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SPECIAL REPORT

Nitroglycerin-induced direct protection of the ischaemic myocardium in isolated working hearts of rats with vascular tolerance to nitroglycerin

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We investigated whether nitroglycerin (NTG) was able to produce an anti-ischaemic effect in isolated working hearts of rats with vascular tolerance to NTG. Hearts isolated from tolerant and non-tolerant rats were subjected to 10 min coronary occlusion in the presence of 10^{-7} M NTG and/or its solvent. NTG alleviated ischaemia-induced deterioration of cardiac function and decreased lactate dehydrogenase release whilst having no effect on coronary flow nor the area of the ischaemic zone both in hearts isolated from NTG-tolerant and non-tolerant rats. The magnitude of the effect was similar in the two groups. These results suggest that the anti-ischaemic effect of NTG involves direct myocardial mechanisms independent of its vascular action and that vascular tolerance to NTG does not affect this direct protective action.

Keywords: Coronary occlusion; nitroglycerin; nitroglycerin tolerance; cardioprotection; rat heart

Introduction The anti-ischaemic effect of nitroglycerin (NTG) is believed to be due to its ability to decrease preload and afterload, improve coronary collateral flow, dilate stenotic coronary arteries and inhibit platelet aggregation (Harrison & Bates, 1993). To exert these effects, NTG is considered to act as a prodrug, that requires enzymatic bioconversion to a pharmacologically active principle, i.e. nitric oxide (NO). However, sodium nitroprusside, a non-enzymatic releaser of NO, has been shown to exert direct effects on myocardial function (Grocott-Mason *et al.*, 1994). Moreover, the protective effect of the NO donor, 3-morpholinosydnonimine-N-ethylcarbamide (SIN-1), against reoxygenation injury in isolated cardiomyocytes (Schlutter *et al.*, 1994), and the loss of ischaemic preconditioning in rabbits with vascular tolerance to NTG (Szilvassy *et al.*, 1994) suggests an unidentified direct cardioprotective effect of NO or NO donors. Therefore, the present study was performed to investigate whether the anti-ischaemic effect of NTG involves a direct myocardial action independent of any vascular action, and whether any direct anti-ischaemic effect of NTG exists in the presence of vascular tolerance to NTG.

Methods Male Wistar rats (300–360 g) were given subcutaneously 100 mg kg^{-1} nitroglycerin (EGIS, Budapest, Hungary) and/or its vehicle (lactose suspended in propyleneglycol) 3 times a day for 3 days to induce vascular tolerance to NTG (Silver *et al.*, 1991). On the fourth day, rats were anaesthetized with diethylether, and injected intravenously with 500 u kg^{-1} heparin. After 30 s, hearts were excised and cannulated through the aorta, and perfused by the Langendorff method at a constant perfusion pressure of 100 cmH_2O (9.8 kPa) for 10 min. During this period, the left atrium was cannulated, and a suture was placed around the left main coronary artery close to its origin, allowing regional ischaemia to be induced as described earlier (Ferdinandy *et al.*, 1995). The heart was then converted to a working preparation perfused at 37°C with oxygenated Krebs-Henseleit bicarbonate buffer for 10 min (Ferdinandy *et al.*, 1993). Preload (17 cmH_2O, 1.7 kPa), and afterload (100 cmH_2O, 9.8 kPa) were kept constant throughout the experiments. Heart rate (HR), coronary flow (CF), aortic flow (AF), and left ventricular end-diastolic pressure (LVEDP) were monitored as described by Ferdinandy *et al.* (1995). Coronary effluents were assayed for lactate dehydrogenase (LDH) activity by means of an automatic analyzer (Hitachi-911) by using Boehringer-Mannheim kits. Relative LDH release was calculated as $(LDH_{ischaemic}-LDH_{preischaemic})xCF/heart$ wet weight and expressed as mu min^{-1} g^{-1}. The ischaemic area of the left ventricle was determined by a dye exclusion method (Curtis & Hearse, 1989). Development of vascular tolerance to NTG was tested in endothelium-free, thoracic aortic rings arranged for isometric tension. Rings of 4 mm length set at a resting tension of 20 mN were precontracted with an EC_{50} concentration of noradrenaline. The rings were then exposed to cumulative NTG concentrations in half-log increments. NTG concentration to produce half-maximum relaxation was 0.075 ± 0.010 μM in rings taken from rats that had been given vehicle (non-tolerant) vs. 1.5 ± 0.2 μM ($P < 0.001$) in those given NTG (tolerant rings).

