PHYSIOLOGY

Effect of L-Arginine on Myogenic Reactions of Vascular Smooth Muscle Cells in Hypercholesterolemia

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In hypercholesterolemia, the reduced vascular response observed during stretching of the vessel wall is probably caused by disorders of endothelial functional activity. L-arginine stimulates synthetic activity of the endothelium, making it possible to use of this amino acid for correction of these impairments.

Key Words: *vascular smooth muscles; L-arginine; nitrogen oxide; hypercholesterolemia*

It has recently been demonstrated that in hypercholesterolemia the endothelium-dependent vasodilation of smooth muscles is diminished and the vascular response to vasoconstrictors is enhanced [6,9]. In many respects these phenomena are due to both a reduced release of the endothelial relaxation factor and an imbalance between endothelium-produced relaxing and constricting factors (endothelin, platelet-activating factor, etc.). It has been shown that nitrogen oxide (NO) or a nitrate which releases NO acts as the endothelial relaxation factor [10,12] and that L-arginine is the precursor in NO synthesis [13,14]. Vasoconstrictive and vasodilative agents are secreted by the endothelium under the influence of various agonists and mechanical factors [1,5,8,15]. Mechanical stimulation of the endothelium results from alterations of intravascular pressure and of the degree of vascular wall stretching, which is accompanied by deformation of endothelial cells. These stimuli lead to the release of vasoactive agents modulating the contractile response of smooth muscles. When endothelial function is impaired (for example, in atherosclerosis, hypertension, and other pathological states [6,7,11]), these influences on vascular smooth muscles from the endothelium may become limited. However, there is evidence that administration of L-arginine, a precursor of NO synthesis, stimulates synthetic function of the endothelium in different states, including hypercholesterolemia [4,6,9].

Our objective was to examine the effect of L-arginine on the length-force relationship for vascular smooth muscles in hypercholesterolemia.

MATERIALS AND METHODS

Experiments were performed on isolated preparations of rabbit portal vein: group I were controls, group II animals received the standard atherogenic diet (0.2 g cholesterol/kg body weight) for 4 months and group III animals were maintained on the atherogenic diet and given L-arginine in an intravenous daily dose of 25 mg/kg for 4 months. Vascular preparations weighing 2-5 mg were placed in a thermostatically controlled perfusion chamber (1 ml), where they were subjected to initial passive stretching with a force of 4-6 mN. The preparations were perfused with normal Krebs solution at 36.6-37°C. The contractile activity of smooth muscles was recorded with a 6MXIC mechanoelec-

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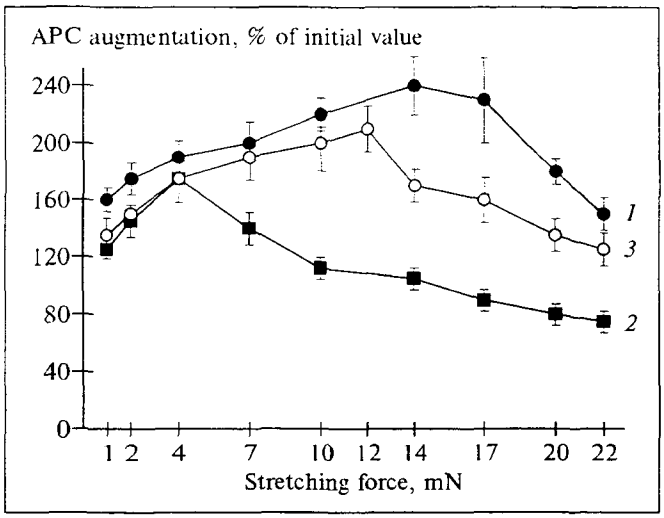

Fig. 1. Effect of hypercholesterolemia on APC of rabbit portal vein stripes upon their stretching and APC changes after L-arginine administration. *1*) control; *2*) after 4 months on atherogenic diet; *3*) after the atherogenic diet with simultaneous administration of L-arginine.

trical converter in a regime close to isometric. The preparations were then stretched with a force of 1-22 mN. Acetylcholine (10^{-7} M) was used to test vascular reactivity. The blood cholesterol content was measured in all animals in the initial state and immediately before the acute experiment.

The results were analyzed using methods of variational statistics and Student's *t* test.

