Loss of preconditioning in rabbits with vascular tolerance to nitroglycerin

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A preceding right ventricular overdrive pacing (VOP) of 500 b.p.m. for 5 min, markedly reduced the severity of global myocardial ischaemia produced by a subsequent 5-min VOP in conscious rabbits. This VOP-induced preconditioning developed in parallel with an increase in cardiac cyclic guanosine 3':5'-monophosphate (cyclic GMP) content. VOP-induced preconditioning was abolished when the animals had been made tolerant to the vasodilator effect of nitroglycerin (NG). In the heart of the NG-tolerant rabbits, neither VOP nor preconditioning increased cyclic GMP content. This suggests that changes by NG tolerance of cyclic GMP metabolism may account for the loss of VOP-induced preconditioning.

Keywords: Ischaemic preconditioning; nitroglycerin tolerance; cyclic GMP; ST-segment elevation; LVEDP; rabbit heart

Introduction The intracellular messenger, cyclic guanosine 3':5'-monophosphate (cyclic GMP) has been suggested to play a crucial role in the mechanism of the anti-ischaemic effect of preconditioning induced by ventricular overdrive pacing (VOP) in conscious rabbits (Szilvassy *et al.*, 1994). Results obtained with anaesthetized dogs (Vegh *et al.*, 1992) also suggest that nitric oxide (NO), a stimulator of soluble guanylate cyclase, contributes to the antiarrhythmic effect of preconditioning produced by brief coronary artery occlusions. Moreover, inhibition of the L-arginine-NO pathway has been found to abolish the antiarrhythmic effect of bradykinin, a putative mediator of preconditioning (Vegh *et al.*, 1993). Several studies have shown that the tolerance to organic nitroesters is accompanied by reduced production and increased breakdown of cyclic GMP in vascular and non-vascular tissues (Axelsson & Ahlner, 1987). Therefore, we examined whether nitroglycerin (NG) tolerance influenced VOP-induced preconditioning in conscious rabbits.

Methods Male New Zealand white rabbits (2500–3000 g) were anaesthetized with 15 mg kg⁻¹ diazepam (Sigma, St Louis, MO, U.S.A.) and 10 mg kg⁻¹ ketamine (EGIS, Budapest, Hungary) given intravenously. A bipolar 'French 4' electrode catheter was introduced into the apex of the right ventricle for pacing and recording the intracavitary electrogram (Szilvassy *et al.*, 1993a,b). Polyethylene catheters were inserted into the left ventricular cavity and the central ear artery to measure intraventricular pressure and mean arterial blood pressure (MABP), respectively. One week after surgery, the rabbits were given 150 µl kg⁻¹ ethanol, the solvent for NG, subcutaneously four times a day for three days. Next morning global myocardial ischaemia was provoked by VOP of 500 b.p.m. for 5 min. Average intracavitary ST-segment elevation during the first five cardiac cycles at sinus rhythm after VOP produced the data for evaluation. Left ventricular end-diastolic pressure (LVEDP) was measured before and immediately after VOP. This VOP was then given for 5 min followed by a second 5-min VOP, and the parameters were determined again. The first VOP served to estimate electrophysiological and haemodynamic changes due to global myocardial ischaemia and to induce preconditioning. The second VOP was to test the preconditioning effect on the parameters measured. The rabbits were then given 50 mg kg⁻¹ NG (EGIS, Budapest, Hungary) subcutaneously four times a day for three days to induce NG tolerance and

