SELECTIVE BLOCKING OF ARTERIAL SENSITIVITY TO BLOOD FLOW RATE BY GLUTARALDEHYDE

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Investigations in recent years have shown that the lumen of the main arteries in mammals depends on the flow rate of blood along it [2, 3]. This property of arteries is due to the ability of their wall to respond to changes in the longitudinal shear force [4]. As Smiesko et al. have shown [8], the element of the arterial wall that is sensitive to shear force is the endothelium. Mechanical removal of the endothelium from a segment of the canine femoral artery causes that segment to lose its sensitivity to flow rate, whereas a segment of artery separated by a distance of 25-30 mm from the area denuded of its endothelium remains sensitive.

Sensitivity of arteries to shear force is evidently determined by deformation of the endothelial cells, the degree of which, other conditions being the same, must depend on the ridigity of these cells. In principle, therefore, it ought to be possible to block the sensitivity of arteries to the blood flow rate by making their endothelial cells less deformable. For this purpose an attempt may be made to utilize the analogy with the action of glutaraldehyde on erythrocytes. We know that treatment of erythrocytes with glutaraldehyde considerably reduces their deformability [6]. If glutaraldehyde also reduces the deformability of endothelial cells, the increase in shear force caused by an increase in flow rate in these cells ought to induce much less deformation of them. This would lead to the reduction or abolition of the stimulus inducing relaxation of the arterial smooth muscles. However, the endothelium exerts on smooth muscles the action not only of a mechanical factor (shear force), but also that of certain vasoactive endogenous substances such as acetylcholine, histamine, bradykinin, and ATP [5]. It is perfectly possible that an agent increasing the rigidity of endothelial cells would damage the chemoreceptors of the endothelium and might even have some action on the contractile system of the smooth muscles.

The aim of this investigation was to discover whether administration of glutaraldehyde could lead to loss of the sensitivity of arteries to blood flow rate and, if so, to establish the strength of the reactions to vasodilator substances mediated under these circumstances by the endothelium.

EXPERIMENTAL METHODS

The diameter of the femoral (17 animals) and common carotid (11 animals) arteries during changes in the blood flow rate along them and in response to intra-arterial injection of acetylcholine before and after exposure to glutaraldehyde, was measured in 28 cats anesthetized with urethane (0.6 g/kg) and chloralose (0.04 g/kg body weight). The measurements were made while the arteries were being perfused at a stabilized pressure. The scheme and method of the experiment were described in detail previously [1].

In each experiment, 30-40 min after a transducer had been placed on the artery to measure its diameter, dilatation of the artery induced, first, by an increase in the flow rate of blood along it from 10-15 to 35-45 ml/min and, second, by intra-arterial injection of acetylcholine chloride (0.1-10 μg), was recorded. Acetylcholine was injected in 0.01 ml of Ringer's solution through a 3-way tube, located immediately before the inlet of the perfusion pump.

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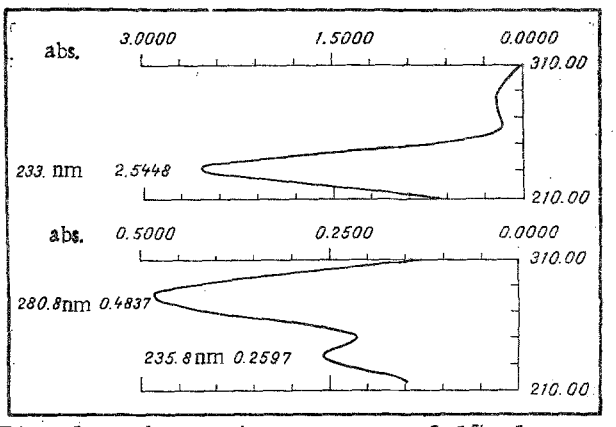

Fig. 1. Absorption spectra of 1% glutaraldehyde solution before and 15 min after addition of 10 µl of 5% NaOH solution to 10 ml of glutaraldehyde solution. Peak at wavelength of 280 nm corresponds to monomer, that at 235 nm to dimer of glutaraldehyde.

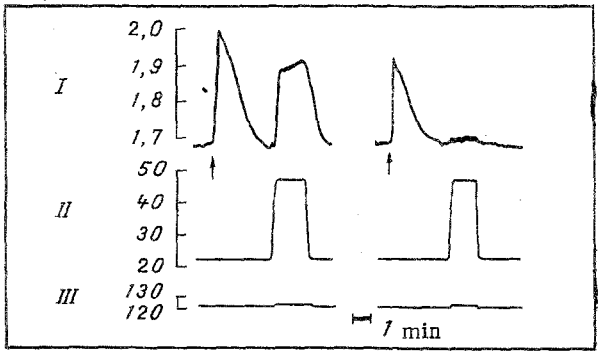

Fig. 2. Reactions of femoral artery to intra-arterial injection of 1 µg acetylcholine (time of injection indicated by arrow) and to increase in volume velocity of blood flow before (on left) and after action of 0.025% solution of glutaraldehyde dimer to the endothelium (on right). I) Diameter of artery (in mm); II) blood flow rate (in ml/min); III) perfusion pressure (in mm Hg).

