creatine phosphate (CP), and inorganic phosphate, and prevented changes in the electrocardiogram of the myocardium in this pathological state [8]. Propylnorantiphein also had a similar prophylactic effect. As Table 1 shows, propylnorantiphein prevented the increase in SDH activity and the decrease in CO activity in the heart, observed immediately after electrical stimulation of the aortic arch, to the same degree as ethimizole.

In neurogenic injury to the myocardium significant changes thus arise in various components of the respiratory chain, leading to a disturbance of the formation of high-energy compounds. It was shown previously that during trophic disturbances of neurogenic character in heart muscle tissue, at different times of observation (3, 24, and 48 h) uncoupling of oxidative phosphorylation and a decrease in the concentrations of ATP and CP are observed [3, 5]. There is no doubt that the changes described are a molecular mechanism of disturbance of myocardial function of the utmost importance during neurogenic injury to the organ.

Administration of ethimizole and propylnorantiphein – preparations stimulating energy metabolism of the CNS – has a beneficial effect on neurogenic injury to the myocardium accompanied by energy deficiency.

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EFFECT OF ISOPROTERENOL ON ADENYLATE CYCLASE ACTIVITY IN ADIPOCYTES OF SPONTANEOUSLY HYPERTENSIVE RATS

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It was shown previously that the cellular response to the action of hormones (insulin, adrenalin, ACTH) in the adipose tissue of spontaneously hypertensive rats (SHR) differs from the response of the same cells in normotensive animals [3]; this difference in the case of adrenalin and insulin, however, appeared only after adrenalectomy. The change in "sensitivity" of fat cells of hypertensive animals to the action of the above-mentioned hormones is evidently connected with a change in the content of cyclic nucleotides, which is controlled mainly by adenylate cyclase (AC), the enzyme controlling synthesis of cyclic AMP, and by phosphodiesterase, which controls the rate of breakdown of cyclic AMP and cyclic GMP.

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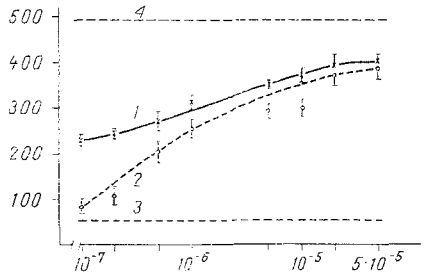

Fig. 1. Effect of isoproterenol on AC activity in ghosts of fat cells. Abscissa: isoproterenol concentration (in M); ordinate: AC activity (in picomoles cyclic AMP/mg protein/min). 1) Control, 2) SHR, 3) basal activity, 4) NaF-stimulated activity.

To determine whether differences exist in the regulation of the cyclic nucleotide level in cells of hypertensive and normotensive animals with respect to the AC system, the investigation was undertaken to study the activity of this enzyme in ghosts of fat cells of intact and adrenalectomized SHR, compared with the corresponding normotensive control.

EXPERIMENTAL METHOD

Inbred male SHR (Kyoto-Wistar) age 12 weeks and weighing 180-200g, with a blood pressure of 190-195 mm Hg were used. Inbred Wistar male rats of the same age, kept under identical conditions from the time of weaning (blood pressure 80-100 mm Hg) served as the control. Half of the experimental animals underwent bilateral adrenalectomy 1 week before the investigation, and these animals received 1% NaCl solution instead of water to drink.

Fat cells were obtained by Rodbell's method [12] from the epididymal adipose tissue of the rats by treatment with collagenase (3 mg/ml, 432 units, from "Worthington," USA) in Krebs–Ringer solution, pH 7.4 [5], with the addition of 2% albumin (fraction V, from Miles Laboratories, USA). Ghosts of fat cells were obtained by the method in [13]. Usually the preparation contained 3.5-4 mg protein/ml and was used at once for a period of 1 h. The protein content was determined by Lowry's method [10], using bovine albumin as the standard.

AC activity was determined by the method described in [4]. The incubation sample (50 μl, 37°C) contained 50 mM Tris-HCl buffer, pH 7.4, 5 mM $MgCl_2$, 1 mM cyclic AMP, 20 mM creatine phosphate; 60 units/ml creatine kinase, and 0.5 mM ATP-^{14}C (500,000-1,000,000 cpm, from "Radiochemical Centre," Amersham, England). Isoproterenol was added in a concentration of between 10^{-7} and 5×10^{-5} M, and NaF in a concentration of 10 mM. Cyclic AMP formation was linear for 20 min and proportional to the protein concentration up to 50 μg per sample.

