performed using either paired or unpaired Student's *t*-test. The present study conforms with the Guide for the Care and Use of Laboratory Animals (U.S. National Institute of Health, publication 8523, revised 1985).

Results

The effects of three NO donors on myocardial contractility were investigated in WT and myo$^{-/-}$ mice. The NO donor SNAP, at a concentration of 100 μM, fully effective in smooth muscle (Henry *et al.*, 1989), did not change significantly force of contraction (F_c) in atrial and ventricular preparations from both groups of animals (Figure 1a, b). The minor decrease of F_c after addition of the drug was not statistically different from that observed if the solvent (DMSO) was applied without drug (Figure 1c). However, concentration of SNAP above 100 μM slightly reduced F_c in atrial preparations from WT mice; this effect was larger in preparations from myo$^{-/-}$ mice (Figure 1d). In the presence of ODQ (100 μM), the effects of SNAP in myo$^{-/-}$ mice were not statistically different from those obtained in WT mice.

The NO donor DEA-NONOate (100 μM) transiently reduced F_c in atrial and ventricular preparations from both

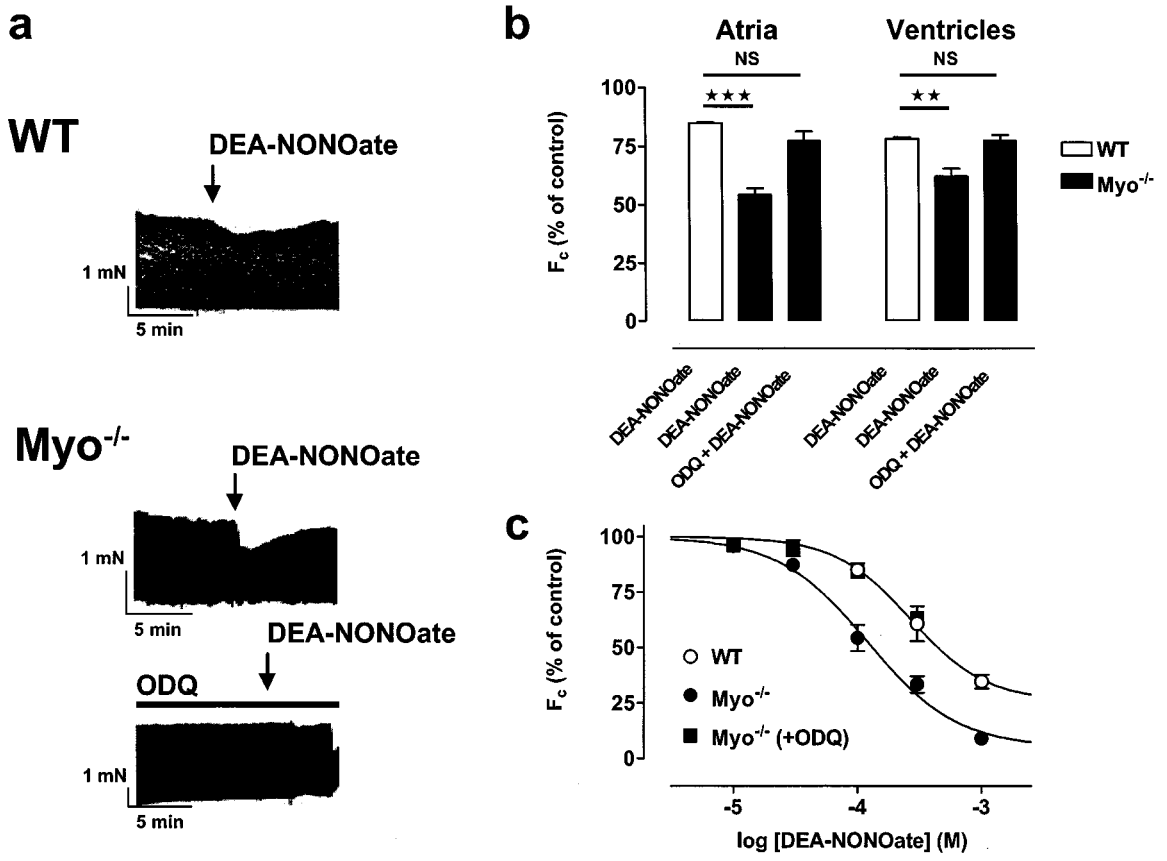

Figure 2 Effects of DEA-NONOate on F_C in cardiac muscle from WT and myo$^{-/-}$ mice. (a) Original recordings of F_C from atrial preparations of WT (upper panel) and myo$^{-/-}$ mice (middle and lower panel). Arrows indicate the times at which DEA-NONOate (100 μM) was added. The bar indicates the presence of ODQ (100 μM). (b) Bars represent means$\pm$s.e.mean ($n=3-14$). Data represent the maximal effects of DEA-NONOate (100 μM) which occurred after 2 to 5 min. ODQ (100 μM) was applied 10 min before DEA-NONOate. NS and asterisks indicate the absence and presence of statistically significant differences, respectively. (c) Concentration-response curves of DEA-NONOate in atria from WT and myo$^{-/-}$ mice in the absence and presence of ODQ (100 μM). Symbols represent means$\pm$s.e.mean ($n=3-6$).