Hearts isolated from NTG-tolerant and non-tolerant rats were each divided into 2 groups and perfused with buffer solution containing the solvent for NTG (ethyl alcohol, 2.2×10^{-3}% v/v), or 10^{-7} M NTG in solvent (Pohl-Boskamp, Hochenlockstedt, Germany), ($n = 7$ in each group) throughout the experiment. After 10 min of aerobic working the hearts were subjected to 10 min coronary occlusion. Measurements were made before ischaemia and at the 10th min of coronary occlusion.

Data are expressed as means ± s.e. They were statistically analyzed by ANOVA. All groups were then compared to the non-tolerant, solvent-perfused group by a *t* test with a Bonferroni correction for multiple comparisons.

Results In the non-tolerant, solvent-treated group, coronary occlusion markedly decreased AF, increased LVEDP, and caused a high LDH release (Figure 1). NTG at 10^{-7} M did not affect preischaemic myocardial function, preischaemic or ischaemic CF, nor the area of the ischaemic zone. However, during ischaemia it improved AF and decreased LVEDP and LDH release. Similar anti-ischaemic effects were produced by 10^{-7} M NTG in hearts isolated from NTG-tolerant rats; the solvent for NTG was without effect (Figure 1 a–c). None of the treatments changed HR or CF.

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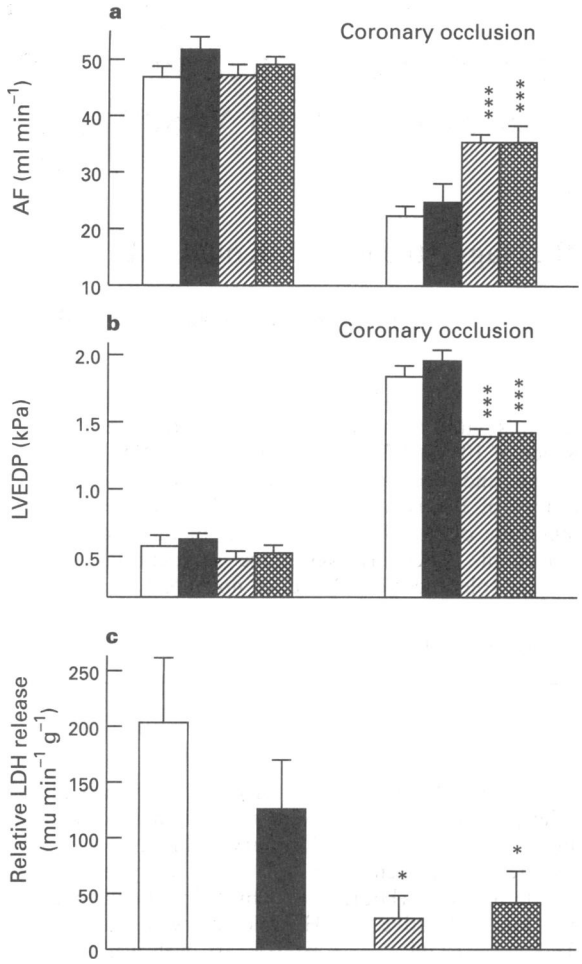

Figure 1 (a) Aortic flow (AF) and (b) left ventricular end-diastolic pressure (LVEDP) before ischaemia and at the 10th min of coronary occlusion and (c) relative lactate dehydrogenase (LDH) release at the 10th min of coronary occlusion in hearts perfused with 10^{-7} M nitroglycerin (NTG) (hatched columns) or solvent (open columns) from rats non-tolerant to NTG and 10^{-7} M NTG (cross hatched columns) or solvent (solid columns) from rats tolerant to NTG. $^{*}P<0.05$, $^{***}P<0.001$ shows significant difference, relative to the non-tolerant, solvent-treated group.

