SOME CHANGES IN ADRENALIN LIPOLYSIS IN ADIPOSE TISSUE IN RATS WITH SPONTANEOUS AND RENAL HYPERTENSION

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Lipolysis was studied in adipose tissue of adrenalectomized and intact rats with hypertension (spontaneous or renal) and in normotensive rats of the corresponding control groups. The degree of lipolysis (in the presence or absence of adrenalin) was judged from the quantity of nonesterified fatty acids in the tissue and the liberation of glycerol into the incubation medium in vitro. The response of adipose tissue to the action of adrenalin in the hypertensive and control animals was the same provided the adrenals were intact. Preliminary adrenalectomy, abolishing the effect of corticosteroid secretion, reduced the lipolytic response of the adipose tissue to adrenalin in control normotensive rats but did not reduce it in rats with hypertension ("facilitation" of the action of the catecholamine on the mechanisms of lipolysis). This phenomenon is examined in connection with the presence of initial changes in the function of the plasma membranes of the fat cells in hypertensive animals. Hypertrophy of the adrenal cortex and potentiation of corticosteroid secretion in these types of hypertension can therefore be regarded as a measure of compensation for disturbance of the function of the cell plasma membranes in the tissues of the internal milieu.

KEY WORDS: hypertension; adrenalectomy; adipose tissue; lipolysis; adrenalin.

In renal and spontaneous hypertension in rats differences in the response of the adipose tissue in vitro to the action of insulin (as reflected in the uptake of [^{14}C]-glucose were found previously. The differences discovered were regarded as evidence of a change in function of the plasma membranes of the adipose cells in these types of chronic arterial hypertension [3].

The object of the present investigation was to study the character of the lipolytic action of adrenalin on adipose tissue in rats with spontaneous and renal hypertension. Since the action of adrenalin on lipolysis in adipose tissue is effected through the cyclic-AMP system [5], the characteristics of the response of adipose tissue to adrenalin in hypertensive animals could give definite information on the state of that system in the cells of adipose tissue in genetic and renal hypertension. Since adrenal hormones correct disturbances of function of the plasma membranes, part of the present investigation was undertaken on adrenalectomized animals (i.e., animals in which the influence of corticosteroid hormones, which stabilize the plasma membranes of fat cells) was eliminated.

EXPERIMENTAL METHOD

The following animals were used in the two series of experiments: in series I, inbred male SHR (spontaneously hypertensive rats, Kyoto-Wistar); in series II rats with renal hypertension. The method of producing renal hypertension, the characteristics of the hypertensive and control animals, and the conditions of adrenalectomy are given in the literature [3]*.

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TABLE 1. Action of Adrenalin on Lipolysis in Adipose Tissue of Intact and Adrenalectomized Rats with Spontaneous Genetic (Series I) and Renal Hypertension (Series II)

Serial No.	Series	Variant of experiment	No. of animals	NEFA content in tissue			Liberation of glycerol into incubation medium		
				without adrenalin	with adrenalin	Δ action of adrenalin	without adrenalin	with adrenalin	Δ action of adrenalin
				μmoles/100 g wet weight of tissue·h					
1	I	Control intact rats	20	40,0±1,5	86,5±2,5	46,5 (116)	1,51±0,06 (10)	4,47±0,11 (10)	2,96 (196)
2	I	Control adrenalectomized rats	19	43,0±1,5	73,5±2,5	30,5 (71)	1,82±0,17 (10)	3,83±0,09 (10)	2,01 (110)
3	I	SHR rats	19	41,0±2,5	91,5±3,0	50,5 (123)	1,66±0,16 (10)	4,66±0,18 (10)	3,0 (181)
4	I	Adrenalectomized SHR rats	19	28,0±1,5	72,5±4,5	44,5 (159)	1,18±0,11 (10)	4,17±0,2 (10)	2,99 (253)
	I	P_{1-2}		N.S.	<0,001	<0,0001	N.S.	<0,001	<0,0001
	I	P_{3-4}		<0,002	<0,001	N.S.	<0,02	N.S.	N.S.
1	II	Control intact rats	25	60,0±5,5	116,0±3,5	56,0 (93)	2,31±0,17 (18)	5,17±0,11 (18)	2,86 (124)
2	II	Control adrenalectomized rats	18	62,5±5,5	104,5±3,5	42,0 (67)	2,29±0,09 (17)	4,59±0,11 (17)	2,30 (100)
3	II	Rats with renal hypertension	22	69,5±4,0	106,5±4,5	37,0 (53)	2,51±0,18 (16)	4,86±0,15 (16)	2,35 (94)
4	II	Adrenalectomized rats with renal hypertension	18	58,0±4,5	105,0±6,0	47,0 (81)	2,44±0,19 (17)	4,89±0,15 (17)	2,45 (100)
	II	P_{1-2}		N.S.	<0,025	<0,001	N.S.	<0,001	<0,001
	II	P_{3-4}		<0,05	N.S.	N.S.	N.S.	N.S.	N.S.

