Parallel tolerance between platelet cyclic GMP and preload effects of nitroglycerin in anaesthetized mini-pigs

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The effects of acute intravenous nitroglycerin (NTG) administration on platelet cyclic GMP in relation to changes in indices of preload (end-diastolic volume) and afterload (effective arterial elastance) were evaluated in the anaesthetized mini-pig, using pressure-volume analysis. NTG ($1\text{--}30\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$, i.v.) elicited a dose-dependent fall in preload and afterload, and an increase in arterial blood platelet cyclic GMP. Repeated doses of NTG ($30\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$) resulted in tolerance to the preload but not afterload effects. The increases in platelet cyclic GMP were also attenuated, being highly correlated with the preload changes. Therefore, platelet cyclic GMP appears to reflect NTG-induced venous tolerance, rather than arterial responsiveness. The measurement of platelet cyclic GMP may represent a simple approach for monitoring the degree of venous tolerance to NTG in animals or patients, facilitating further mechanistic investigations.

Keywords: Nitroglycerin; platelet cyclic GMP; tolerance; preload; afterload; nitric oxide

Introduction Nitroglycerin (NTG) causes both venous and arterial peripheral vasodilatation resulting in reductions in preload and afterload (Imhof *et al.*, 1980). Continuous exposure to NTG leads to an attenuation of its haemodynamic effects, i.e. tolerance, which is primarily exhibited by the venous circulation (Ghio *et al.*, 1992). The vasodilator actions of NTG are due to its metabolic conversion, by vascular smooth muscle cells, to nitric oxide (NO) which activates vascular soluble guanylate cyclase leading to an accumulation of intracellular guanosine 3':5'-cyclic monophosphate (cyclic GMP) (Ignarro *et al.*, 1981). Platelets also contain soluble guanylate cyclase, and although they do not appear to possess the enzyme required for the direct conversion of NTG, it is believed that sufficient NO can be released from NTG by the vascular tissue enzyme to enable activation of the platelet guanylate cyclase (Gerzer *et al.*, 1989). Thus platelet cyclic GMP might be an index of the tone, or state of relaxation of the vessels (Watanabe *et al.*, 1993; Fung *et al.*, 1993) following intravenous NTG.

The aim of the present study, in the anaesthetized mini-pig, was to quantify the effects of acute intravenous NTG administration on platelet cyclic GMP in relation to changes in indices of preload (end-diastolic volume) and afterload (effective arterial elastance). Pressure-volume analysis (Kass *et al.*, 1988) was chosen as a sensitive approach to quantify changes in left-ventricular preload, afterload and contractility.

Methods Male Göttinger mini-pigs (10.5–15.5 kg) were anaesthetized with sodium pentobarbitone (75 mg kg^{-1} , i.p.; followed by $0.2\text{--}0.25\text{ mg kg}^{-1}\text{ min}^{-1}$, i.v.) and ventilated with oxygen-enriched air. Left ventricular pressure was measured with a 5F micromanometer-tipped pressure catheter (Millar), and ventricular volume with an 8-electrode 6F conductance catheter, calibrated as previously described (Kass *et al.*, 1988). A 5F Swan-Ganz thermodilution catheter was used to measure cardiac output for calibration purposes. The left femoral artery was catheterized with a fluid-filled catheter (tip in thoracic aorta) for measurement of arterial blood pressure and sampling of blood.

Protocol Intravenous doses of $1, 3, 10$ and $30\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$ NTG ($n=10$), or an equivalent volume of saline ($n=6$), were each infused for 3 min; 15 min separated each infusion. Pilot studies had shown $30\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$ NTG to

Figure 1 Effects of intravenous infusion of nitroglycerin (hatched columns) or saline vehicle (open columns) on arterial elastance (afterload index), left ventricular end-diastolic volume (preload index), and arterial blood platelet cyclic GMP. There were dose-related changes in all parameters between 1 and $30\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$. Repeated doses caused a reproducible fall in arterial elastance. However, there was a parallel tolerance to the effects of NTG on end-diastolic volume and platelet cyclic GMP.