WT and myo$^{-/-}$ mice. The effect was more pronounced in preparations from myo$^{-/-}$ than in those from WT mice (Figure 2). F_c was maximally reduced after 2–5 min and reached control values within 10 min. Pre-incubation of the preparations with ODQ (100 μM) for 10 min prevented the effects of DEA-NONOate on F_c in myo$^{-/-}$ mice (Figure 2). The concentration-dependence of the effects was studied in atrial preparations: the EC_{50} of DEA-NONOate was calculated to 0.3 and 0.1 mM in preparations from WT and myo$^{-/-}$ mice, respectively (Figure 2d). However, a major part of the preparations developed a contracture at concentrations higher than 1 mM and was rejected from the analysis.

The NO donor DETA-NONOate was without effect in atrial and ventricular preparations from WT mice but reduced F_c in those from myo$^{-/-}$ mice (Figure 3). The effect of 100 μM DETA-NONOate developed slowly and reached a steady state within 30 min (Figure 3a). The effects of DETA-NONOate were absent, if the preparations were pre-incubated with ODQ (100 μM) for 10 min (Figure 3). The concentration-dependence of the effects were studied in atrial preparations from myo$^{-/-}$ mice: the EC_{50} amounted to 0.1 mM (Figure 3c).

Discussion

The present study has shown that NO donors of the NONOate group, DEA-NONOate and DETA-NONOate, were more effective in reducing contractility in cardiac preparations from myo$^{-/-}$ than from WT mice. The S-nitrosothiol-type NO donor SNAP, although effective in smooth muscle preparations (Henry *et al*., 1989), did barely change cardiac F_c in WT mice but reduced weakly F_c in myo$^{-/-}$ mice, albeit at concentrations greater than 100 μM. In line with these findings, DEA-NONOate but not SNAP have been reported to reduce β-adrenergic-stimulated Ca^{2+} current in rat ventricular cardiomyocytes (Abi-Gerges *et al*., 2001). A possible explanation for this difference may be the way how the substances release NO. NONOates are relatively stable as the pH is raised but release NO spontaneously at physiological or low pH, albeit at different time constants (Keefer *et al*., 1996). S-nitrosothiols are believed to decompose non-enzymatically to give NO but also to transfer the nitroso group to other molecules (Williams, 1996). In addition, the generation of NO from S-nitrosothiols has been shown to depend on protein-bound Cu^{2+} sources (Dicks & Williams, 1996). However, it remains

