difficult problem is that of the mechanisms of adaptive (i.e., determined by changes in metabolism in skeletal muscles) vasodilator reactions. However, it can be postulated on the basis of the results that disturbance of vasodilator influences, due to α-adrenergic stimulation in effector tissues, and counteracting the direct α-adrenoreceptor constrictor effect on SMC of the vessel wall, may lead to systemic disturbance of vascular tone. Further investigation of adrenergic influences on vasodilator adaptive reactions, and also of Ca- and α-adrenergically dependent relations of SMC of the vessel wall with the endothelium and blood cells, may shed some light on the mechanisms of the hypotensive action of Ca in a high proportion of patients with hypertension [2, 7].

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ROLE OF THE ENDOTHELIUM IN CONTRACTILE RESPONSES OF VASCULAR SMOOTH MUSCLES WITH DIMINISHED OXYGENATION

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KEY WORDS: vascular smooth muscles; noradrenalin; endothelium; hypoxia.

Many new data have been obtained to broaden significantly our traditional views on the functional role of the vascular endothelium. Participation of the endothelium in relaxation of smooth muscles (SM) of arteries in response to injection of acetylcholine [6], histamine [10], ATP and ADP [4], bovine thromin and arachidonic acid [5], bradykinin [3], substance P [11], the calcium ionophore A 23187 [3], etc., has been established. Information on the role of the endothelium in the formation of vascular responses in hypoxic states of varied genesis is scanty and contradictory [2, 5].

The aim of this investigation was to study the role of the endothelium in the development of contractile reactions of vascular SM (VSM) when their oxygenation is diminished.

EXPERIMENTAL METHOD

Experiments were carried out on isolated preparations of the rat thoracic aorta. The endothelial layer was removed mechanically, by gently rolling the vascular preparation on filter paper [6]. This is one of the gentlest ways of removing endothelium, for it causes no damage to the muscular layer and the inner elastic membrane is preserved [5]. Complete removal of the endothelial layer was tested by noting the absence of relaxation of the preactivated aortic SM on application of acetylcholine (from Merck, West Germany) in a concentration of 10^{-7} M. Segments weighing 1-2 mg were placed in a constant temperature chamber (36-37°C), perfused

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with Krebs' bicarbonate solution, and subjected to passive stretching by a force of 10^{-2}-1.5•10^{-2} N. Contractile activity of SM was recorded under isometric conditions by means of a mechanotron transducer. Changes in oxygenation of the testing solution were brought about by saturating it with a gas mixture containing up to 1% O_2 in nitrogen. The pO_2 of the solution close to the surface of the preparation was monitored polarographically. The effect of lowering the oxygenation of the perfusion fluid from 147 to 30-40 mm Hg on contractile responses of the intact and de-endothelized segments, evoked by L-noradrenalin (from Serva, West Germany), in a concentration of 10^{-6} M was investigated. In some experiments the preparations were treated with the Ca^{++} antagonist verapamil, in a concentration of $5•10^{-6}$ M (from LEK, Yugoslavia), and also with calcium-free solution with the addition of the Ca^{++} chelating agent EGTA, in a concentration of 1 mM (from Serva, West Germany).

EXPERIMENTAL RESULTS

Noradrenalin in a concentration of 10^{-6} M caused the development of persistent tonic contractions of SM of intact segments of the rat thoracic aorta with an amplitude which averaged 2.99 ± 0.7•10^{-3} M/mg wet weight of the preparation (Fig. 1a). A decrease in oxygenation of the testing solution, containing noradrenalin in the same concentration, to 30-40 mm Hg led to a biphasic change in amplitude of the tonic contraction of VSM. During the first 1-2 min an increase in amplitude of contraction of SM was observed (on average by more than 20%), but later this was followed by their definite relaxation, which amounted to 60-70%.

After removal of the endothelial layer, as shown by absence of relaxation of the aortic SM in response to application of acetylcholine (Fig. 2) the preparations were kept for about 30 min. Under these circumstances no regular differences in the time course and amplitude of contraction induced by noradrenalin in a concentration of $1•10^{-6}$ M could be determined. In de-endothelized segments the mean amplitude of tonic contractions was 3.06 ± 0.8•10^{-3} N/kg wet weight of preparation. However, the character of the response to a fall of pO_2 of the incubation solution to 30-40 mm Hg differed significantly. In de-endothelized segments as a rule the initial transient rise of amplitude of tension of SM was not reproduced (Fig. 1b). The relaxing effect of restriction of oxygenation was preserved in this case, it developed at about the same periods of time, but it was more marked (up to 80% of the initial amplitude of contraction when noradrenalin was used).