the pacing protocol was repeated. The effect of an intravenous bolus of 30 µg kg⁻¹ NG on MABP was tested before and after the three-day NG administration. In the second set of experiments, two groups of electrode catheter-instrumented rabbits (15 of each) underwent the three-day treatment with NG or solvent, respectively. Anaesthetized, open-chest, artificially ventilated rabbits were subjected to VOP of 500 b.p.m. for 5 min as described (Szilvassy *et al.*, 1993a). Normal and NG-tolerant rabbits were divided into three subgroups. One was subjected to a single VOP, then immediately after VOP, samples were taken from the apex of the left ventricle for cyclic GMP determination. Another five rabbits underwent two periods of VOP with an interpacing period of 5 min, and samples were taken immediately after the second VOP. A group was killed for measuring baseline cyclic GMP values. Cardiac cyclic GMP content was measured by means of radioimmunoassay using Amersham kits as described (Szilvassy *et al.*, 1993a). Data expressed as means ± s.d. were statistically analyzed by ANOVA followed by a modified *t* test for multiple comparisons according to Bonferroni.

Results In non tolerant rabbits, 30 µg kg⁻¹ intravenous NG decreased MABP from 88 ± 5.8 mmHg to 72 ± 5.1 mmHg ($P < 0.01$), whereas it did not change MABP in tolerant animals (91 ± 4.4 mmHg vs 93 ± 5.2 mmHg). A single VOP induced an intracavitary ST-segment elevation of 1.1 ± 0.16 mV in non tolerant rabbits (Figure 1a); nevertheless, when this was preceded by a preconditioning VOP, the ST-segment elevation decreased to 0.6 ± 0.07 mV ($P < 0.001$). In tolerant rabbits, ST-segment elevation induced by a single VOP was more marked than in controls (1.5 ± 0.22 mV vs 1.1 ± 0.16 mV, $P < 0.05$), and it remained unchanged after a preconditioning VOP. The increase in LVEDP produced by a single VOP was markedly decreased by a preconditioning VOP in non tolerant but not in NG tolerant rabbits (Figure 1b). In non tolerant animals, a single VOP significantly increased cardiac cyclic GMP content as compared to resting values (Figure 1c) and preconditioning slightly amplified the VOP-induced cyclic GMP increase. In tolerant animals, however, neither a single VOP nor consecutive periods of VOP changed cardiac cyclic GMP content. Resting LVEDP and cardiac cyclic GMP of NG tolerant rabbits did not significantly differ from that of non tolerant ones (data not shown).

Discussion These results confirm previous findings that the global myocardial ischaemia induced by VOP is markedly

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Figure 1 Effect of ventricular overdrive pacing (VOP) on (a) intracavitary ST-segment elevation and (b) left ventricular end-diastolic pressure (LVEDP) in conscious rabbits ($n = 6$) and (c) on cardiac cyclic GMP content in anaesthetized rabbits ($n = 5$). Open columns show resting, hatched columns non preconditioned values measured after a single VOP (500 b.p.m. for 5 min), solid columns designate values measured after a 5 min VOP preceded by a preconditioning VOP of the same rate and duration with an interpacing period of 5 min. 'Normal' values are obtained from solvent-treated animals. 'Nitroglycerin tolerant' values derived from rabbits treated with 50 mg kg⁻¹ nitroglycerin (NG) four times a day for three days. Data are means $\pm$ s.d.; #significant difference between resting and postpacing; *between non preconditioned and preconditioned values, $P < 0.05$.

attenuated by a preceding ischaemic challenge provoked by VOP (Szilvassy *et al.*, 1994). The results also indicate that this protection is lost when tolerance to the vasodilator effect of NG develops. Ischaemic preconditioning is believed to be due to the increased formation and release of endogenous cardioprotective substances. These substances may derive from the vascular endothelium or cardiac myocytes and stimulate soluble guanylate cyclase either directly like NO (Moncada *et al.*, 1991), or indirectly like bradykinin via releasing NO (Vegh *et al.*, 1992; 1993). An increased formation of cyclic GMP may therefore be considered an important step in the signal transduction pathways involved in preconditioning. This concept is in accord with our previous finding that an increase in cardiac cyclic GMP content correlates with the time course of the anti-ischaemic effect of VOP-induced preconditioning (Szilvassy *et al.*, 1994). Pharmacological inhibition of cyclic GMP breakdown has also been found to have an anti-ischaemic effect in conscious rabbits (Szilvassy *et al.*, 1993b). The present data, showing that VOP-induced preconditioning is lost in NG tolerance in association with the inability of VOP to increase myocardial cyclic GMP content, suggest that cyclic GMP signalling contributes to the protection of the ischaemic myocardium.