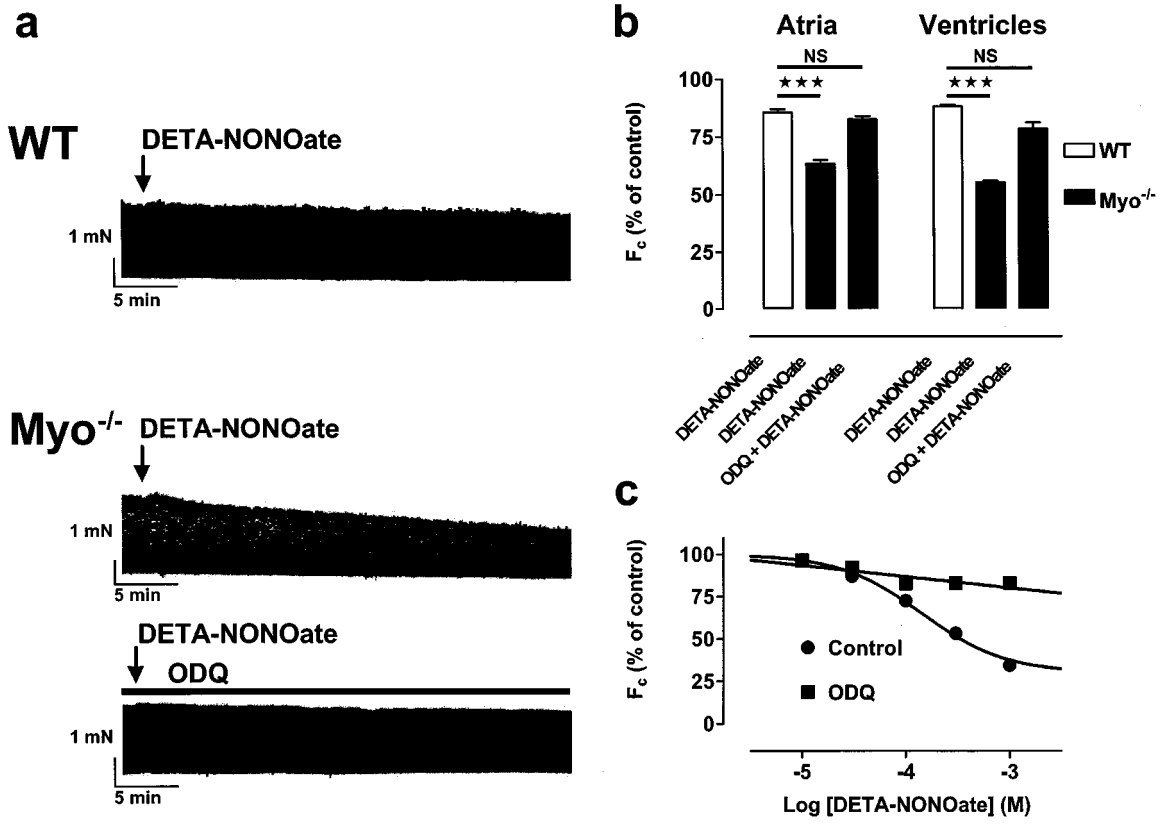

Figure 3 Effects of DETA-NONOate on F_C in cardiac muscle from WT and myo$^{-/-}$ mice. (a) Original recordings of F_C from atrial preparations of WT (upper panel) and myo$^{-/-}$ mice (middle and lower panel). Arrows indicate the times at which DETA-NONOate (100 μM) was added. The bar indicates the presence of ODQ (100 μM). (b) Bars represent means$\pm$s.e.mean ($n=3-9$). Data were obtained 30 min after the addition of DETA-NONOate (100 μM). ODQ (100 μM) was applied 10 min before DETA-NONOate. NS and asterisks indicate the absence and presence of statistically significant differences, respectively. (c) Concentration-response curves of DETA-NONOate in atria from myo$^{-/-}$ mice in the absence and presence of ODQ (100 μM). Symbols represent means$\pm$s.e.mean ($n=3-5$).

open, whether these reactions have a bearing on the weak cardiac effects of SNAP.