Discussion These results suggest that the anti-ischaemic action of NTG involves a direct protective effect on the ischaemic myocardium, independent of any vascular effects. The results also show, that the anti-ischaemic effect of NTG is preserved when a marked tolerance to the vasodilator effect of NTG has developed. The fact that the present experiments were performed on crystalloid-perfused hearts of the rat, a species deficient in coronary collaterals, excludes effects of NTG on the peripheral circulation, platelets, and on coronary collaterals. NTG selectively dilates coronary vessels of >100 μm, and therefore has minimal effect on coronary resistance (Harrison & Bates, 1993). Accordingly, NTG did not change the area of the ischaemic zone and failed to affect CF. Thus, in the rat isolated, working heart, the anti-ischaemic effect of NTG seems to involve a direct action on the myocardium. Recently, sodium nitroprusside has been shown to exert a direct, myocardial relaxant effect in rat isolated hearts (Grocott-Mason *et al.*, 1994). In our study, NTG significantly attenuated the ischaemia-induced increase in LVEDP even in the presence of profound impairment of NTG-induced vascular relaxation due to tolerance development. This suggests either that the NTG-NO-cyclic GMP-relaxation pathway is different in vascular and cardiac muscle, or that NTG exerts an NO-independent protective effect on the ischaemic heart. The latter is supported by the results of Laustiola *et al.* (1983) who showed that NTG directly alters anaerobic metabolism in the ischaemic myocardium, and by those of Schlutter *et al.* (1994) who showed that the NO donor, SIN-1, protects myocardial cells from reoxygenation injury by an NO-independent mechanism. The fact that the anti-ischaemic effect of NTG was similar in isolated hearts taken from NTG-tolerant and non-tolerant animals not only confirms that NTG exerts a direct myocardial action independent of its vascular effects, but also suggests that the myocardial effect of NTG does not depend on the vascular metabolism of NTG, or vascular cyclic GMP signalling, since the mechanism of NTG tolerance comprises diminished conversion of NTG to NO and/or alteration of cyclic GMP metabolism (Bassenge & Zanzinger, 1992). The myocardial effect of NTG may however, be underlain by myocardial metabolism of NTG to NO.

Development of vascular tolerance to NTG results in an impairment of the drug induced preload/afterload reduction and coronary dilatation which is manifest as a diminished antianginal effect at therapeutic doses of NTG (Harrison & Bates, 1993). Nevertheless, the present observation that NTG exerts a direct, cardioprotective effect even in the presence of vascular tolerance to NTG suggests that the overall protective effect of organic nitrates on the ischaemic heart may still be substantial even in the presence of vascular tolerance to NTG.

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References

BASSENGE, E. & ZANZINGER, J. (1992). Nitrates in different vascular beds, nitrate tolerance, and interactions with endothelial function. *Am. J. Cardiol.*, **70**, 23B–29B.

CURTIS, M.J. & HEARSE, D.J. (1989). Ischaemia-induced and reperfusion-induced arrhythmias differ in their sensitivity to potassium: implications for mechanisms of initiation and maintenance of ventricular fibrillation. *J. Mol. Cell. Cardiol.*, **21**, 21–40.

FERDINANDY, P., DAS, D.K. & TOSAKI, A. (1993). Pacing-induced ventricular fibrillation leading to oxygen free radical formation in aerobically perfused rat heart. *J. Mol. Cell. Cardiol.*, **25**, 683–692.

FERDINANDY, P., SZILVASSY, Z., KOLTAI, M. & DUX, L. (1995). Ventricular overdrive pacing-induced preconditioning and no-flow ischemia-induced preconditioning in isolated working rat hearts. *J. Cardiovasc. Pharmacol.*, **25**, 97–104.

GROCOTT-MASON, R., FORT, S., LEWIS, M.J. & SHAH, A.M. (1994). Myocardial relaxant effect of exogenous nitric oxide in isolated ejecting hearts. *Am. J. Physiol. Heart Circ. Physiol.*, **266**, H1699–H1705.

HARRISON, D.G. & BATES, J.N. (1993). The nitrovasodilators. New ideas about old drugs. *Circulation*, **87**, 1461–1467.

LAUSTIOLA, K., VOURINEN, P., VAPAATALO, H. & METSA-KETELA, T. (1983). Effects of cysteine and nitroglycerin on bovine heart guanylate cyclase and on tissue cyclic GMP and lactate of rat atria. *Eur. J. Pharmacol.*, **91**, 301–304.

SCHLUTTER, K.D., WEBER, M., SCHRAVEN, E. & PIPER, H.M. (1994). NO donor SIN-1 protects against reoxygenation-induced cardiomyocyte injury by a dual action. *Am. J. Physiol.*, **267**, H1461–H1466.

SILVER, P.J., PAGANI, E.D., DE GARAVILLA, L., VAN ALLER, G.S., VOLBERG, M.L., PRATT, P.F. & BUCHHOLZ, R.A. (1991). Reversal of nitroglycerin tolerance by the cGMP phosphodiesterase inhibitor zaprinast. *Eur. J. Pharmacol.*, **199,** 141–142.

SZILVASSY, Z., FERDINANDY, P., BOR, P., JAKAB, I., SZILVASSY, J., NAGY, I., LONOVICS, J. & KOLTAI, M. (1994). Loss of preconditioning in rabbits with vascular tolerance to nitroglycerin. *Br. J. Pharmacol.*, **112,** 999–1001.

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