The mechanism of NG tolerance is unclear, although different hypotheses have been advanced, suggesting (i) the

importance of pharmacokinetic factors, (ii) the oxidation of critical sulphhydryl groups in the receptor inducing a low affinity state (Axelsson & Ahlner, 1987), and (iii) alterations in the formation and breakdown of cyclic GMP. Cyclic GMP is an important mediator of vasorelaxation induced by NG. Keith *et al.* (1982) found an inhibition of NG-induced cyclic GMP generation in aortic strips of NG-tolerant rats; however, the relaxation induced by 8-bromo-cyclic GMP, a stable, membrane-penetrating cyclic GMP analogue, was unaltered, indicating that, once it is formed, the intracellular action of cyclic GMP is unimpaired. In NG tolerance, Axelsson & Andersson (1983) have shown that guanylate cyclase activity is decreased, while cyclic GMP hydrolysis is enhanced. These findings might explain the impairment of preconditioning in NG-tolerant rabbits. Besides theoretical considerations, our results emphasize the potential risk of nitrate tolerance in clinical patients maintained on prolonged nitrate therapy.

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References

- AXELSSON, K.L. & AHLNER, J. (1987). Nitrate tolerance from a biochemical point of view. *Drugs*, **33** (Suppl. 4), 63–68.
- AXELSSON, K.L. & ANDERSSON, R.G.G. (1982). Tolerance towards nitroglycerin, induced in vivo, is correlated to reduced cGMP response and an alteration in cGMP turnover. *Eur. J. Pharmacol.*, **88**, 71–79.
- KEITH, R.A., BURKMAN, A.M., SOKOLOSKI, T.D. & FERTEL, R.H. (1982). Vascular tolerance to nitroglycerin and cyclic GMP generation in rat aortic smooth muscle. *J. Pharmacol. Exp. Ther.*, **221**, 525–531.
- MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.*, **43**, 109–142.
- SZILVASSY, Z., FERDINANDY, P., BOR, P., JAKAB, I., LONOVICS, J. & KOLTAI, M. (1994). Ventricular overdrive pacing-induced myocardial anti-ischemic effect: a conscious rabbit model of preconditioning. *Am. J. Physiol.* (in press).
- SZILVASSY, Z., JAKAB, I., BOR, P., KOLTAI, M., TARRADE, T., ESANU, A., BRAQUET, P.G. & LONOVICS, J. (1993a). Cicletanine attenuates overdrive pacing-induced global myocardial ischemia in rabbits: possible role of cyclic nucleotides. *Coron. Artery Dis.*, **4**, 443–452.
- SZILVASSY, Z., JAKAB, I., FERDINANDY, P., KOLTAI, M., LONOVICS, J., TARRADE, T. & BRAQUET, P.G. (1993b). Zaprinas, cicletanine, and verapamil attenuate overdrive pacing-induced myocardial ischemia in conscious rabbits. *Life Sci.*, **53**, PL13–PL18.
- VEGH, A., PAPP, J.Gy., SZEKERES, L. & PARRATT, J.R. (1993). Prevention by an inhibitor of the L-arginine-nitric oxide pathway of the antiarrhythmic effect of bradykinin in anesthetized dogs. *Br. J. Pharmacol.*, **110**, 18–19.
- VEGH, A., SZEKERES, L. & PARRATT, J.R. (1992). Preconditioning of the ischaemic myocardium: involvement of the L-arginine nitric oxide pathway. *Br. J. Pharmacol.*, **107**, 648–652.

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