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Changes in the Coronary Vessel Tone Resulting from Immobilization StressA

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Coronary perfusion pressure at increasing flow of Krebs-Henseleit buffer in the hearts of animals exposed to stress is decreased 23%, a decrease which is eliminated by administering the NO-synthase blocker N^G-monomethyl-L-arginine. Stimulated vasodilation of coronary vessels (administration of sodium nitroprusside and acetylcholine) decreases markedly in stressed animals; the concentrations of sodium nitroprusside and acetylcholine inducing a half-maximum coronary response increase.

Key Words: *coronary blood flow; endothelium; nitrogen oxide; stress*

Immobilization stress causes pronounced alterations in the coronary circulation [1]: the volume of coronary blood flow increases, whereas intraventricular pressure decreases. A similar disparity between the demand for and the supply of the myocardium with blood is largely associated with a reduced tone of coronary vessels, which may ensue from alterations both of the contractile function of coronary vessels and of the functional activity of erythrocytes. The baseline activity of the nitrogen oxide system in coronary endotheliocytes has an important role in the regulation of coronary tone [7]. The amount of constantly released NO, which reduces coronary tone, is determined by the activity of the constitutional NO-synthase [5]. The role of this system in stress-induced changes in coronary blood flow is unknown. In this study we investigated the role of the NO-synthase system of rat coronary vessels in the post-stress alterations in coronary tone. For this purpose we studied: first, the effect of the specific competitive NO-synthase blocker N^G-monomethyl-L-arginine (N^G-MMLA) on stress-induced alterations in the tone of coronary vessels; second, the response of coronary vessels to administration of the exogenous NO source sodium nitroprusside; and, third, the acetylcholine-stimulated, endothelium-dependent dilatation of coronary vessels.

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MATERIALS AND METHODS

Experiments were performed on isolated hearts of female rats weighing 180-230 g. The control and experimental series included 96 and 65 animals, respectively. Hearts were excised under urethane anesthesia (1 g/kg), and Langendorff perfusion was performed at a constant flow of carbogen-aerated (5% CO_2/95% O_2) Krebs-Henseleit buffer in a thermostatically controlled chamber. When the dependence between coronary perfusion pressure (PP)

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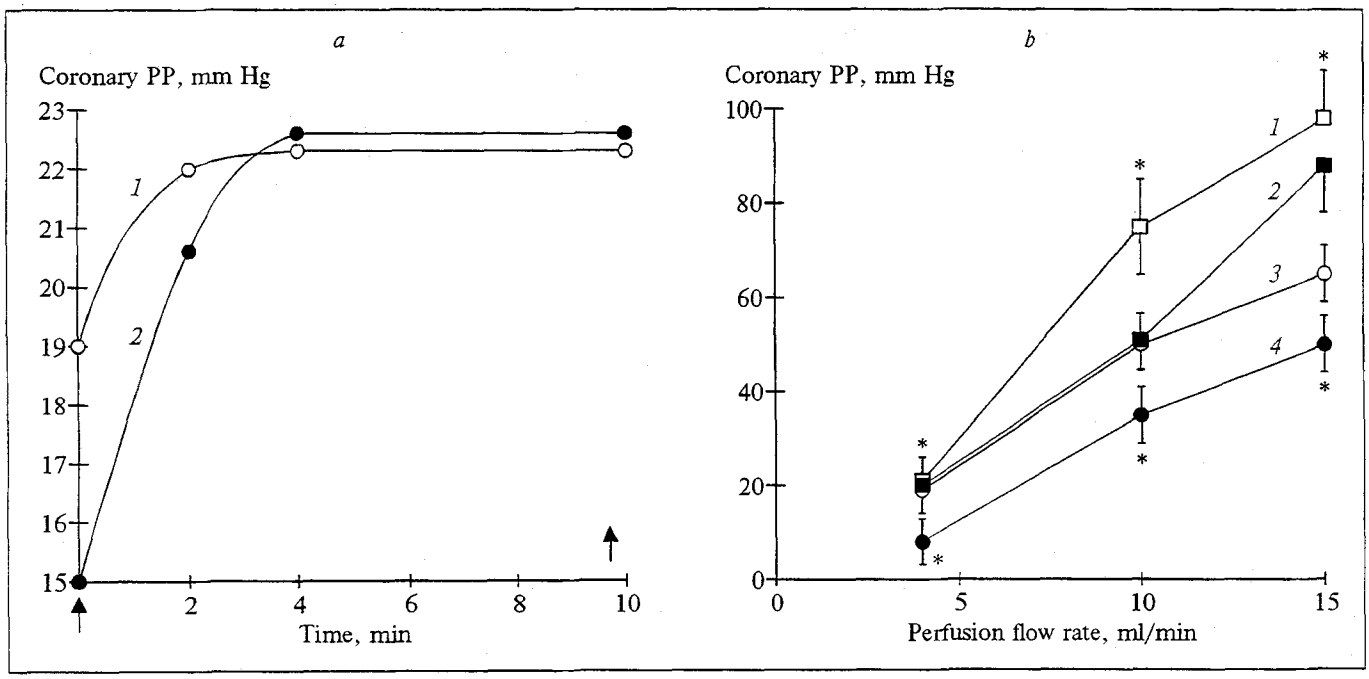

