REMOVAL OF ENDOTHELIUM ABOLISHES STRESS-INDUCED DEPRESSION OF AORTIC ADRENOREACTIVITY

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Blood vessels are a very important target for sympathetic nervous influences. Besides with smooth muscles, catecholamines also interact with another type of vascular cells, namely endothelial cells. We know that the endothelium not only is responsible for relaxation of vascular smooth muscles induced by acetylcholine, bradykinin, thrombin, ATP, ADP, and several other endogenous and exogenous vasodilators [8], but it also modulates vasoconstrictor reactions to many agonists, including adrenergic [9]. The writer showed previously that experimental myocardial infarction and emotional-painful stress (EPS) lead to considerable potentiation of endothelium-dependent relaxation of smooth muscle of the isolated rat aorta, which is accompanied by a sharp fall of blood pressure [3]. It is also known that severe stress significantly depresses the adrenoreactivity of isolated blood vessels [2]. This potentiation of endothelium-dependent relaxation, combined with depression of adrenoreactivity, may play a role in the reduction of vascular tone, the lowering of blood pressure, and increased storage of blood in the venous bed, and it may ultimately enable the development of states resembling collapse and cardiogenic shock accompanying myocardial infarction in man. To understand the mechanisms of these disturbances, we decided to study the role of potentiation of the inhibitory action of the endothelium on smooth muscle in stress-induced changes in vascular adrenoreactivity. In the investigation we described below the effect of an experimental myocardial infarct and of EPS was studied on sensitivity of the adrenoreceptors of the isolated intact and de-endothelized rat aorta to noradrenalin (NA).

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 220-250 g. An experimental myocardial infarct was produced by the method in [12] by ligation of the descending branch of the left coronary artery. Intact rats and rats undergoing a mock operation served as the control. The mock operation consisted of thoracotomy and the insertion of a ligature beneath the artery, but without tying it. EPS was produced in the form of a so-called "anxiety" neurosis by the method in [6] for a period of 6 h. The study of the dynamics of the effect of a myocardial infarct and of EPS on contractility of vascular smooth muscle showed previously that maximal disturbances of this function develop 3 h after the infarct and 2 h after EPS [3]. In the present investigation the animals were therefore killed by decapitation at these times after the experimental procedures. Immediately after decapitation of the animals the thoracic aorta was removed, stripped of its adipose and connective tissue, and a ring preparation 3 mm wide was placed in a thermostatically controlled (37°C) working chamber, filled with continuously oxygenated (95% O_2 + 5% CO_2) Krebs' solution. The initial stretching load was 1200 mg. The preparation was kept under these conditions for 1 h before the beginning of the experiment (stabilization period). Contractions of the intact and de-endothelized preparations were recorded simultaneously on a two-channel recorder ("Ugo Basile," Italy). Integrity of the endothelium was tested by measuring relaxation of the preparation under the influence of a standard dose of acetylcholine (10^{-5} M), against the background of contraction induced by NA ($5 \cdot 10^{-7}$ M). The de-endothelized preparations did not relax in response to acetylcholine. The endothelium was removed by insertion of a special catheter into the lumen of the vessel. Contraction of the preparation was

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TABLE 1. Effect of Experimental Myocardial Infarction, EPS, and Mock Operation on Adrenoreactivity of Aortic Smooth Muscle from Rat with Intact and De-endothelized Aorta ($M \pm m$)

Group of animals	Preparation	Number of animals	$K_д$
Control	Intact	$n=14$	2,5±0,2
	De-endothelized	$n=14$	1,5±0,1
Exptl. myocardial infarct	Intact	$n=14$	3,3±0,3*
	De-endothelized	$n=13$	0,9±0,09***
EPS	Intact	$n=18$	3,9±0,5**
	De-endothelized	$n=8$	0,7±0,07***
Mock operation	Intact	$n=8$	3,0±0,3
	De-endothelized	$n=8$	1,0±0,1***

Legend. $*p < 0.05$; $**p < 0.02$; $***p < 0.01$.

induced by NA (10^{-8}-10^{-6} M). Adrenoreactivity of the aortic smooth muscle was evaluated as the reciprocal of the apparent dissociation constant (K_d) of the NA – adrenoreceptor complex [1], calculated by the method of least squares. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Table 1 shows that an experimental myocardial infarct and EPS led to a significant increase in K_d, i.e., to a decrease in the adrenoreactivity of smooth muscle of the aorta with intact endothelium. For animals undergoing the mock operation this depression of adrenoreactivity was not significant, but was evidence in support of the stress-induced nature of this effect.