The results indicate that the endothelium of segments of the rat thoracic aorta evidently does not participate in the formation of the relaxing effect of a fall in oxygenation of the medium, but it plays a significant role in the development of the phase of an increase in amplitude of tension of the previously activated SM.

Previously [5] only an increase in amplitude of contraction of the noradrenalin-activated segments of dogs' arteries was recorded during temporary anoxia, and its magnitude was reduced after removal of the endothelial layer. On this basis, the authors cited postulated that the endothelium may make a definite contribution to contractile responses of VSM to anoxia. In another investigation [2], on the other hand, during a fall of oxygenation only endothelium-dependent relaxation of segments of the caudal artery of rats and femoral artery of dogs, previously contracted by noradrenalin, was found. Since indomethacin, as an inhibitor of cyclooxygenase, weakens the dilator effect (by about 60-80%), the authors cited linked relaxation of VSM with the effect of prostacycline, synthesized in the endothelial layer.

It is difficult to say at present whether the responses of VSM to a change in their oxygenation, discovered by ourselves and by other workers, and dependent on endothelium, are a regular feature characteristic of the whole vascular bed, or whether regional heterogeneity, connected with differences in the morphological organization of the vessels, their caliber, and metabolism exists.

To determine the precise nature of the increase in amplitude of contraction of aortic SM during lowering of their oxygenation, discovered in this investigation, experiments were carried out with blocking of the Ca current, and also with total removal of Ca^{++} from the testing solution combined with the addition of EGTA (1 mM).

During incubation of intact vascular preparations, previously activated with noradrenalin, in Krebs' solution containing verapamil ($5•10^{-6}$ M), no definite changes were found in the response of aortic SM to a fall in pO_2 (Fig. 3a). In the presence of the blocker, a transient increase in the amplitude of contraction (by more than 20%) also was observed during the first minutes of hypoxia, and this was followed by significant relaxation of SM, although rather less marked than in the control.

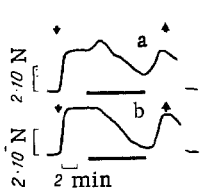

Fig. 1

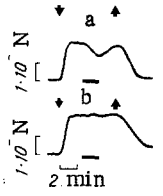

Fig. 2

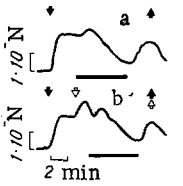

Fig. 3

Fig. 1. Effect of diminishing oxygenation of perfusion fluid on noradrenalin-induced contractions of SM of intact (*a*) and de-endothelized (b) segments of rat thoracic aorta. Arrows indicate beginning and end of action of L-noradrenalin (10^{-6} M). Line below trace shows period of lowering of pO_2 (30-40 mm Hg). Breaks in traces correspond to period of 6 min.

Fig. 2. Effect of acetylcholine on noradrenalin-induced contractions of SM of intact (*a*) and de-endothelized (b) segments of rat thoracic aorta. Line below curve shows period of action of acetylcholine (10^{-7} M). Remainder of legend as to Fig. 1.

Fig. 3. Effect of blockade of Ca channels by verapamil (*a*) and of complete removal of Ca^{++} from perfusion fluid (b) on noradrenalin-induced contractions of SM of rat thoracic aorta during lowering of their oxygenation. Empty arrows indicate beginning and end of perfusion with calcium-free solution with addition of 1 mM EGTA. Remainder of legend as to Fig. 1.

Similar data also were obtained by the use of an incubation solution from which Ca^{++} was completely absent (Fig. 3b). Under these circumstances, perfusion of VSM, contracted beforehand with noradrenalin, with calcium-free solution for 1 min itself led to yet another significant (by more than 70%) increase in the amplitude of the tonic component of contraction of SM. This was evidently due to release of the Ca^{++} fraction sequestrated on the inner surface of the plasma membrane, as a result of disturbance of equilibrium between the concentrations of extracellular and intracellular calcium.