The effects of DEA-NONOate and DETA-NONOate on F_c had completely different time courses. The effect of DEA-NONOate reached a maximum within 2 min and returned to control values within 10 min. In contrast, the effect of DETA-NONOate reached its maximum within 30 min. An explanation for this difference may be the different time course of the NO release by each drug. The decomposition of DEA-NONOate and DETA-NONOate to release NO shows a half-life (at 37°C) of about 2 min and 20 h, respectively (Keefer *et al*., 1996). Using these values, the concentration-time profile of the NO release from DEA-NONOate and from DETA-NONOate was calculated taking into account NO auto-oxidation as described (Schmidt *et al*., 1997). According to this calculation, NO released by 100 μM DEA-NONOate produced a peak concentration of 10 μM within 80 s and returned to about 2 μM after 5 min. NO released by 100 μM DETA-NONOate produced a steady-state concentration of 0.6 μM within 1000 s during the calculated time interval of 40 min. These calculated profiles of [NO] fit well to and are, therefore, assumed to determine the time courses of the cardiac effects of DEA-NONOate and DETA-NONOate in myo$^{-/-}$ mice observed in the present study. Similarly, fast and slow time courses were also shown for the vasorelaxant effects of DEA-NONOate and SPER/NO ($t_{1/2}=39$ min) and related to the different time courses of NO release by each drug (Morley *et al*., 1993).

Recently, myoglobin has been suggested to be involved in the inactivation of NO and to substantially determine the NO effects on coronary blood flow (Flögel *et al*., 2001). In the latter study, NO decreased dp/dt in a Langendorff-type preparation, and this effect was slightly pronounced in hearts from myo$^{-/-}$ mice. The present study shows a more complete dissociation of the effects of NO donors in normal and myoglobin-deficient hearts. Most probably, the different techniques to assess contractility (whole heart vs isolated strips in isometric conditions) account for these differences. It seems likely, therefore, that the level of myoglobin (around 100–200 μM; (Wittenberg, 1970)) being present in the cardiomyocytes from WT mice acts as an intracellular scavenger for NO, as has been shown for extracellular haemoglobin (Martin *et al*., 1985), reducing the effective NO concentration in the cardiomyocytes. As a consequence, only high levels of exogenously applied NO may reach intracellular target molecules to induce physiological effects, as seen for 100 μM DEA-NONOate (corresponding to about 10 μM NO) in the preparations from WT mice (Figure 2). On the

other hand, low levels of myoglobin may make it easier for exogenously derived NO to reach intracellular receptors in cardiomyocytes. Indeed, different levels of myoglobin were taken as evidence for the differences in NO donor-induced increases in cGMP levels in atrial and ventricular preparations from the rabbit (Ishibashi *et al*., 1993) and the differences of NO donor-dependent effects in cardiac muscle from neonatal and adult rats (Vulcu *et al*., 2000).

The effects of the NO donors DEA-NONOate and DETA-NONOate were absent in the presence of ODQ, an inhibitor of soluble guanylyl cyclase (sGC) (Garthwaite *et al*., 1995). These results suggest that the cGMP/sGC signalling pathway is involved in mediating the effects of NO in the murine myocardium. Intracellular application of cGMP has also been shown to induce similar effects on L-type Ca^{2+} current (I_{Ca}) in rat cardiomyocytes (Vandecasteele *et al*., 2001) as did the NO donor SIN-1 (Méry *et al*., 1993). DEA-NONOate (100 μM) has been reported to reduce contractility in rat cardiomyocytes which was, however, not inhibited by 25 μM ODQ (Sandirasegarane & Diamond, 1999). Since ODQ is able to interact with other heme containing proteins besides sGC (Feelisch *et al*., 1999; Zhao *et al*., 2000), e.g. with intracellular myoglobin (100–200 μM) as suggested (Wegener *et al*., 1999), the ODQ concentration used in the former study may have been too low to result in a sufficient inhibition of sGC. However, concentrations of SNAP and DEA-NONOate above 100 μM reduced F_c in preparations from myo$^{-/-}$ mice in the presence of 100 μM ODQ and even from WT mice. This finding indicates that, at these concentrations, effects besides activation of sGC are involved in the reduction of F_c, as for example inhibition of mitochondrial energy supply (Stumpe *et al*., 2001).