De-endothelization caused an increase of adrenoreactivity of the preparations in all experimental groups, in agreement with results obtained by other workers [9]. However, the most interesting fact is that this increase in adrenoreactivity differed for the different groups of animals: it was much greater after myocardial infarction, EPS, and the mock operation than in the control. Changes in K_d (ΔK_d) due to de-endothelization of the aorta for all groups tested are shown in Fig. 1. Clearly on the whole the difference in adrenoreactivity between the intact and de-endothelized preparation corresponded to the severity of the stress: the greatest value of ΔK_d was found in animals subjected to EPS, the lowest value after the mock operation. As a result, evaluation of adrenoreactivity of the de-endothelized preparations showed that it was not depressed after stress, as in the intact preparations but, on the contrary, it was increased compared with the control.

It can be concluded from these results first, that stress leads to depression of adrenoreactivity of the aorta and de-endothelization in all the experimental series led to an increase of adrenoreactivity above the normal level. This means that the effect on the endothelium significantly limits adrenoreactivity. Second, de-endothelization of the aorta in animals exposed to stress led to a significantly greater increase in adrenoreactivity than in the control; consequently, the inhibitory effect of the endothelium in these stress situations was enhanced. This indicates the need for discussion of the nature of the inhibitory effect of the endothelium on vascular adrenoreactivity and on the mechanism by which this effect is increased in stress situations. One possible mechanism of enhancement of the response of smooth muscle to NA after de-endothelization is that α_2-adrenoreceptors located on the endothelium mediate the release of endothelium-derived relaxing factor adrenoreactivity EDRF) [7]. This means that activation of α_2-adrenoreceptors leads to the lowering of vascular tone, i.e., under the influence of NA, a nonselective agonist of α-adrenoreceptors, they are brought into antagonistic relations with α_1-adrenoreceptors located on smooth muscle, and responsible for its contraction [15].

It has also been shown that during immobilization stress there is not only an increase in sensitivity in the peripheral organs, but also a sharp increase in the number of α_2-adrenoreceptors, accompanied by relative stability of the density of α_1-adrenoreceptors [13]. The possibility cannot be ruled out that this effect is linked with the predominant induction by high plasma catecholamine concentrations, of α_2-adrenoreceptor formation at the genetic level [5].

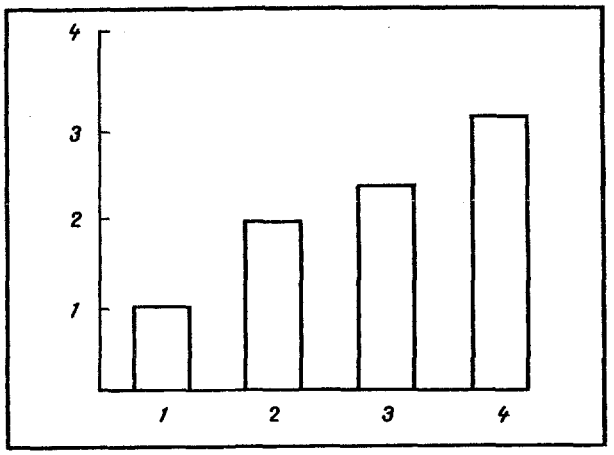

Fig. 1. Effect of stress on change in adrenoreactivity (in $K \cdot 10^{-8}$ M) induced by de-endothelization. Abscissa: 1) control, 2) mock operation, 3) experimental myocardial infarction, 4) EPS.

It can thus be tentatively suggested that potentiation of the inhibitory effect of the endothelium, which we found, is due to a stress-induced increase in α_2-adrenoreactivity, mediating EDRF release and relaxation of the preparation, and its predominance over α_1-adrenoreactivity, mediating contractile responses. De-endothelization abolishes the inhibitory effect of the increased α_2-adrenoreactivity and reveals a small increase in α_1-adrenoreactivity. Consequently, the depression of adrenoreactivity of the vessels observed in stress is due, not to desensitization of the α_1-adrenoreceptors, which are comparatively resistant to it [11], but to strengthening of the inhibitory effect of the α_2-adrenoreceptors. Since selective activation of the endothelial α_2-adrenoreceptors can abolish the spastic effect of α_1-adrenoreceptors, these results raise the question of identifying selective α_2-agonists capable of abolishing peripheral vascular spasm.

Under normal conditions intact endothelium plays an important role in the prevention of excessive vasoconstriction and vascular spasm [14]. Potentiation of the inhibitory action of endothelium on contraction of the vascular wall, revealed in the present experiments as excessive activation of endothelial α_2-adrenoreceptors, may, together with other factors, play a role in the development of states of collapse and cardiogenic shock, in association with the severe stress that often accompanies myocardial infarction in man. Meanwhile, in a disturbance of integrity of the endothelium observed in atherosclerosis, hypertension, and several other pathological states, weakening of the inhibitory effect of the endothelium may facilitate the development of vascular spasm [10, 14], which has in fact been proved for these states.

