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Research Articles

On the mechanism of the involvement of endothelium in reactive hyperemia

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Received 9 August 1989; accepted 11 December 1990

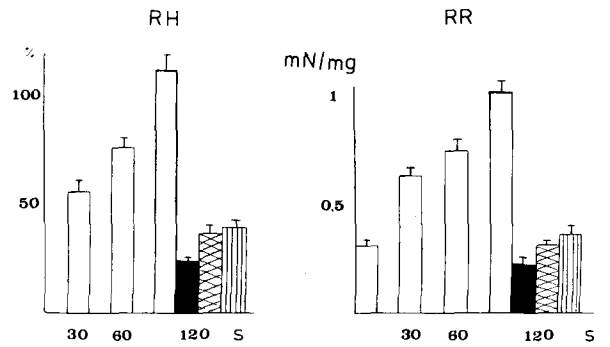
Abstract. Experiments on anesthetized dogs and on vascular test-preparations demonstrated that reactive hyperemia (RH) was accompanied by the appearance of vasodilator in the blood, and that the level increased with the duration of occlusion of the artery. Removal of the endothelium of the part of the vascular bed studied using saponin, decreased the RH and relaxation of a test-preparation. A rise of pressure in the vascular bed, and a decrease in the deformability of the endothelium resulting from pretreatment with dimerized glutaraldehyde, affected both the hyperemia and the reaction of the vascular preparation in a similar way. It was concluded that the RH resulted from the secretion of vasoactive substances by the endothelium in response to a fall in intravascular pressure.
Key words. Reactive hyperemia; endothelium.

The capacity of the endothelium, epithelial and neuronal cells to synthesize a potent substance with a relaxing effect on smooth muscle, called endothelium-derived relaxing factor (EDRF), is an important discovery of the last decade[1-3]. The endothelium plays a significant role in the development of vascular reactions following the effect of various stimulants, producing its modulating effect through the release of relaxing and contracting factors, whose nature has recently been elucidated[4,5]. Quite recently a decisive role has been attributed to endothelium in the development of reactive hyperemia (RH)[6,7]. This effect is brought about by the release of EDRF[6].

The purpose of the present investigation was to ascertain the participation of humoral factors of endothelial origin in RH and to identify the stimulus which initiates the release of this factor by the endothelium.

Methods

RH was reproduced in the femoral vascular bed of dogs weighing 16–23 kg under chlorasol-urethane anesthesia (0.05 and 0.5 g/kg i.v., respectively) through the restoration of blood flow in the femoral artery after 30-, 60- and 120-s periods of occlusion. The presence of vasoreactive substance in the venous blood during the development of RH was determined by biological testing, as follows. A blood sample (100 µl) was taken from the femoral vein when RH reached its maximum, and instantly transferred to a tissue organ bath containing the segment (3–5 mg) of the femoral artery of the dog which was used for the bioassay of dilator activity. Depending on the mass and size, the vascular preparations were subjected to initial passive distension (5–10 mN). The vascular preparations were perfused with Krebs solution containing (mmol/l): 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, t = 37 °C. Contractile responses of the vascular apparatus were registered with a mechanoelectric transducer. Chemical removal of the endothelium of the femoral vessels was performed with saponin applied to the femoral vascular bed (1 mg/ml, after a 5-min occlusion)[8]. Endothelium removal was checked by mor-



Reactive hyperemia (RH) in the bed of canine femoral vessels and reaction of relaxation (RR) of isolated preparation of femoral artery vascular wall on the venous blood after occlusion of varying duration. □, control; ■, after endothelium removal; □, occlusion without fall of mean intravenous pressure; □, after dimerized glutaraldehyde pretreatment.

phological examination. In order to maintain pressure in the distal part of the vessel during the occlusion it was connected with a bottle located high enough (1.3 m) to stabilize pressure at the initial level.

A series of 10 experiments was performed to study the effect of dimerized glutaraldehyde on RH development. This agent was used to prevent the endothelium from responding to mechanical stimuli and to make the arteries insensitive to the blood flow rate[9]. 25% glutaraldehyde (Merck, FRG) was diluted to 0.05% in Ringer solution. For dimerization, 5 µl of 5% NaOH were added to 10 ml of 1% glutaraldehyde. The solution was diluted to a final level of 0.05% aldehyde and then administered into the femoral artery during a 2-min period of occlusion.

Results and discussion

The magnitude of RH depended on the duration of the preceding occlusion of the femoral artery (fig.). In all cases the addition of venous blood to the bath resulted in relaxation of the isolated preparation of the vascular wall of the femoral artery. The addition of blood taken at the peak of RH was accompanied by a substantial increase in the relaxation of the vascular preparation, which may signify a marked increase in the concentration of biologically active dilators in these blood portions. Moreover, the observed relaxation of the vascular preparation increased significantly with the increase of duration of occlusion (fig.) and correlated with the degree of hyperemia. This testifies to the increased release of biologically active dilators to the blood with an increased duration of occlusion.

Endothelium is the source of the dilating substances, which is evident from the fact that the removal of the endothelium from the femoral vessels by saponin-treatment is accompanied by about a 5-fold decrease in the degree of RH, and also by a similar decrease in the relaxation reaction of the vascular preparation, expressed as a less marked decrease of its tension when it received blood after a 2-min occlusion (1.01 ± 0.044 mN/mg vs 0.23 ± 0.033 mN/mg) (fig.). Similar changes in the degree of hyperemia and in the reaction of the vascular test segment were noted after acetylcholine administration under conditions of endothelium denudation.

