MECHANISMS OF INVOLVEMENT OF THE ENDOTHELIUM IN REACTIVE HYPEREMIA

V. F. Sagach and M. N. Tkachenko

UDC 616-018.7-092-07

Key Words: reactive hyperemia; endothelium.

It has been shown in recent years that the endothelium plays an essential role in the development of vascular reactions to various stimuli [3, 5, 14]. The endothelium exerts its modulating action on vascular responses by secreting relaxing and constrictor factors, whose nature likewise has recently been discovered [9, 15]. It has been shown that the endothelium plays a decisive role in the development of reactive hyperemia [2, 6], and realizes its action through secretion of an endothelial relaxation factor [2].

The aim of this investigation was to demonstrate the involvement of humoral factors of endothelial origin in reactive hyperemia and to determine the stimulus triggering secretion of this factor by the endothelium during the development of reactive hyperemia.

EXPERIMENTAL METHOD

Reactive hyperemia was induced in the femoral vascular system of dogs (16-23 kg) under chloralose-urethane anesthesia (0.05 and 0.5 g/kg, respectively), intravenously), by restoring the blood flow in the femoral artery after its occlusion for 30, 60, and 1200 sec. The appearance of vasoactive substances in the venous blood during the development of reactive hyperemia was identified by biological testing. A sample of 100 μl of blood was taken from the femoral vein for this purpose at the peak of reactive hyperemia and immediately transferred into a thermostatted cell containing a circular isolated preparation of the vascular wall of the femoral artery from the same dog, weighing on average from 3 to 5 mg. Depending on the mass and size, the vascular preparations were subjected to preliminary passive stretching with a force of between 5 and 10 mN. The vascular preparations were superfused with Krebs' solution of the following composition (in mM): NaCl – 133, KCl – 4.7, $NaHCO_3$ – 16.3, $NaHPO_4$ – 1.38, $CaCl_2$ – 2.5, $MgCl_2$ – 1.2, glucose – 7.8, pH 7.4 at 37°C. Contractile responses of the vascular preparation were recorded by means of a mechanical to electrical transducer.

Chemical de-endothelization of the femoral vessels was carried out by injecting a solution of saponin [12] (1 mg/kg, blood flow arrested for 5 min) into the system of the femoral arteries. The de-endothelization was verified morphologically.

To prevent the pressure from falling in the distal segment during occlusion, it was connected to a reservoir located at a height ensuring stabilization of the pressure in the distal segment at its previous level after occlusion of the femoral artery and vein.

The effect of dimerized glutaraldehyde, used to prevent the response of the endothelium to mechanical stimulation, and causing the arteries to lose their sensitivity to the velocity of the blood flow [1], on the development of reactive hyperemia was studied in 10 experiments. The glutaraldehyde (25% solution, from "Merck," West Germany) was diluted to the necessary concentration in Ringer's solution. For dimerization, 5 μl of 5% NaOH solution was added to 10 ml of a 1% solution of glutaraldehyde, and the resulting solution was diluted to 0.05% concentration of the aldehyde. The glutaraldehyde solution was injected into the femoral artery while the blood flow was arrested for 2 min.

The results were subjected to statistical analysis.

Department of Physiology of the Circulation, A. A. Bogomolets Institute of Physiology, Academy of Sciences of the Ukrainian SSR, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR P. G. Kostyuk.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 5, pp. 420-422, May, 1990. Original article submitted June 16, 1989.

 0007-4888/90/0005-0552$12.50

TABLE 1. Reactive Hyperemia in Territory of Distribution of Dog's Femoral Vessels and Reaction of Relaxation of Isolated Preparation of Vascular Wall of Femoral Artery to Venous Blood after Occlusion of Varied Duration

No. of expt.	Duration of occlusion	Degree of reactive hyperemia, % (relative to initial blood flow)	Magnitude of response of relaxation of vascular preparation to venous blood, mN/kg
1	0 (control)	—	0,31±0,025
2	30	+55±5,6	0,63±0,041*
3	60	+76±5,2	0,75±0,049*
4	120	+112±7,2	1,01±0,044*
	After de-endotherlization with saponin		
5	120	+24±2,0**	0,23±0,033**
	After occulsion without significant fall of intravascular pressure		
6	120	+36±3,5**	0,32±0,022**
	After treatment with dimerized glutaraldehyde		
7	120	+38±3,6**	0,39±0,04**

Legend. *) Difference significant relative to data for experiment No. 1; **) to data of experiment No. 4, at $p < 0.001$.