The optimal state of the endothelium is therefore one of the local regulatory mechanisms which plays a role both in an increase and a decrease of vascular tone.

LITERATURE CITED

1. B. N. Manukhin, Physiology of Adrenoreceptors [in Russian], Moscow (1968).
2. E. B. Manukhina, Byull. Éksp. Biol. Med., No. 2, 5 (1983).
3. F. Z. Meerson, E. B. Manukhina, A. V. Lapshin, and E. E. Ustinova, Byull. Éksp. Biol. Med., No. 7, 21 (1989).
4. É. A. Shirinyan, O. M. Martirosyan, T. K. Atayan, and S. A. Arutyunyan, Ontogenetic and Genetic-Evolutionary Aspects of Neuroendocrine Regulation of Stress [in Russian], Novosibirsk (1988), pp. 197-198.
5. T. Brand, S. Rohmann, H. S. Sharma, and W. Schaper, J. Molec. Cell. Cardiol., **21**, Suppl. III, 3 (1989).
6. O. Desiderato, J. R. MacKinnon, and H. J. Hissom, J. Comp. Physiol., **87**, 208 (1974).
7. C. Egleme, T. Godfraind, and R. C. Miller, Brit. J. Pharmacol., **81**, 16 (1984).
8. R. F. Firchgott, Circulat. Res., **53**, 557 (1983).
9. T. Godfraind, C. Egleme, and I. H. Alosachie, Clin. Sci., **68**, Suppl. 10, 65S (1985).
10. R. Gryglewski, R. M. Botting, and J. R. Vane, Hypertension, **12**, 530 (1988).
11. C. A. Hamilton and J. L. Reid, Eur. J. Pharmacol., **152**, 179 (1988).
12. H. Selye, E. Bajusz, S. Grasso, and P. Mendell, Angiology, **11**, 398 (1960).

13. D. C. U'Prichard and R. Kvetnansky, Catecholamines and Stress: Recent Advances, New York (1980), pp. 299-308.
14. P. M. Vanhoutte, J. Molec. Cell. Cardiol., **16**, 679 (1986).
15. P. A. van Zwieten, Arzneimittel-Forsch., **35**, No. 11, 1904 (1985).

ATHEROGENIC LIPOPROTEINS FOUND IN THE BLOOD OF PATIENTS WITH CORONARY ATHEROSCLEROSIS ARE DESIALYLATED LOW-DENSITY LIPOPROTEINS

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The writers showed recently that low-density lipoproteins (LDL) isolated from the blood of most patients with coronary heart disease (CHD) and with angiographically verified coronary atherosclerosis verified differ from the LDL obtained from healthy human blood in their ability to induce intracellular cholesterol accumulation in a culture of smooth-muscle cells from the unaffected intima of the human aorta. It was suggested that LDL from CHD patients are modified lipoproteins, differing in their chemical composition from LDL isolated from normal individuals. The investigation described below established a significant decrease in the sialic acid content of LDL isolated from the blood of CHD patients with coronary atherosclerosis compared with LDL from normal individuals. The desialylation of native LDL obtained from normal subjects by neuraminidase makes these lipoproteins atherogenic, i.e., capable of causing an increase in the intracellular cholesterol concentration in cultures of smooth-muscled cells from the intima of the unaffected human aorta.

Sialic acid is known to be a component of native LDL [13] and to perform an important function in their metabolism [1, 4, 5].

EXPERIMENTAL METHOD

Experiments were carried out on blood from 22 healthy subjects with no clinical features of CHD of classes II-IV according to the Canadian classification [2] and with angiographically documented atherosclerosis of 1 to 3 coronary arteries [8]. Blood was taken from the cubital vein of the fasting subject before breakfast. LDL (d = 1.030-1.050 g/cm^3) were obtained by ultracentrifugation [3]. Protein B was determined as described previously [3]. Lipids were extracted from the cells with a mixture of chloroform and methanol (1:2, v/v [6]), and then fractionated by thin-layer chromatography on silica gel 60 plates (Merck, West Germany). The lipid content was determined by scanning densitometry [10]. The sialic acid level in LDL was measured by the method [14]. Total protein was determined by the method in [9]. LDL were treated with neuraminidase (Sigma, USA) as described in [12]. Intimal smooth-muscle cells (SMC) were isolated from the aorta of persons dying suddenly from myocardial infarction between the ages of 40 and 51 years, 1-3 h after death. The cells were isolated by dispersion of the tissue with elastase and collagenase ("Sigma") and were cultured as described previously [10]. The atherogenecity of the LDL was assessed by accumulation of cholesterol in cultured SMC compared with control cells, as described previously [11].

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