In summary, the present study has shown the absence of myocardial effects of exogenously applied NO, at least at concentrations below 10 μM. This has been explained by the presence of intracellular myoglobin acting as scavenger for NO. However, the *in vitro* findings do not unequivocally deny a physiological role for NO in the myocardium, if liberated from the cardiovascular endothelium or cardiomyocytes (Shah & Maccarthy, 2000). An intra-myocardial source for NO may be able to induce physiological functions in spite of the presence of myoglobin, if NO is generated in close vicinity to its target molecules. Indeed, an isoform of the NO synthase (endothelial NO synthase; eNOS) has been found closely associated to membrane caveolae in cardiomyocytes (Feron *et al*., 1999) and reported to mediate the stretch dependence of Ca^{2+} release (Petroff *et al*., 2001). In addition, it has been shown that eNOS co-purifies with ryanodine receptor channel-containing sarcoplasmatic reticulum fractions from the myocardium (Zahradnikova *et al*., 1997). In cerebellar neurones, an NO synthase is co-localized with the NMDA receptor *via* an anchoring protein (Christopherson *et al*., 1999). A compartimentalization would escape the scavenge of NO by myoglobin in cardiomyocytes and may lead to local NO concentrations sufficient to exert effects on β-adrenergic signalling, Ca^{2+} handling, or oxygen consumption.

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References

ABI-GERGES, N., FISCHMEISTER, R. & MERY, P.F. (2001). G protein-mediated inhibitory effect of a nitric oxide donor on the L- type Ca2+ current in rat ventricular myocytes. *J. Physiol.,* **531,** 117–130.

CHRISTOPHERSON, K.S., HILLIER, B.J., LIM, W.A. & BREDT, D.S. (1999). PSD-95 assembles a ternary complex with the N-methyl-D-aspartic acid receptor and a bivalent neuronal NO synthase PDZ domain. *J. Biol. Chem.,* **274,** 27467–27473.

DICKS, A.P. & WILLIAMS, D.L. (1996). Generation of nitric oxide from S-nitrosothiols using protein-bound Cu2+ sources. *Chem. Biol.,* **3,** 655–659.

FEELISCH, M., KOTSONIS, P., SIEBE, J., CLEMENT, B. & SCHMIDT, H.H. (1999). The soluble guanylyl cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3,-a] quinoxalin-1-one is a nonselective heme protein inhibitor of nitric oxide synthase and other cytochrome P-450 enzymes involved in nitric oxide donor bioactivation. *Mol. Pharmacol.,* **56,** 243–253.

FERON, O., ZHAO, Y.Y. & KELLY, R.A. (1999). The ins and outs of caveolar signaling. m2 muscarinic cholinergic receptors and eNOS activation versus neuregulin and ErbB4 signaling in cardiac myocytes. *Ann. NY Acad. Sci.,* **874,** 11–19.

FLESCH, M., KILTER, H., CREMERS, B., LENZ, O., SUDKAMP, M., KUHN REGNIER, F. & BOHM, M. (1997). Acute effects of nitric oxide and cyclic GMP on human myocardial contractility. *J. Pharmacol. Exp. Ther.,* **281,** 1340–1349.

FLÖGEL, U., MERX, M.W., GODECKE, A., DECKING, U.K. & SCHRADER, J. (2001). Myoglobin: A scavenger of bioactive NO. *Proc. Natl. Acad. Sci. USA,* **98,** 735–740.

GARTHWAITE, J., SOUTHAM, E., BOULTON, C.L., NIELSON, E.B., SCHMIDT, K. & MAYER, B. (1995). Potent and selective inhibition of nitric oxide-sensitive guanylyl cyclase by 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one. *Mol. Pharmacol.,* **48,** 184–188.

GÖDECKE, A., FLÖGEL, U., ZANGER, K., DING, Z., HIRCHENHAIN, J., DECKING, U.K. & SCHRADER, J. (1999). Disruption of myoglobin in mice induces multiple compensatory mechanisms. *Proc. Natl. Acad. Sci. USA,* **96,** 10495–10500.