EXPERIMENTAL RESULTS

The degree of reactive hyperemia depended on the duration of preceding occlusion of the femoral artery (Table 1). In all cases the isolated preparation of femoral artery reacted to the addition of venous blood to the cell by relaxation. The action of blood collected at the peak of reactive hyperemia was accompanied by a significant increase in relaxation of the vascular preparation, evidence of the appearance de novo or of a substantial increase in the concentration of biologically active substances with dilator action in these blood samples. Under these circumstances the response of relaxation of the vascular preparation increased significantly in magnitude with an increase in the duration of occlusion (Table 1) and correlated with the degree of hyperemia. This is evidence of increased release of biologically active substances with dilator action into the bloodstream with an increase in the duration of occlusion. The source of these substances is the endothelium. This is shown by the fact that de-endothelization of the femoral vessels with saponin was accompanied not only by a considerable (almost fivefold) reduction in the intensity of reactive hyperemia, but also by the same degree of diminution of the response of relaxation of the vascular preparation. This response was reduced under the influence of blood after occlusion for 2 min from 1.01 ± 0.044 to 0.23 ± 0.033 mN/mg (Table 1). Similar changes in the degree of hyperemia and the response of the vascular test preparation were noted to injection of acetylcholine after de-endothelization.

Analysis of the possible stimuli capable of inducing synthesis of relaxation factor and its secretion by the endothelium in response to vascular occlusion suggested that lowering of the intravascular pressure might be such a stimulus. This hypothesis was supported by the close correlation found between the degree of postocclusion hyperemia and the mean intravascular pressure observed during arterial, venous, and combined vascular occlusion (Table 2). Prevention of a sharp fall of intravascular pressure by combined occlusion of the femoral artery and vein, by connecting the distal vascular segment with a reservoir of physiological saline kept at a height of 1.3 m, significantly reduced the degree of reactive hyperemia and the relaxation response of the vascular test preparation (Table 1). This fact confirms that stimulation of this kind not only inhibits the hyperemic response, but also significantly reduces the release of substances with dilator properties into the bloodstream. By performing the experiment in this way, the effect of hypoxia, which can stimulate release of endothelial relaxation factor [4, 10], on the blood vessels cannot be excluded. At the same time, the pressure drop is excluded and a comparatively high intravascular pressure is maintained, and this has recently been proposed as a factor inhibiting the release of endothelial relaxation factor and endothelium-dependent relaxation reactions [8, 11]. Inhibition of such reactions in animals with hypertension is well known [7]. Some workers, in order to prove the ability of endothelium to respond to mechanical stimuli, have used for this purpose the reduction of deformability of endothelial cells as a result of their treatment with dimerized glutaraldehyde [1]. In the present experiments preliminary treatment of the vascular bed with dimerized glutaraldehyde significantly reduced both the degree of reactive hyper-

TABLE 2. Reactive Hyperemia (RH) to Initial Blood Flow (in %) and Mean Pressure (MP; in mm Hg) in Territory of Distribution of Dog's Femoral Vessels during Arterial, Mixed, and Venous Occlusion of Varied Duration

Occulsion of	Duration of arrest of limb perfusion, sec					
	30		60		120	
	RH	MP	RH	MP	RH	MP
Artery	+67±9,2	4,6±0,6	+83±9,9	4,7±0,7	+118±6,8	4,9±0,3
Artery and vein	+45±3,9	39±3,1	+70±2,0	47±3,5	+100±7,7	53±4,1
Vein	+21±5,3	62±6,3	+35±11,0	68±5,0	+43±8,7	74±6,9

emia and the magnitude of the response of relaxation of the vascular test preparation (Table 1), i.e., the procedure was accompanied by significant inhibition of the release of vasodilator substances into the bloodstream. Meanwhile the response to acetylcholine was not significantly reduced after administration of dimerized glutaraldehyde, i.e., the response to chemical endothelium-dependent stimuli was not impaired under those conditions.

Reactive hyperemia is thus largely the result of secretion of biologically active substances with a vasodilator action by endothelial cells. The stimulus which triggers the biosynthesis and secretion of these substances by the endothelium under conditions of vascular occlusion is a fall of intravascular pressure. The ability of endothelium to respond by secreting a dilator factor to acceleration of the blood flow is also well known [3, 13], and it undoubtedly takes place when the vascular occlusion is removed. However, the fact that such acceleration does not depend on the duration of occlusion, whereas the degree of hyperemia is strictly dependent of it, is evidence against this manner of triggering the secretory activity of the endothelium and of the subsequent development of reactive hyperemia.

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