EFFECT OF HYPERCHOLESTEROLEMIA ON ELECTRICAL AND CONTRACTILE PROPERTIES OF THE VASCULAR WALL

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Atherosclerosis has a significant influence on vascular reactivity to regulatory factors, and may lead to the appearance of paradoxical vascular responses [3, 5, 8, 10]. However, it is not known whether this is the result of structural changes in the vascular wall (thickening of the intima, the appearance of large numbers of collagen fibers) or reflects changes in the physiological properties of the smooth-muscle cells (SMC).

In the investigation described below changes in the contractile and electrical properties of vascular smooth muscles and their reactivity to acetylcholine (ACh) were studied during alimentary hypercholesterolemia (HChE), of varied duration (2 and 4 months).

EXPERIMENTAL METHOD

Circular strips from the arch of the rabbit aorta were used as the test object. The preparation was perfused with standard Krebs' solution [2], oxygenated with a mixture of 95% O_2 and 5% CO_2. The temperature of the solution was maintained at 36°C. Phenylephrine (PhE, 10^{-5} M) was used to increase the initially low tone of the vascular strip. Electrical activity of SMC was recorded by the "sucrose gap" method [1] and contractile activity of the strip was recorded at the same time by means of a 6MKhIS mechanical to electrical transducer. Changes in the degree of polarization of the SMC membrane were produced with a polarizing current. The potassium current was blocked by tetraethylammonium (TEA) in a concentration of 5 mM. HChE was induced by keeping the animals on an atherogenic diet (0.5 g cholesterol/kg body weight daily). The blood cholesterol level was determined in the initial state and after 2 and 4 months of cholesterol feeding; its mean values were 2.5 ± 0.4, 20 ± 0.8, and 30 ± 1.2 mmoles/liter, respectively. ACh and nitroglycerin (NG) were used in a concentration of 10^{-5}M.

EXPERIMENTAL RESULTS

The results indicate significant weakening of the dilator action of ACh on the vascular wall under the influence of HChE. This was manifested as a progressive decrease in ACh-induced relaxation of the strip, previously contracted by PhE, with an increase in the duration of HChE. Whereas in normal vessels, under the influence of ACh the strips relaxed by about 60% compared with the level of previous contraction in response to PhE, taken as 100%, after HChE for 2 and 4 months the degree of relaxation decreased to 40 and 20%, respectively; in some cases the response became biphasic in character, with the late development of a constrictor component of the response (Fig. 1).

The character of the electrical and contractile responses of vascular strips from the rabbits with HChE to the direct action of ACh (after mechanical removal of the endothelium) showed no significant change. Membrane depolarization was observed, combined with the development of tonic contraction. Meanwhile HChE significantly altered the character of the indirect effect of ACh through the endothelium. This was manifested as weakening of the dilator response, the appearance of a constrictor phase of the response, and inhibition of hyperpolarization induced by ACh. The vascular response to ACh is known to be integral in character and to reflect competition between its direct constrictor effect on SMC and

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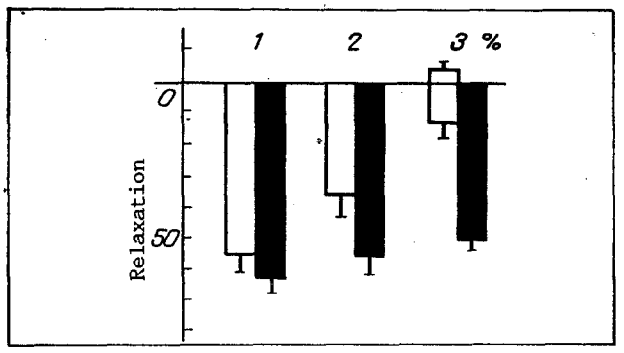

Fig. 1. Degree of relaxation of aortic SMC in response to ACh and PhE (in % of maximal contraction induced by PhE). Zero on ordinate corresponds to maximal tension of muscle before application of ACh; 1, 2, 3) normal state and after 2 and 4 months of HChE, respectively. Unshaded columns – ACh, black columns – PhE.

its indirect dilator effect through the endothelium [2, 10]. A very important property of the vascular endothelium is its ability to produce an endothelium-dependent relaxation factor (EDRF) in response to the action of agonists of different types of receptors with a tonic effect on smooth muscle (ACh, noradrenalin, histamine). This factor weakens the constrictor response or may even convert it into relaxation of SMC. The data showing weakening of the relaxing action of ACh during HChE can therefore be interpreted as the result either of a disturbance of endothelial function or a change in the sensitivity of SMC to EDRF.