Fig. 1. Effect of immobilization stress and N^G-MMLA on coronary PP in isolated rat hearts at constant (4 ml/min, *a*) and increased (*b*) perfusion flow rates. Arrows indicate the beginning and the end of N^G-MMLA administration. *1*) control; *2*) immobilization stress; 3) N^G-MMLA; *4*) stress + N^G-MMLA. Here and in Fig. 2 an asterisk indicates $p<0.05$ compared with the control.Coronary PP, mm HgTime, minPerfusion flow rate, ml/min

and perfusate flow was studied, the animals in each series were divided into two groups: group 1, perfusate contained N^G-MMLA and group 2, perfusate contains no N^G-MMLA. Each group consisted of 7 rats. Coronary blood flow (4, 10, and 15 ml/min) was provided with an NP-1M peristaltic pump. Coronary response was assessed by changes in PP recorded with the aid of an EMT-34 sensor (Mingograf-81) and an Lp-50 potentiometer. A heart rate of 240 beats/min was paced with an ESL-2 eletrostimulator. The response of coronary vessels to a bolus (100 μl) intracoronary injection of increasing doses of sodium nitroprusside or acetylcholine was studied on isolated hearts stopped with KCl (34 mM) and perfused at a constant flow rate of 4 ml/min. Each dose of both preparations was tested on 6-11 isolated hearts. Stress was induced by immobilization on the back for 6 h. Studies were carried out 60 min after stress. N^G-MMLA was injected in an aortic cannula for 15 min in a volume equal to 1/40 of the coronary flow rate (final concentration 100 μM). Endothelial damage was produced by administration of saponin (44 μg/ml for 2 min) [8]. The results were analyzed using the nonparametric Wilcoxon-Mann-Whitney test.

RESULTS

In isolated hearts of animals exposed to stress the coronary PP at a constant perfusion rate of 4 ml/min was 15% lower than in the control (Fig. 1, *a*). The perfusion pressure increased after administration of N^G-MMLA , the increase being greater (36±2%) in experimental than in control rats (15±3%, $p<0.05$).

When the perfusion flow rate was increased (4-15 ml/min), PP in the hearts of stressed animals decreased by 23% compared with the control (Fig. 1, *b*). Administration of the NO-synthase blocker under these conditions into control animals increased the coronary PP by 32%. After administration of N^G-MMLA in the coronary bed of stressed animals PP did not differ from the control at various rates coronary flow, i.e., administration of N^G-MMLA almost completely eliminated the post-stress decrease in coronary tone.

The responses to sodium nitroprusside and acetylcholine were studied in coronary vessels constricted with KCl. After administration of KCl the coronary PP in stopped isolated hearts of stressed animals (101±6 mm Hg) did not differ significantly from that in the control (95±2 mm Hg). The decrease in coronary PP under the influence of higher doses of sodium nitroprusside was significantly smaller in the experimental animals than in the control (Fig. 2), while its concentration inducing the half-maximum response of coronary vessels was higher than in the control: 92±25 *vs.* 18±5 μM.