The artery was then rinsed for 30 sec with 5% glucose solution to remove blood and filled for 15-30 sec with 0.01-0.05% glutaraldehyde solution. All these procedures were carried out with the pressure in the artery stabilized at 100 or 120 mm Hg. After 10-15 min the diameter of the artery was again measured while the rate of the blood flow through it was increased, and during intra-arterial injection of acetylcholine. Glutaraldehyde (25% solution, from Sigma, USA) was diluted to the required concentration in Ringer's solution of the following composition: NaCl 9 g/liter, KCl 200 mg/liter, $CaCl_2$ 200 mg/liter, NaH_2PO_4 12 mg/liter, and Na_2HPO_4 56.8 mg/liter. In five experiments (3 on the femoral and 2 on the carotid artery) glutaraldehyde monomer was used. In the remaining experiments the dimer of the aldehyde was used. Dimerization was carried out by adding 10 µl of 5% NaOH solution to 10 ml of a freshly prepared 1% solution of glutaraldehyde, after which the resulting solution was diluted to the required concentration of aldehyde. An alkaline medium sharply increases the rate of dimerization of glutaraldehyde [7], and 15 min after the addition of alkali a 1% solution of aldehyde already consists virtually entirely of the dimer. The degree of dimerization of the aldehyde was judged from absorption spectra in ultraviolet light, obtained on a "Beckman DU-8" spectrophotometer. The glutaraldehyde monomer has a maximum of absorption at 280 nm, the dimer at 235 nm. Absorption spectra of the glutaraldehyde monomer and dimer used in the experiments (1% solution) are illustrated in Fig. 1. The pH of the aldehyde solution (0.01-0.05%) injected into the artery was 7.4-7.5.

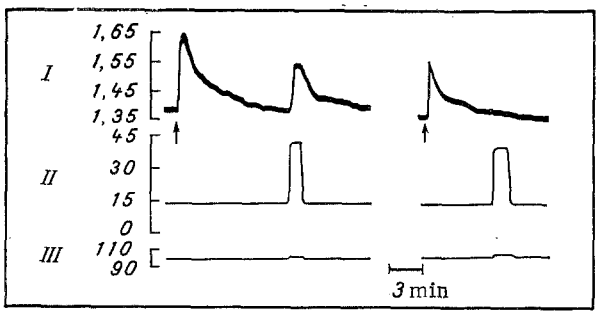

Fig. 3. Reactions of carotid artery to intra-arterial injection of 1 μg acetylcholine and to increase in volume velocity of blood flow before and after action of 0.025% solution of glutaraldehyde dimer on endothelium. Legend as to Fig. 2.

RESULTS

Experiments in which glutaraldehyde monomer was used showed that the action of this substance on the endothelium of the arteries causes disappearance (or considerable weakening) of the dilator responses both to an increase in the blood flow rate and to intra-arterial injection of acetylcholine. Smooth muscle tone is preserved under these circumstances, as shown by the marked dilatation of the arteries in response to application of a 2% solution of no-shpa [a vasodilator of plant origin used in Russian folk medicine] to the adventitia. A marked constrictor response of the arteries to intra-arterial injection of noradrenalin (10 μg) also was preserved.

The action of glutaraldehyde dimer (0.01-0.025% solution, 30 sec; 0.03-0.05% solution, 20 sec) on the endothelium of the arteries caused loss of sensitivity to the blood flow rate, whereas the dilator response to acetylcholine still remained. Reactions of the femoral and carotid arteries respectively to an increase in blood flow rate and to injection of acetylcholine before and after the action of glutaraldehyde on the endothelium (0.025% solution, 30 sec) are compared in Figs. 2 and 3. Treatment of the endothelium with glutaraldehyde dimer led to disappearance of the dilator response of the arteries to an increase in blood flow rate, without producing any significant change in the intensity of the response to acetylcholine. Loss of sensitivity of the arteries to the blood flow rate in response to administration of glutaraldehyde dimer was accompanied in these experiments by a decrease in dilatation of the arteries in response to acetylcholine by 25.6 ± 5.9% (M ± m); in all the experiments, when the response to an increase in blood flow rate was abolished, dilatation induced by acetylcholine still remained.

Treatment of arteries with glutaraldehyde dimer thus leads to loss of their sensitivity to the blood flow rate without abolishing or, indeed, causing any significant change in the endothelium-dependent dilatation induced by acetylcholine. If it can be shown that glutaraldehyde dimer does not abolish dilatation of large arteries induced by other substances acting through the endothelium, and that it acts equally selectively on arterial microvessels, it will be possible to use this substance to determine the role of arterial sensitivity to blood flow rate in vascular responses of organs.

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