RESULTS

An additional stretching of intact muscle stripes of rabbit portal vein with a force of 1-10 mN led to an increase in the amplitude of phase contractions (APC) by 60±5-120±10% of the initial value (Fig. 1). When the stretching force was increased to 14 mN, the APC augmentation reached the maximum (140±12%). A further increase in the stretching force (to 17 mN) led to a certain decrease in APC, which, however, still remained high. The APC augmentation started to decrease at an additional stretching force of 20 and 22 mN.

The contraction force (absolute values) of isolated vascular preparations obtained from animals receiving the atherogenic diet for 4 months was 7- to 10-fold lower than in the controls. A further stretching of portal vein stripes with a force of 1 and 2 mN increased APC by 25±4 and 45±8% of the initial value, respectively. The maximum APC augmentation was observed at an additional stretching force of 4-5 mN (75±7%). The augmentation dropped as the stretching force was further increased. The response to acetylcholine in these animals decreased by 78±8.3%. In isolated preparations of portal vein, acetylcholine is known to increase vascular tone and the frequency of spontaneous contractions [2].

Thus, in animals given the atherogenic diet during a 4-month period the APC augmentation for the same stretching force was smaller than that in the controls. In the experimental animals, the maximum APC augmentation was achieved at a smaller stretching force, being 2-fold lower than in the control group. In group II animals, a reduction in APC was observed upon application of a much smaller stretching force. These results indicate that in rabbits fed an atherogenic diet for 4 months the myogenic mechanisms responsible for alterations in the contractile activity of stretched muscles are less efficient than in intact controls.

The augmentation of APC was greater in rabbits given L-arginine together with the atherogenic diet compared with that observed in group II (Fig. 1). The maximum APC augmentation (110±8% of the initial value) was reached at a stretching force of 11-12 mN. In group III animals, the response of vascular stripes to acetylcholine was much more intense than that in group II.

Our results indicate that in hypercholesterolemia APC of portal vein preparations is markedly decreased upon stretching, and the stretching force at which the maximum contraction occurs is considerably smaller than in health. Previously, we showed that APC of vascular preparations upon their stretching is strongly dependent on the functional activity of the endothelium and is determined by its secretion of biologically active substances stimulating the contraction of vascular smooth muscles [3]. At the same time, numerous studies demonstrate that endothelial function is inhibited during atherogenesis [6,7,9]. Therefore, it can be assumed that the inhibition of the length-force reaction in hypercholesterolemic animals is due to insufficient endothelial function. This assumption is confirmed by the influence of arginine on the length-force dependence in these animals. It is known that L-arginine, a precursor of NO biosynthesis, stimulates both the synthesis of NO and the endothelium-dependent vascular responses [4,13,14]. The restoration of the length-force curve observed for L-arginine administration in hypercholesterolemia may result from the stimulating activity of this agent on endothelial function. On the other hand, the restorative activity of L-arginine confirms the involvement of endothelial factors in these reactions. This suggests that L-arginine can be used to correct impaired vascular reactivity to changes in intravascular pressure accompanied by alterations in the length of vascular smooth muscles.

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The Contribution of Dopamine Autoreceptors to the Reactivating Effect of Opioid Antagonists

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The dopamine-opioid interaction is analyzed in amnesia and forgetfulness with the use of passive avoidance conditioning in experiments on mice. Naloxone or ICI174.864 administration restored the conditioned response in both learning situations against the background of saline. Pretreatment with (+)3PPP eliminated the reactivating effects of μ- and δ-opiate receptor blockade in the case of amnesia. The activation of dopamine autoreceptors in forgetfulness disrupted the memory restoration induced by ICI174.864, but not that by naloxone. The findings attest that reactivation of an amnestied memory trace by opioid receptor blockade depends on dopaminergic system functioning, whereas in the case of forgetfulness dopamine-opioid interactions are probably determined by the functional heterogeneity of the μ- and δ-opiate receptors and diminished contribution of the dopamine system to the process.

Key Words: *amnesia; forgetfulness; opioid antagonists; dopamine autoreceptors*

The activation of the dopaminergic (DA) system and blockade of the μ- and δ-opiate receptors help counteract amnesia [1,7,11]. Investigations of DA and opioid receptor interaction in behavioral reactions studied in detail during analysis of the reinforcing system of the brain and motor activity [5,9], are now being pursued intensively. DA-receptor blockade effectively changes the modifying effect of the μ-, δ-, and κ-receptor agonists on habituation [4]. The role of DA-opioid interaction in recalling the memory trace is virtually unknown, although the modulating effects of neuropeptides on learning depending on the functional state of monoaminergic systems have been examined [3,6].

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