The response of KCl-treated coronary vessels to acetylcholine was dose-dependent. At low con-

centrations (0.1 and 1.0 μM) the coronary PP only dropped, while at higher concentrations (10 and 100 μM) the response became biphasic. An initial increase in coronary PP (vasoconstriction), the maximum reaching 25-30 sec after administration of acetylcholine, was followed by a decrease (vasodilation), with the maximum reached by the 60th sec. After the coronary endothelium had been damaged with saponin, the acetylcholine-induced decrease in coronary PP was eliminated in control hearts, and only the second phase of the response (increase) was observed (Fig. 3). Presumably, saponin did not damage smooth muscle cells, since injection of sodium nitroprusside (a vasodilatory agent whose effect is not mediated by endothelium) in saponin-treated coronary vessels induced essentially the same decrease in coronary PP (26±5%) as in intact vessels (25±4%). N^G-MMLA almost completely blocked the decrease induced by acetylcholine (100 μM) in coronary PP (3.2±1.4 *vs.* 22.6± ±5.4% in the control, $p<0.01$) and induced no statistically significant changes in the response to sodium nitroprusside (17±2 *vs.* 24±9% in the control, $p>0.05$). Consequently, the dilatation of coronary vessels caused by acetylcholine is endothelium-dependent and is probably mediated by NO release.

In the hearts of stressed animals, the decrease in PP induced by higher doses of acetylcholine was significantly smaller than in the control (Fig. 3). The sensitivity of coronary vessels to acetylcholine also diminished, since the concentration of acetylcholine inducing the half-maximum dilatory response markedly increased (61±17 *vs.* 21±8 μM in the control).

The magnitude of the initial constrictor reaction of cardiac vessels to the administration of 10 and 100 μM acetylcholine in the coronary bed of stressed animals did not change (7.2±3.2 and 17.6±4.7% *vs.* 8.5±3.7 and 19.2±5.1% in the control, respectively).

Our results suggest that the baseline release of NO by the coronary endothelium increases, which may reduce coronary tone and the sensitivity of coronary smooth muscle cells to sodium nitroprusside, since the stepped-up release of endogenous NO diminishes the effect of exogenous NO (nitrovasodilator). This is due to the involvement in both cases of the same effector mechanism operating in smooth muscles of coronary vessels (activation of soluble guanylate cyclase [2,3]) and to an increase in their cGMP content [6].

Concerning the acetylcholine-stimulated dilatation of coronary vessels, it is unlikely that its decrease by stress is due to depletion of the functional reserve of the L-arginine/NO system, since the baseline and stimulated syntheses of NO by endotheliocytes are different [7] and, besides, there are substantial sources of L-arginine in the cell [4]. The post-stress decrease of stimulated endothelium-dependent dilatation of coronary vessels may be associated with, first, a decrease in the choline reactivity of endothelial cells and, second, an altered sensitivity of coronary smooth muscles (as a result

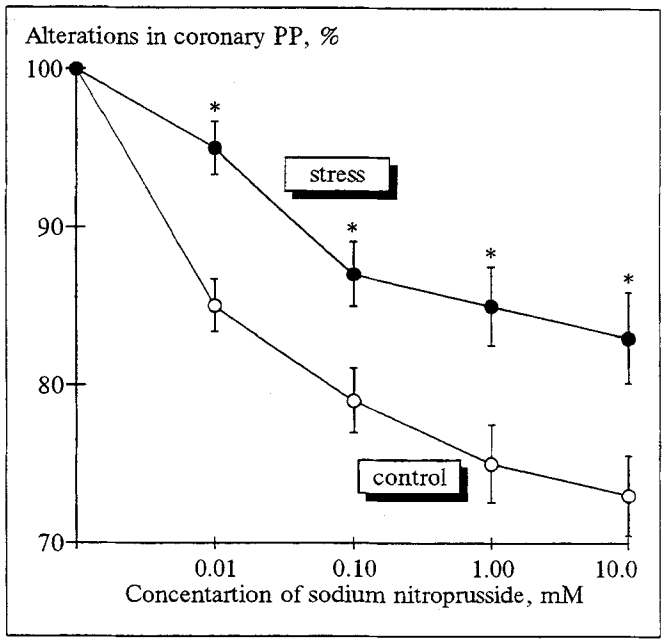

Fig. 2. Effect of immobilization stress on dilatory response of coronary bed of isolated rat heart to sodium nitroprusside. Coronary PP prior to administration of sodium nitroprusside was taken as 100%.

