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Interaction between capsaicin and nitrate tolerance in isolated guinea-pig heart

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Abstract

Capsaicin-induced increases in heart rate and coronary flow were blocked by N^G-nitro-L-Arg-methyl ester (30 mM) in Langendorff-perfused guinea-pig hearts. Neither heart rate nor coronary flow changed by capsaicin in hearts from animals made tolerant to the hypotensive effect of 30 μg/kg nitroglycerin by the administration of 50 mg/kg nitroglycerin subcutaneously 4 times a day over 3 days. We conclude that the effector function of sensory nerves may deteriorate in nitrate tolerance.

Keywords: Capsaicin; Coronary flow; Nitrate tolerance

Nitric oxide (NO) in concert with calcitonin gene-related peptide (CGRP) have been shown to be involved in capsaicin-induced coronary vasodilation and reduction in coronary perfusion pressure in rabbits and pigs (Mitchell et al., 1997; see for review Franco-Cereceda, 1988). If NO was of dominant influence in neurogenic coronary artery dilation, we postulated that either pharmacologic blockade of NO synthesis or desensitization of the effects of endogenous NO by nitrate tolerance would influence capsaicin-induced coronary dilation. We therefore sought whether development of haemodynamic tolerance to nitroglycerin influenced the decrease in coronary resistance and positive chronotropy evoked by capsaicin (see for review Franco-Cereceda, 1988) in Langendorff-perfused guinea-pig hearts.

These experiments conform with the European Community guiding principles for the care and use of laboratory animals and the experimental protocol applied has been approved by the ethical committee of our university.

Adult male guinea-pigs weighing 350–450 g were randomized into two groups. Six animals were given 50 mg/kg nitroglycerin s.c. (EGIS, Budapest, Hungary) 4 times a day over 3 days to induce haemodynamic nitrate tolerance (Szilvássy et al., 1994). The development of tolerance was confirmed by the lack of decrease in mean arterial blood pressure in response to an intravenous bolus of 30 μg/kg nitroglycerin (Szilvássy et al., 1994) in pentobarbitone (30 mg/kg i.p.)-anaesthetized animals. In the control group, (12 guinea-pigs) 150 ml/kg ethanol was diluted with distilled water (2 ml); the solvent for nitroglycerin was administered in the same way.

Six hearts from normal animals were then excised and mounted on a Langendorff apparatus as described (Tosaki et al., 1993). Following a 20 min period of aerobic perfusion with Krebs solution, the hearts were exposed to 0.1 mM capsaicin for 5 min. Separate hearts (*n*:6) were perfused with 30 mM N^G-nitro-L-Arg-methyl ester, a NO synthase inhibitor over 30 min preceding the exposure to capsaicin (0.1 μM). This was followed by perfusion with L-Arg (3 mM). The hearts from 'tolerant' animals were exposed to capsaicin after the 20 min equilibration period. Changes in heart rate and coronary flow were measured as described (Tosaki et al., 1993). All drugs and chemicals

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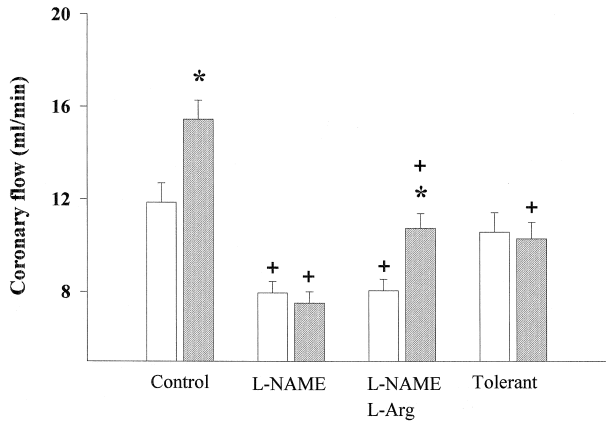

Fig. 1. Effect of capsaicin (0.1 μM) on coronary flow in isolated Langendorff-perfused guinea-pig hearts. Open columns represent values 'without capsaicin', hatched columns indicate values 'with capsaicin'. Control refers to as perfusion with Krebs solution; L-NAME: after perfusion with 30 mM N^G-nitro-L-Arg-methyl ester; L-NAME, L-Arg: additional perfusion with 3 mM L-Arg; Tolerant: the hearts were excised from animals made tolerant to the hypotensive effect of 30 mg/kg nitroglycerin i.v. The data are means ± S.E.M. obtained with 6 preparations. * designates a significant difference between values with vs. without capsaicin at $P < 0.05$, +, significantly different from corresponding control at $P < 0.05$.

were purchased from Sigma (St. Louis, MO) except nitroglycerin which was a gift from EGIS (Budapest, Hungary). The data expressed as means ± S.E.M. were analyzed with one-way analysis of variance (ANOVA) followed by Bonferroni's *t*-test. Level of significance was $P < 0.05$.

The capsaicin-induced increase in heart rate (25 ± 3.9%) and coronary flow were blocked by N^G-nitro-L-Arg-methyl ester in hearts from the normal animals (Fig. 1). These inhibitory effects of were reversed by L-Arg. Interestingly, L-Arg did not restore baseline coronary flow decreased by N^G-nitro-L-Arg-methyl ester. Neither heart rate nor coronary flow changed by capsaicin in hearts from the 'tolerant' animals (Fig. 1).

The results confirm previous findings in that capsaicin increases heart rate and coronary flow in Langendorff-perfused guinea-pig heart (see for review Franco-Cereceda, 1988). These effects are NO-mediated as indicated by the effect of N^G-nitro-L-Arg-methyl ester reversible by L-Arg excess. The major original finding of this work, however, is that the capsaicin effects are lost in haemodynamic nitrate tolerance.

Capsaicin-sensitive sensory nerves influence cardiovascular function due to their neurotransmitters such as CGRP and NO underlying their local effector function (see for review Franco-Cereceda, 1988). These nerve endings act as sensors for ischaemia, hypoxia, lactate, extracellular K^+, responding to signals with neurotransmitter release (see for review Franco-Cereceda, 1988). We have shown that depletion of the CGRP and NO contents of these nerves blocks preconditioning, the most effective cardioprotective mechanism described to date (see for review Ferdinandy et al., 1998). Nitric oxide seems to be the dominant mediator in this respect, since either blockade of NO synthesis or desensitization of the effects of endogenous NO by development of nitrate tolerance also blocked preconditioning (Szilvássy et al., 1994; see for review Ferdinandy et al., 1998). Here we show that the effect of pharmacological activation of capsaicin-sensitive nerves is also vulnerable to both blockade of NO synthesis and nitrate tolerance. Tolerance to nitroglycerin in vivo may modulate vascular effects of endogenous NO due to endothelial superoxide production that scavenges NO yielding peroxynitrate. However, no deficiency in NO production from nitroglycerin has been found in rats with tolerance to nitroglycrin in vivo (Laursen et al., 1996), suggesting the possible implication of mechanisms distal to NO metabolism. Moreover, baseline coronary flow was not altered by nitrate tolerance in our study suggesting effective compensatory mechanisms by endothelium-dependent vasodilators other than NO such as endothelium derived hyperpolarizing factor or prostacyclin. This might explain the virtual contradiction to results by Yaoita et al. (1994) who found that capsaicin-sensitive neuropeptides substantially contributed to baseline coronary flow in rats. Since the effect of capsaicin is selective for sensory neurons (Caterina et al., 1997), it is likely that beyond direct vascular effects, nitrate tolerance may cause profound cardiovascular regulatory disorders implying sensory nerves.

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