Calcium-Dependent and Endothelium-Dependent Mechanisms for a Constrictor Response of the Saphenous Vein

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Acute experiments on narcotized cats (method of venous resistography) were performed to study a constrictor response of the great saphenous vein in the hindlimb to a bolus intravenous injection of norepinephrine and/or angiotensin II. The saphenous vein exhibited an increased or decreased constrictor response to norepinephrine and angiotensin II, which depended on the order of exposure to adverse factors (calcium channel blocker nifedipine and chemical deendothelialization-inducing agent hemoglobin).

Key Words: *saphenous vein; constrictor response; calcium channels; endothelial factors*

Studying the mechanisms for vascular tone regulation are associated with the estimation of Ca^{2+} action on contraction and relaxation of smooth muscles [3,11-13] and evaluation of the endothelium-dependent regulation of blood vessel lumen under the influence of humoral factors [7,9]. *In vitro* experiments with rings of large arteries [1] resulted in the identification of some endothelium-derived relaxing (EDRF) and contracting factors (EDCF). Much attention is paid to the search for substances that are secreted by vascular endotheliocytes under basal conditions and exposure to humoral agents [2,4]. The interaction of calcium-dependent and endothelial mechanisms has an important role in the regulation of vascular tone in smooth muscles [6,8].

Previous studies revealed that extracellular calcium antagonists cause a blockade of endothelium-dependent reactions [6]. Experiments with the isolated aortic segments of rats showed that EDRF release is not mediated by the calcium-dependent mechanisms (as differentiated from EDCF) [10]. The contractile response was suppressed under conditions of calcium channel blockade, but returned to normal or exceeded the baseline value after mechanical deendothelialization.

Here we studied the constrictor response of the great saphenous vein (GSV) in cat hindlimb after calcium channel blockade and induction of endothelial dysfunction.

MATERIALS AND METHODS

Experiments were performed on 16 cats of both sexes weighing 2.5-4.0 kg and anesthetized with urethane (1 g/kg intravenously, treatment with 1500 U/kg heparin). Blood flow resistance of GSV in rat hindlimb was measured as follows. The GSV segment was separated in the ankle joint. A catheter was introduced into the lumen of the vein. The vessel was perfused with arterial autoblood after stabilization of pulsatile blood flow (pulsation frequency 134 cpm, pulse pressure 15-20 mm Hg). Perfusion pressure was maintained at 20-25 mm Hg, which did not exceed a normal physiological value of venous pressure in this region (taking into

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account the decrease in catheter pressure by 10-15 mm Hg). Variations in blood flow resistance under the influence of various factors were evaluated from perfusion pressure (measurement with an electromanometer).

A calcium channel blocker nifedipine (1 mg in 1 ml 6% dextran solution per min) was infused. Functional integrity of the venous endothelium was impaired by infusion of 1% horse hemoglobin [6]. Norepinephrine and angiotensin II (5 mg in 0.1 ml 6% dextran solution, Poliglyukin) served as the vasoactive test substances.

The results were analyzed by Student's *t* test (significance level p=0.05).

RESULTS

Norepinephrine and angiotensin caused contraction of GSV smooth muscles in the hindlimb under normal physiological conditions. Perfusion pressure increased by 41±4 and 16±5 mm Hg after treatment with norepinephrine and angiotensin, respectively (Fig. 1). Endothelial dysfunction of the venous wall due to infusion of 1% hemoglobin solution was followed by a greater response to norepinephrine than to angiotensin. Administration of norepinephrine and angiotensin was accompanied by an increase in perfusion pressure by 59±8 and 27±7 mm Hg, respectively (Fig. 1). These changes probably result from a decrease in the effect of endotheliocyte-derived relaxing factors [1,2,4]. The constrictor response of GSV to norepinephrine and angiotensin was significantly reduced after blockade of extracellular calcium influx with nifedipine (29±3 and 8±3 mm Hg, respectively). The observed responses were much smaller (p=0.05) than those induced by test substances under conditions of endothelial dysfunction due to infusion of 1% hemoglobin solution (Fig. 1). The data indicate that a calcium channel blocker nifedipine abolishes the constrictor response of GSV, which does not depend on functional integrity of venous endotheliocytes.

The order of exposure to adverse factors was modified in the next series. Endothelial integrity was impaired after treatment with nifedipine (antagonist of extracellular calcium influx).

Under normal conditions, norepinephrine and angiotensin caused contraction of GSV smooth muscles (similarly to the previous series). Perfusion pressure increased by 48±5 and 16±3 mm Hg after treatment with norepinephrine and angiotensin, respectively (Fig. 2). The responses decreased significantly after infusion of a calcium channel blocker nifedipine (1 mg/kg, p=0.05). The reactions to norepinephrine and angiotensin were 24±3 and 8±3 mm Hg, respectively (Fig. 2). Deendothelialization was followed by a sharp increase in the amplitude of constrictor responses (particularly to norepinephrine). Under these conditions the reactions to norepinephrine and angiotensin were 51±7 and 25±5 mm Hg, respectively (Fig. 2). The response to norepinephrine was much greater than that observed after blockade of calcium channels (p=0.05, Fig. 2).