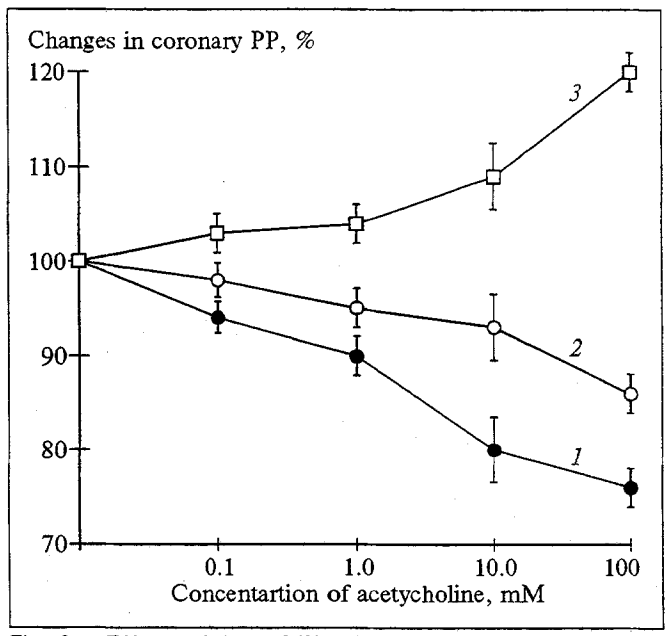

Fig. 3. Effect of immobilization stress on coronary PP alterations induced by acetylcholine. Coronary PP prior to administration of acetylcholine was taken as 100%. *1*) control; *2*) stress; *3*) control after endothelial damage.

of the potentiated baseline synthesis of NO) to the NO released under the influence of acetylcholine.

Thus, stress stimulates the baseline synthesis of NO by coronary endothelium. This probably causes a decrease in coronary tone and lowers sensitivity to sodium nitroprusside, and it is also accompanied by a reduction of stimulated endothelium-dependent coronary dilatation.

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Dynamics of Aseptic Inflammation against the Background of α-Tocopherol

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Administration of α-tocopherol before the induction of inflammation reduces the vascular response and inhibits the leukocyte phase, which limits the development of secondary alterations in tissues. During the reparative period fibroblast proliferation is suppressed and differentiation is accelerated, whereas the synthetic activity is lowered. As a result, the formation of the fibroblast capsule is slowed down.

Key Words: *inflammation; α-tocopherol acetate*

Activation of free-radical oxidation (FRO) is an integral component of any inflammatory process [8,9]. On the one hand, the products of lipid peroxidation play an important role in membrane renewal and in the regulation of cell functions [3], while on the other hand, excessive accumulation of FRO products is an important pathogenic link in the development of inflammation: it increases vascular permeability, aggravates DNA and plasma membrane damage, and affects the metabolism [2], i.e., it potentiates secondary alterations. Consequently, elucidation of the regularities and mechanisms governing inflammation under conditions where the organism's antioxidant potential is high would be conducive toward optimizing the inflammatory process. The objective of this study was therefore to investigate cell and vascular reactions in inflammation initiated against the background of the naturally occurring antioxidant α-tocopherol (α-TPH).

MATERIALS AND METHODS

Experiments were performed on 90 male rats. Inflammation was induced by subcutaneous insertion of a 1×5 mm celloidin plate in the shank area. The animals were assigned to two groups: α-TPH was injected once to group 1 (100 mg/kg intraperitoneally) one day before the induction of in-

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