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be equivalent to an ED_{50} for lowering of arterial blood pressure. Fifteen min following completion of the dose-response curve, $3 \times 30 \mu\text{g kg}^{-1} \text{min}^{-1}$, i.v. NTG or saline were each infused for 5 min, allowing 15 min between infusions for parameters to return to baseline.

Arterial blood samples were withdrawn prior to and 1 min following each infusion. Cyclic GMP was extracted according to Watanabe *et al.* (1993) and the aqueous phase analysed using a [^3H]-cyclic GMP radioimmunoassay kit (Amersham). All data were calculated as the mean $\pm$ s.e.mean.

Materials Sodium pentobarbitone was purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.). NTG (in 96% ethanol) was diluted 1 in 100 with saline immediately prior to use. All solvents and buffer constituents for extraction were obtained from Merck (Darmstadt, Germany).

Results NTG elicited dose-related decreases in arterial elastance and end-diastolic volume (max. fall $38.4 \pm 4.2\%$; $P < 0.001$, and $29.6 \pm 5.1\%$; $P < 0.01$, respectively) and increases in arterial platelet cyclic GMP ($112.8 \pm 23.1\%$; $P < 0.01$) (Figure 1). However, whereas the maximum fall in arterial elastance was reproducible with repeated $30 \mu\text{g kg}^{-1} \text{min}^{-1}$ doses, a significant attenuation of the maximum effect of NTG on EDV was observed. This was mirrored by a similar attenuation of the increase in platelet cyclic GMP. In fact, the changes in cyclic GMP were highly correlated with end-diastolic volume ($r = 0.743$, $P < 0.05$) but not arterial elastance ($r = 0.597$, $P > 0.1$). Contractility, as measured by end-systolic elastance was not affected by NTG.

The intravenous infusion of NTG caused a dose-related reduction in mean arterial blood pressure. The maximum fall (from 106 ± 3 to 64 ± 4 mmHg; $P < 0.001$), like arterial elastance, was found to be reproducible with repeated $30 \mu\text{g kg}^{-1} \text{min}^{-1}$ doses. The decrease in pressure was accompanied by a transient rise in heart rate (HR) with a peak increase from 88 ± 5 to 96 ± 3 beats min^{-1} ($P < 0.05$), fol-

lowing the $10 \mu\text{g kg}^{-1} \text{min}^{-1}$ dose. Saline had no effect on any measured parameter.

Discussion NTG dose-dependently reduced both preload and afterload: the peak effect of NTG on preload was significantly attenuated following repeated dosing whilst afterload was still reduced to the same extent. This suggests that tolerance develops to NTG in the venous circulation in agreement with similar findings (Ghio *et al.*, 1992) in patients.

Kukovetz & Holzmann (1986) showed that the *in vitro* exposure of vascular tissue to NTG caused a dose-related increase in intracellular cyclic GMP which correlated with the simultaneous relaxation of the preparation, with both effects being impaired after prolonged incubation with NTG. Similar findings were reported *in vivo* (Watanabe *et al.*, 1993) where the increase in platelet cyclic GMP correlated with epicardial coronary artery diameter. Both platelet cyclic GMP and the coronary artery changes were less pronounced in those patients receiving nitrate therapy. The present study further extends these findings by showing that repeated doses of NTG resulted in tolerance to the preload but not afterload effects. Additionally, the increases in platelet cyclic GMP were also attenuated, being highly correlated with the preload changes. This would also suggest that clinical tolerance to NTG, i.e. reduced venodilatation is, at least partly, due to biochemical tolerance at the cellular level.

In conclusion, platelet cyclic GMP appears to reflect NTG-induced venous tolerance, rather than arterial responsiveness. The measurement of platelet cyclic GMP may represent a simple approach for monitoring the degree of venous tolerance to NTG in animals or patients, facilitating further mechanistic investigations.

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