Analysis of possible stimulants, capable of inducing synthesis and release of relaxation factor by the endothelium in response to vascular occlusion, suggested that a drop in intravascular pressure may be such a stimulant. The close relationship between the degree of post-occlusional hyperemia and the level of mean intravascular pressure, which exists under conditions of arterial, venous and combined vascular occlusion, adds support to this notion (table). In addition, if the rapid fall of intravascular pressure following combined occlusion of femoral artery and vein was prevented by connecting the distal part of the vessel to a bottle containing saline, suspended 1.3 m above the artery, there was a significant decrease in the degree of RH and of the relaxation-reaction of the femoral artery segment (fig.). The latter observation seems to indicate that along with inhibition of the hyperemic reaction, the above effect significantly decreases the supply of dilators to the blood.

One disadvantage of this experimental procedure is that it might expose intact vessels to the effect of hypoxia, which may play a certain role in RH development[10]. It has been observed that hypoxia can induce stimulation of EDRF release[11,12]. However, the sharp decrease of the release of vasodilators in the zone studied, and the observed attenuation of RH caused by high occlusion-induced intravascular pressure, seem to indicate that mechanisms other than hypoxia are involved. In addition, the experimental procedure used here provides for the maintenance of relatively high intravascular pressure, which is considered as a factor which inhibits both EDRF release and endothelium-dependent relaxation reactions[13,14]. Also well known is the inhibition of such reactions in hypertensive animals[15].

Reactive hyperemia (RH) and mean pressure in the femoral vascular bed of the dog after arterial, venous and combined occlusion of varying duration

	Duration of occlusion 30 s RH (%)	Mean pressure (mm Hg)	60 s RH (%)	Mean pressure (mm Hg)	120 s RH (%)	Mean pressure (mm Hg)
Arteries	+ 67 ± 9.2	4.6 ± 0.6	+ 83 ± 9.9	4.7 ± 0.7	+ 118 ± 6.8	4.9 ± 0.3
Veins	+ 21 ± 3.9	62 ± 6.3	+ 35 ± 11	68 ± 5	+ 43 ± 8.7	74 ± 6.9
Combined	+ 45 ± 3.9	39 ± 3.1	+ 70 ± 2	47 ± 4.5	+ 100 ± 7.7	53 ± 4.1

The decrease of deformability of glutaraldehyde-pretreated endothelial cells has been used by some authors as evidence for the capacity of endothelium to respond to mechanical stimulation[9]. In our experiments, glutaraldehyde pretreatment of the vascular bed significantly decreased the levels of both RH and of the relaxation of the test segment of femoral artery (fig.), i.e. the effect was accompanied by substantial inhibition of the release of vasodilators into the blood. However, the response to acetylcholine against the background of dimerized glutaraldehyde remained unchanged. In control experiments, the shift of the response to acetylcholine in the bed of the femoral artery was $+ 164 \pm 10.0\%$ of initial blood flow level, compared with $+ 122.0 \pm 12.2\%$ following glutaraldehyde pretreatment. It may be assumed therefore that the response to chemical stimuli was not affected by the pretreatment.

In conclusion, RH is largely a result of the release of biologically active vasodilators by the endothelial cells. The stimulus which initiates the occlusion-related synthesis and release of such vasodilators by the endothelium has been shown to be the fall of intravascular pressure. The capacity of endothelium to respond with the release of dilator factor as a flow-dependent vascular reaction is also well known[16,17]. Undoubtedly, this does occur when the blood flow is restored after occlusion. However, the fact that the acceleration of blood flow does not depend on the duration of occlusion, whereas the degree of hyperemia is strictly dependent on the latter, may be used as an argument against such a mechanism for the initiation of endothelium secretory activity and the subsequent development of RH.

In the development of vascular reactions and of RH in particular, a special role has been attributed to adenosine[10]. Apart from the direct effect of adenosine on smooth muscle cells, the possible involvement of EDRF in the vasodilatory effect of adenosine should not be excluded. Such a possibility is supported by data on the participation of the endothelium in the adenosine effect[18,19].

The data presented testify to the involvement of endothelium in the development of RH, which can be achieved as a result of various factors which stimulate the synthetic activity of the endothelium.

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0014-4754/91/080828-03$1.50 + 0.20/0
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Effect of vasoactive amines on Weibel-Palade bodies in capillary endothelial cells

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Received 17 April 1990; accepted 4 February 1991

Abstract. The presence and distribution of Weibel-Palade bodies in stomach and colonic mucosal microvessels after the administration of vasoactive amines (serotonin and histamine), the serotonin depletor reserpine, and the von Willebrand factor secretagogue thrombin, was studied by transmission electron microscopy. These agents elevated the number of Weibel-Palade bodies in all microvascular endothelial cells and especially in capillaries. It is concluded that vasoactive amines enhance the synthesis and secretion of large von Willebrand protein multimers by endothelial cells.
Key words. Capillary; endothelial cell; mucosa; Weibel-Palade body; von Willebrand factor; electron microscopy; rat.