EXPERIMENTAL RESULTS

In all variants of the experiment, both basal and NaF-stimulated AC activity of the fat cells was equal, namely 50 ± 3.5 and 488 ± 8 picomoles cyclic AMP/mg protein/min. However, a study of the activity of isoproterenol-stimulated AC revealed differences between the normotensive and hypertensive animals. First, given the same maximal velocity of cyclic AMP formation in the experiment and control, the isoproterenol concentration required to achieve half the maximal effect was three times higher in SHR than in the corresponding experiment with normotensive animals (Fig. 1; Table 1). Second, after adrenalectomy on normotensive rats the maximal velocity (V_{max}) of the reaction of cyclic AMP synthesis in the fat cells was reduced by 27%, and its value in fat cells of SHR was identical with that of V_{max} in intact animals (Table 1). These observations suggest that the number of combining sites for catecholamines on the membrane of the fat cells was the same in both SHR and the control rats, but that affinity for them was considerably greater in normotensive animals. After adrenalectomy the number of β-adrenoreceptors decreased in the control but was unchanged in the animals with hypertension.

TABLE 1. Kinetic Parameters of Isoproterenol-Stimulated AC in Ghosts of Rat Adipocytes (M ± m)

Variant of experiment	V_{max}	$K_a \cdot 10^{-7}$ M (for isoproterenol)
1. Intact control rats	410±30 (n=30)	1,8±0.2
2. Control rats after adrenalectomy	300±30 (n=10)	1,5±0.1
3. Spontaneously hypertensive rats	370±35 (n=11)	5,7±0,9
4. Spontaneously hypertensive rats after adrenalectomy	460±60 (n=11)	4.4±0.4
P_{1-2}	<0,03	<0,5
P_{1-3}	<0,5	<0,001
P_{3-4}	<0,5	<0,5

Legend. ATP concentration 0.5 mM. V_{max} given in picomoles cyclic AMP/mg protein/min. K_a) Activation constant.

Data on AC activity in the different tissues of hypertensive animals are contradictory [7, 9, 14]. AC activity in the plasmalemma of the fat cells has not been investigated from this standpoint, although adipose tissue is one of the best objects in which to study the mechanisms of interaction of hormones with the cell membrane and regulation of the cyclic nucleotide level in the cell in this pathology. The decrease in affinity of AC for isoproterenol discovered in SHR and the decrease in AC activity in the fat cells of normotensive rats after adrenalectomy were evidently due to a change in the intracellular distribution of Ca^{++}, which has been demonstrated in fat cells both of SHR [2] and of adrenalectomized normotensive rats [1]. The Ca^{++} concentration is known to be the main factor which determined equilibrium between active and inactive forms of AC. Meanwhile, the reduction in the activity of hormone-stimulated AC in the adipocytes after adrenalectomy was perhaps due to the action of adenosine, the metabolism of which is disturbed after adrenalectomy [8]. Adenosine can regulate the transmission of the signal from hormone to cell, either by directly affecting AC activity in the membrane [15] or by acting as antagonist of prostaglandins and thus participating in the redistribution of intracellular Ca^{++} [6]. The absence of change in AC activity in the fat cells after adrenalectomy is difficult to explain, for the multireceptor AC system of fat cells is characterized by a complex process of regulation at the level of both regulatory and catalytic units. All that can be said is that steroid hormones may be involved in these processes through their action on the system for synthesis either of the regulatory unit of AC or of membrane proteins, including proteins forming permeability channels for monovalent and bivalent ions [11]. Preservation of the magnitude of the lipolytic response of the adipose tissue in SHR after adrenalectomy, demonstrated previously, is thus connected with absence of a decrease in AC activity, such as is normally observed after adrenalectomy. This is probably not the only mechanism, for according to observations made in the writers' laboratory, preservation of the level of lipolysis in fat cells of SHR after adrenalectomy is also connected with a decrease in activity of membrane-bound cyclic AMP phosphodiesterase, the Michaelis constant of which has a low value.

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