AGE DIFFERENCES IN THE EFFECT OF INSULIN ON SMOOTH-MUSCLE CELLS OF THE FEMORAL ARTERY

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Hitherto the question of the direct effect of insulin on vascular smooth-muscle cells (SMC) has attracted little attention of research workers. Diabetes mellitus, in which the blood insulin level is low, is known to be accompanied by a disturbance of activity of the cardiovascular system. In old age this pathology arises more often [1, 3, 4], so that investigation of the effect of insulin on SMC in old animals is particularly interesting. The aim of the present investigation was to study this problem.

EXPERIMENTAL METHOD

Experiments were carried out on isolated spiral strips of femoral arteries of adult (6-8 months) and old (24-26 months) rats. The preparations were perfused with Krebs' solution at 36 and 38°C. The membrane potential (MP) was measured by means of intracellular microelectrodes. Preliminary stretching of the preparation was carried out with a load of 600-800 mg. Contractile reactions were recorded by means of a 6 MKh1S electronic transducer. Exposure to insulin was carried out after stabilization of the tone of the preparation. After the end of the experiment the muscle strip was weighed. Changes in tone were calculated per milligram of vascular tissue tested.

EXPERIMENTAL RESULTS

Perfusion of preparations from the femoral artery of the mature animals with solution containing insulin in a concentration of 400 milliunits/ml (mU/ml) at 36°C caused an increase in MP of SMC (Table 1). The increase in MP under the influence of this insulin concentration amounted to 8.8 ± 1.5% of the initial MP level of the test cells (68.5 ± 5.9 mV). Hyperpolarization of the cell membranes was accompanied by lowering of the tone of the preparations (relaxation of the vascular smooth muscles) by 110 ± 10 mg. With an increase in the insulin concentration in the solution to 600 mU/ml hyperpolarization of the cells increased (Table 1) to 14.6 ± 1.2% of the initial level of MP. Relaxation of the preparations also became more marked and reached 176 ± 12 mg. The effect of the temperature of the perfusate on the intensity of

TABLE 1. Increase in MP (hyperpolarization) of SMC of Femoral Artery from Mature and Old Rats under the Influence of Insulin (in mV)

Insulin concentration in perfusate, mU/ml	Rats aged 6-8 months		Rats aged 24-26 months	
	temperature of perfusate			
	36 °C	38 °C	36 °C	38 °C
400	6±1,4	9,9±0,97	—	4,4±1,1
600	10,4±1,1	14,2±1,2	4,2±0,9	7,8±1,3

Legend. n = 14-16.

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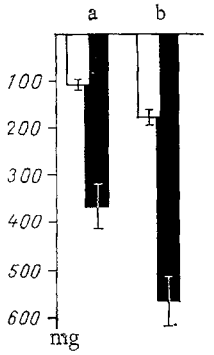

Fig. 1

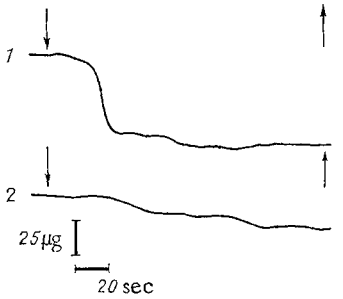

Fig. 2

Fig. 1. Effect of temperature of perfusate on relaxation of smooth muscles from femoral artery of mature rats under the influence of insulin in a concentration of 400 mU/ml (a) and 600 mU/ml (b). Unshaded columns — magnitude of response at 36°C, black columns — at 38°C.

Fig. 2. Lowering of tone of smooth muscles from femoral artery of mature (1) and old (2) rats under the influence of insulin in a concentration of 400 mU/ml. Temperature of perfusate 38°C. Arrows mark beginning and end of exposure.

responses of SMC of the femoral artery to insulin is noteworthy. When the temperature was increased to 38°C the degree of hyperpolarization of the SMC (Table 1) and of their relaxation (Fig. 1) was increased. Since a rise of temperature stimulates metabolic processes, the more marked responses to insulin under these conditions could be further confirmation of the close connection which has been postulated between changes developing under the influence of insulin and metabolism of the SMC. Insulin still had an effect even during perfusion of the preparations with solution not containing glucose. This is evidence that the responses which developed were not connected with increased transport of glucose into the SMC (under the influence of insulin).

There is evidence that the first stage of the action of insulin on the cell is binding of the hormone with specific receptors of the plasma membranes [2, 8]. It has been shown that during the action of insulin on liver cells and striated muscle cells, Na,K-ATPase is activated [6, 10], and this leads to an increase in the intracellular K^+ concentration [7, 13], the equilibrium potential of which largely determines the MP level. As a result of such changes, the hyperpolarization of the cell membranes observed in SMC probably develops. It has also been postulated that insulin, as a result of activation of protein biosynthesis, stimulates the formation of intracellular macromolecular anions, and this may also make a contribution to the increase in MP [13]. The developing hyperpolarization may cause, in particular, lowering of the tone of the vascular smooth muscles. In striated muscles the intracellular Ca^{++} concentration falls under the influence of insulin [12], which affects the level of muscle tone.

The effect of insulin was next tested on SMC from the femoral artery of old animals. It was shown previously that with age the sensitivity of vascular SMC to humoral influences is altered; for example, the sensitivity of SMC from the rat portal vein to noradrenalin increases with age [5].

The mean level of MP of SMC of the blood vessels studied from mature and old animals differed only a little, amounting to 65.8 ± 5.9 and 71.4 ± 2.9 mV, respectively, ($P > 0.01$). On the addition of insulin to the perfusate in a concentration of 400 mU/ml (36°C) no change was recorded in the MP level or tone of the smooth muscles of preparations from the femoral artery of old rats. If the temperature was raised to 38°C a response to the hormone appeared and hyperpolarization of SMC developed (Table 1), accompanied by relaxation. However, hyperpolar-

ization amounted to only 5 ± 1.1% of the initial MP level. As will be clear from Fig. 2, relaxation of the vascular smooth muscles of old rats was much weaker and the maximum of the response was reached later than in the mature animal. With an increase in the insulin concentration to 600 mU/ml responses of SMC of the old rats to insulin also appeared at 36°C, but they were much weaker than in the mature animals. If the magnitude of the responses of SMC of the mature animals to insulin is taken as 100%, electrical responses of SMC of the old rats amounted to 40-48%. Changes in tone of smooth muscles from the femoral artery of old rats amounted to between 6 and 20% only, i.e., the dilator effect of insulin in vascular smooth muscles of old animals is modified by a far greater degree than the changes produced by insulin in the electrical characteristics of SMC. This is evidence that the vascular smooth muscles of old animals are less able to relax, possibly due to a reduction of elasticity of the vascular wall and also to a disturbance of relaxation and of electromechanical coupling in vascular smooth muscles in old age.

The results are evidence of lowering of the sensitivity of SMC from the femoral artery of old animals to insulin. The intensity of responses to an increase in the insulin concentration in the perfusate and to a rise of temperature also is less. With age ability of insulin to bind with receptors of the plasma membranes of liver cells and fibroblasts is known to diminish [9, 11]. The fall in sensitivity of SMC to insulin in old age and the weakening of the intensity of reactions to insulin may also be linked with a decrease in the number of insulin receptors present on the membrane and with a decrease in the affinity of the receptors for insulin.

Insulin has a relaxing action on vascular smooth muscles of mature and old animals. A fall in its concentration in the blood probably leads to changes in vascular tone. Lowering of the sensitivity of the vascular wall to insulin in old age may be one of many causes of the vascular disturbances characteristic of old age.

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