Despite the adverse effect of calcium channel blockade and chemical deendothelialization on smooth muscles, we revealed an increase in the amplitude of GSV constriction in response to norepinephrine and angiotensin. The observed changes were most significant for norepinephrine (p=0.05). It should be emphasized that the constrictor response of GSV to constricting factors returned to normal (baseline value) after exposure to two adverse agents. Calcium channel blockade with nifedipine had little effect after chemical deendothelialization by hemoglobin. The constrictor response of the vein increased significantly under these conditions. This phenomenon is probably related to opening of nifedipine-independent calcium channels. Our hypothesis is consistent with published data [5]. Experiments with segments of the aorta and arterial vessels showed that the constrictor response returns to normal after calcium channel blockade and deendothelialization. A similar phenomenon was observed in experiments on GSV of cat hindlimb (close to *in vivo* conditions).

Our results indicate that calcium-dependent and endothelium-dependent mechanisms can occur independently of each other in mediating the con-

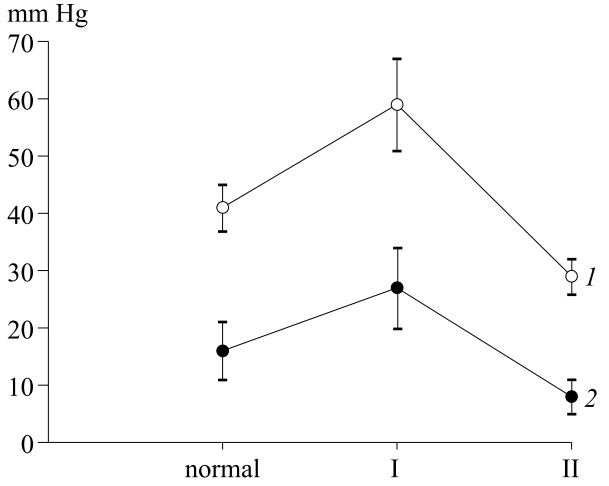

Fig. 1. Mean changes in blood flow resistance of the saphenous vein to norepinephrine (5 mg) and angiotensin II (5 mg) before and after deendothelialization with hemoglobin and infusion of nifedipine. Ordinate: change in blood flow resistance of the vein. Here and in Fig. 2: norepinephrine (*1*) and angiotensin II (*2*). Infusion of 1% horse hemoglobin (I); and infusion of nifedipine (1 mg/min, II).

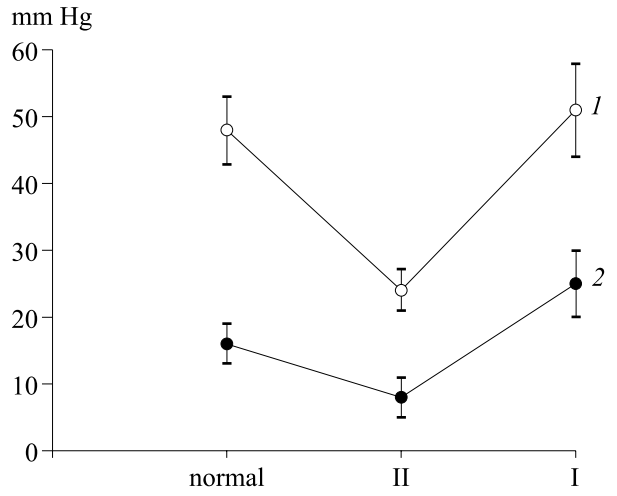

Fig. 2. Blood flow resistance of the saphenous vein to norepinephrine (5 mg) and angiotensin II (5 mg) before and after infusion of nifedipine and deendothelialization with hemoglobin.

strictor response of GSV. A blocker of extracellular calcium influx reduces the constrictor response, which does not depend on functional integrity of the venous epithelium. However, the induction of chemical deendothelialization after treatment with a calcium channel blocker returns the constrictor response of GSV to the baseline value. We conclude that no action of endothelial factors on smooth muscles of the vascular wall abolishes the effect of a calcium channel blocker. Hence, extracellular calcium for venous constriction is released into smooth muscle cells through newly opened channels. Therefore, the calcium-dependent and endothelium-dependent mechanisms interact with each other under specific conditions. The order of exposure to adverse factors is of particular importance in this respect. It should be emphasized that a calcium channel blocker reduces the constrictor response of GSV after the impairment of functional integrity in the endothelium. However, all parameters return to a normal physiological level after blockade of extracellular calcium influx into smooth muscle cells and subsequent chemical deendothelialization. The observed changes are probably associated with extracellular calcium influx into smooth muscle cells through new channels. It remains unclear why new calcium channels do not open in another order of combined exposure to these factors. Further physiological and biochemical studies are required to answer this question.

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