HENRY, P.J., DRUMMER, O.H. & HOROWITZ, J.D. (1989). S-nitrosothiols as vasodilators: implications regarding tolerance to nitric oxide-containing vasodilators. *Br. J. Pharmacol.,* **98,** 757–766.

ISHIBASHI, T., HAMAGUCHI, M., KATO, K., KAWADA, T., OHTA, H., SASAGE, H. & IMAI, S. (1993). Relationship between myoglobin contents and increases in cyclic GMP produced by glyceryl trinitrate and nitric oxide in rabbit aorta, right atrium and papillary muscle. *Naunyn Schmiedeberg's Arch. Pharmacol.,* **347,** 553–561.

JI, G.J., FLEISCHMANN, B.K., BLOCH, W., FEELISCH, M., ANDRESSEN, C., ADDICKS, K. & HESCHELER, J. (1999). Regulation of the L-type Ca2+ channel during cardiomyogenesis: switch from NO to adenylyl cyclase-mediated inhibition. *FASEB J.,* **13,** 313–324.

KEEFER, L.K., NIMS, R.W., DAVIES, K.M. & WINK, D.A. (1996). ''NONOates'' (1-substituted diazen-1-ium-1,2-diolates) as nitric oxide donors: convenient nitric oxide dosage forms. *Methods Enzymol.,* **268,** 281–293.

KOJDA, G., KOTTENBERG, K., NIX, P., SCHLUTER, K.D., PIPER, H.M. & NOACK, E. (1996). Low increase in cGMP induced by organic nitrates and nitrovasodilators improves contractile response of rat ventricular myocytes. *Circ. Res.,* **78,** 91–101.

MARTIN, W., VILLANI, G.M., JOTHIANANDAN, D. & FURCHGOTT, R.F. (1985). Selective blockade of endothelium-dependent and glyceryl trinitrate- induced relaxation by hemoglobin and by methylene blue in the rabbit aorta. *J. Pharmacol. Exp. Ther.,* **232,** 708–716.

MÉRY, P.F., PAVOINE, C., BELHASSEN, L., PECKER, F. & FISCHMEISTER, R. (1993). Nitric oxide regulates cardiac Ca2+ current. Involvement of cGMP-inhibited and cGMP-stimulated phosphodiesterases through guanylyl cyclase activation. *J. Biol. Chem.,* **268,** 26286–26295.

MITTAL, C.K., ARNOLD, W.P. & MURAD, F. (1978). Characterization of protein inhibitors of guanylate cyclase activation from rat heart and bovine lung. *J. Biol. Chem.,* **253,** 1266–1271.

MONCADA, S., PALMER, R.M. & HIGGS, E.A. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.,* **43,** 109–142.

MORLEY, D., MARAGOS, C.M., ZHANG, X.Y., BOIGNON, M., WINK, D.A. & KEEFER, L.K. (1993). Mechanism of vascular relaxation induced by the nitric oxide (NO)/nucleophile complexes, a new class of NO-based vasodilators. *J. Cardiovasc. Pharmacol.,* **21,** 670–676.

NAWRATH, H., BÄUMNER, D., RUPP, J. & OELERT, H. (1995). The ineffectiveness of the NO-cyclic GMP signaling pathway in the atrial myocardium. *Br. J. Pharmacol.,* **116,** 3061–3067.

O'BRIEN, P.J., SHEN, H., MCCUTCHEON, L.J., O'GRADY, M., BYRNE, P.J., FERGUSON, H.W., MIRSALIMI, M.S., JULIAN, R.J., SARGEANT, J.M., TREMBLAY, R.R. & BLACKWELL, T.E. (1992). Rapid, simple and sensitive microassay for skeletal and cardiac muscle myoglobin and hemoglobin: use in various animals indicates functional role of myohemoproteins. *Mol. Cell. Biochem.,* **112,** 45–52.