It can be postulated on the basis of these experiments that the increase in amplitude of contraction of aortic SM in response to lowering of their oxygenation is not due to the inflow of extracellular calcium. Its formation is evidently connected with release of ionized Ca^{++} from intracellular binding sites: mitochondria, sarcoplasmic reticulum, and plasma membrane.

Analysis of the possible factors inducing an endothelium-dependent transient increase in amplitude of contraction of VSM in response to hypoxia suggested that one of the strongest candidates for this role is evidently cGMP. Investigations of the cellular function of cyclic nucleotides have shown that their influence on the cardiovascular system is reciprocal. According to these views cGMP, unlike cAMP, is a mediator of the effect of vasoactive factors, capable of inducing a constrictor effect [1]. It has been shown experimentally that after removal of the endothelial layer the basal cGMP level may be lowered: in the rat thoracic aorta, for example, from 0.8 to 0.5 pmole/mg protein [8] and in the rabbit aorta from 0.20 to 0.06 pmole/mg [7].

In the light of this fact it is perfectly reasonable to suggest the possible existence of a relationship of cause and effect between this decrease in the basal cGMP level after de-endothelization, its vasoconstrictor action, and the absence of the transient increase in amplitude of contraction of aortic segments denuded of their endothelium, in response to hypoxia, which was found in the present investigation. Confirmation of this hypothesis requires further study.

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EFFECT OF GUANETHIDINE DESYMPATHIZATION ON CARDIAC FUNCTION IN YOUNG RATS

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Guanethidine inhibits release of noradrenalin from terminals of adrenergic neurons [10-12] and thereby disturbs equilibrium in the catecholamine concentrations in the tissues of many organs [4]. It is accordingly interesting to study functional changes in the sympathetic regulatory apparatus of the heart in the growing animal before puberty.

In the present investigation the effect of chemical desympathization by guanethidine on the functional state of the sympathico-adrenal system and activity of the developing heart was studied.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats aged 3 weeks. Starting from the 1st day of life, the animals were given guanethidine (Isobarin, from Pliva, Yugoslavia) by daily subcutaneous injection in a dose of 30 mg/kg, which was sufficient to block sympathetic neurons completely [3]. Physiological saline was injected into rats of the control group. The rats were used in the acute experiments on the 21st day of life, and were anesthetized with urethane (600 mg/kg). To determine the chronotropic sensitivity of the heart, solutions of adrenalin and noradrenalin in concentrations of 10^{-6}-10^{-5} M were injected into the jugular vein through a vinyl chloride catheter, and the ECG was then recorded. The heart rate was calculated by measurement of 10 R-R intervals. The sensitivity of the heart was judged by the maximal changes in heart rate (HR) from its initial values before injection of the drugs. To determine the state of the catecholamines in the blood and tissues the rats were decapitated. Blood was collected in small jars, into each of which 5 ml of an 8% solution of perchloric acid had first been poured. The heart and both adrenals were removed, dried with filter paper, and used in the fresh state for investigation of their catecholamine content. The concentration of adrenalin and noradrenalin was determined fluorometrically [5] and expressed in nanograms/ml of blood and in micrograms/gram wet weight of tissue in the heart and adrenals.

EXPERIMENTAL RESULTS

HR in 3-week-old rats chemically desympathized with guanethidine was higher by 21.4 beats/min ($P < 0.05$), than in the control (Table 1). Relative tachycardia has been observed by other workers also in older desympathized rats [7, 9]. However, this fact has not been explained. HR in the growing organism depends on relations between the autonomic nerves. High functional activity of the sympathetic nervous system in the growing organism gives rise to high values of HR [1, 8]. In this case, in desympathized 3-week-old rats the influence of the sympathetic nerves on the heart was greatly weakened, and HR, on the contrary, was considerably higher than in the control animals. How can the correlation between these parameters be explained? In desympathized rats the total peripheral vascular resistance of the systemic circulation was 1.7 times less than in normal rats [7]. Consequently, the tone of their blood vessels was depressed. Under these conditions the heart must contract more frequently and expel more blood in order to maintain the necessary blood pressure in the peripheral ves-

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