To test the ability of SMC to relax under the influence of EDRF we used NG, which shares with EDRF a common final stage of action, realizing its effect through stimulation of guanylate cyclase and an increase in intracellular cGMP activity [4, 6, 7, 9]. Comparison of responses of the strips to ACh and NG during HChE thus enables the role of changes in disturbances of the functional properties of the vascular wall in the endothelium and in SMC to be compared.

The results of this comparison show that the dilator action of NG is weakened during the development of HChE by a much lesser degree than the effect of ACh. For instance, by the end of the 2nd month of HChE the response of the strip to NG was virtually unchanged; toward the end of the 4th month some weakening of the response was observed, but it was moderate in degree and did not exceed 20% of the normal response (Fig. 1). These data thus indicate that the principal factor, and the one which acts earliest, disturbing the normal response of the vascular wall to ACh during HChE is a change in the physiological activity of the endothelium; changes in reactivity of SMC themselves are less marked and arise in the later stages of HChE.

In an attempt to explain these facts it might be suggested that during HChE, cholesterol inserted into the phospholipid layer of the SMC membrane changes its electrical excitability, and in that way it modifies both the direct effects of ACh and its indirect effects through the endothelium. To test this hypothesis, electrophysiological properties of SMC of normal vessels and of vessels of rabbits with HChE were investigated.

The experiments showed that SMC of the normal rabbit aorta possess neither spontaneous nor evoked electrical activity. Application of a depolarizing current to SMC of the strip led to the appearance of a catelectrotonic potential, but this did not develop into an AP and, correspondingly, did not induce contraction of the strip (Fig. 2A, a-c, 1). Blocking the outward potassium current with 5 mM TEA caused a slight degree of depolarization and an increase in resistance of the membrane. However, even against this background, catelectric depolarization did not lead to AP generation or to the onset of contraction; only a local response to the depolarizing stimulus appeared (Fig. 2A, a-d, 2).

HChE significantly modified the physiological properties of the SMC membranes. By the end of the 4th month of HChE, for instance, with the same TEA concentration in the Krebs' solution, even weak depolarizing pulses of current caused the generation of lengthy APs, accompanied by contraction of the strip (Fig. 2B, a-c), although preparations not treated with TEA, like preparations of the normal aorta, did not exhibit evoked activity.

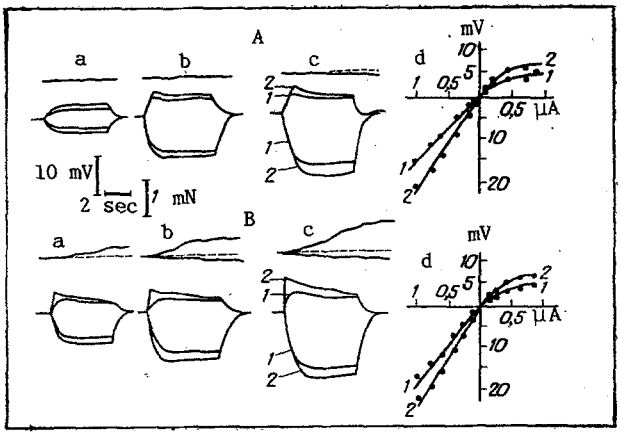

Fig. 2. Action of polarizing current on electrical and contractile activity of aortic strips before (a) and during HChE (B). A (a-c) and B (a-c) an- and catelectrotonic potentials before and during HChE. A, B, d) Current-voltage characteristics of SMC membrane; 1, 2) an- and catelectrotonic potentials before and during application of TEA.

HChE thus disturbs the function of the endothelium and increases the electrical excitability of the vascular SMC, and one cause of this may evidently be a change in the normal ratio between calcium and potassium conductance of the membrane. A combination of these factors ultimately leads to weakening of the endothelium-dependent relaxing action of ACh.

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