PETROFF, M.G., KIM, S.H., PEPE, S., DESSY, C., MARBAN, E., BALLIGAND, J.L. & SOLLOTT, S.J. (2001). Endogenous nitric oxide mechanisms mediate the stretch dependence of Ca2+ release in cardiomyocytes. *Nat. Cell. Biol.,* **3,** 867–873.

SANDIRASEGARANE, L. & DIAMOND, J. (1999). The nitric oxide donors, SNAP and DEA/NO, exert a negative inotropic effect in rat cardiomyocytes which is independent of cyclic GMP elevation. *J. Mol. Cell. Cardiol.,* **31,** 799–808.

SCHMIDT, K., DESCH, W., KLATT, P., KUKOVETZ, W.R. & MAYER, B. (1997). Release of nitric oxide from donors with known half-life: a mathematical model for calculating nitric oxide concentrations in aerobic solutions. *Naunyn Schmiedeberg's Arch. Pharmacol.,* **355,** 457–462.

SHAH, A.M. & MACCARTHY, P.A. (2000). Paracrine and autocrine effects of nitric oxide on myocardial function. *Pharmacol. Ther.,* **86,** 49–86.

SMITH, J.A., SHAH, A.M. & LEWIS, M.J. (1991). Factors released from endocardium of the ferret and pig modulate myocardial contraction. *J. Physiol. Lond.,* **439,** 1–14.

STUMPE, T., DECKING, U.K. & SCHRADER, J. (2001). Nitric oxide reduces energy supply by direct action on the respiratory chain in isolated cardiomyocytes. *Am. J. Physiol. Heart Circ. Physiol.,* **280,** H2350–H2356.

VANDECASTEELE, G., VERDE, I., RUCKER-MARTIN, C., DONZEAU-GOUGE, P. & FISCHMEISTER, R. (2001). Cyclic GMP regulation of the L-type Ca(2+) channel current in human atrial myocytes. *J. Physiol.,* **533,** 329–340.

VULCU, S.D., WEGENER, J.W. & NAWRATH, H. (2000). Differences in the nitric oxide/soluble guanylyl cyclase signalling pathway in the myocardium of neonatal and adult rats. *Eur. J. Pharmacol.,* **406,** 247–255.

WEGENER, J.W., CLOSS, E.I., FORSTERMANN, U. & NAWRATH, H. (1999). Failure of 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) to inhibit soluble guanylyl cyclase in rat ventricular cardiomyocytes. *Br. J. Pharmacol.,* **127,** 693–700.

WEGENER, J.W. & NAWRATH, H. (1997). Cardiac effects of isoliquiritigenin. *Eur. J. Pharmacol.,* **326,** 37–44.

WEYRICH, A.S., MA, X.L., BUERKE, M., MUROHARA, T., ARMSTEAD, V.E., LEFER, A.M., NICOLAS, J.M., THOMAS, A.P., LEFER, D.J. & VINTEN JOHANSEN, J. (1994). Physiological concentrations of nitric oxide do not elicit an acute negative inotropic effect in unstimulated cardiac muscle. *Circ. Res.,* **75,** 692–700.

WILLIAMS, D.L. (1996). S-nitrosothiols and role of metal ions in decomposition to nitric oxide. *Methods Enzymol.,* **268,** 299–308.

WITTENBERG, J.B. (1970). Myoglobin-facilitated oxygen diffusion: role of myoglobin in oxygen entry into muscle. *Physiol. Rev.,* **50,** 559–636.

ZAHRADNIKOVA, A., MINAROVIC, I., VENEMA, R.C. & MESZAROS, L.G. (1997). Inactivation of the cardiac ryanodine receptor calcium release channel by nitric oxide. *Cell Calcium,* **22,** 447–454.

ZHAO, Y., BRANDISH, P.E., DIVALENTIN, M., SCHELVIS, J.P., BABCOCK, G.T. & MARLETTA, M.A. (2000). Inhibition of soluble guanylate cyclase by ODQ. *Biochemistry,* **39,** 10848–10854.

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