Legend. Percentage shown in parentheses.

Rats were deprived of food but given water ad lib for 48 h, after which they were decapitated, the adipose tissue of the epididymis was removed, and the thin distal part (about 250 mg from each epididymis) was cut off and placed in incubation medium without adrenalin (adipose tissue from the right epididymis) and with adrenalin (tissue from the left epididymis).

The study of how the rate of synthesis of nonesterified fatty acids (NEFA) in adipose tissue and the liberation of glycerol into the incubation medium depend on the concentration of adrenalin (L-epinephrine, from Calbiochem, USA) added to the sample showed that dependence of the degree of lipolysis on the dose of adrenalin was identical in the normotensive and hypertensive animals, and that the maximal effect of the hormone was obtained when it acted in concentrations of 0.7-1.0 μg/ml. The concentration of adrenalin chosen for the subsequent investigations was 1 μg/ml.

Samples containing 2 ml of Krebs–Ringer-phosphate buffer, pH 7.4 [4] were incubated with shaking for 1 h in a constant-temperature shaker at 37°C. At the end of incubation the samples were placed in ice, 100-mg samples were weighed, and the content of NEFA in them was determined by Duncombe's method [6]. An aliquot (0.1 ml) was taken from the incubation sample and the quantity of glycerol in it was determined by an enzymatic method [7]. To determine the initial content of NEFA pieces of adipose tissue of the epididymis (100 mg) were placed on ice immediately after the animal was killed and the NEFA content in them was determined by the method indicated above. A special investigation showed that the NEFA content in the adipose tissue of the same animal before and after incubation for 1 h in medium without adrenalin was identical. This showed that the incubation medium itself did not affect the degree of initial lipolysis.

EXPERIMENTAL RESULTS AND DISCUSSION

The weight of the adrenals was greater in the rats with hypertension. For instance, in the series with spontaneous hypertension the weight of the two adrenals was 28.8 ± 0.4 mg compared with 21.2 ± 0.5 mg in the control ($P < 0.001$).

The initial level of lipolysis, judging from the content of NEFA and glycerol in the adipose tissue, was identical in the intact hypertensive and the control rats in each series of experiments (Table 1). It showed no significant change after adrenalectomy in the normotensive control rats but was considerably reduced in the SHR rats, as shown by determination of both NEFA and glycerol (Table 1). The initial level of lipolysis also was somewhat reduced in the adipose tissue of rats with renal hypertension after adrenalectomy, as shown by determination of NEFA.

Adrenalin, added to the incubation medium, stimulated lipolysis in the adipose tissue of all groups of animals, but the highest values of lipolysis (its "ceiling") in the adrenalectomized control animals and SHR were rather lower – by 15% in the control and by 20.7% in the hypertensive animals, according to determination of NEFA (Table 1). However, as regards the absolute value of the response of the adipose tissue to adrenalin (Δ) the groups of adrenalectomized rats in the two series of experiments differed significantly. In the experiments with spontaneous hypertension adrenalectomy caused an appreciable reduction in the absolute magnitude of the response of the adipose tissue to adrenalin in the control normotensive animals, which was not found in the rats with hypertension: With a low initial level of lipolysis the value of Δ for NEFA and glycerol was higher in absolute terms in the adrenalectomized rats with spontaneous hypertension than in the control adrenalectomized rats. In the series with renal hypertension the results on the whole were similar to those obtained previously. The magnitude of the lipolytic response of the adipose tissue to adrenalin, as regards both the absolute quantity of NEFA and glycerol liberated and the relative value of the increase in the adrenalectomized rats with hypertension, was not reduced compared with the response of the adipose tissue of the intact rats, as was the case in the control (Table 1).

It will be clear from Table 1 that lipolysis in the adipose tissue of hypertensive rats is more dependent in vitro on the secretion of corticosteroid hormones than in control animals: Removal of adrenal secretion lowered their initial intensity of lipolysis, which was not observed in the control groups.

Since the absolute magnitude of the lipolytic response of the adipose tissue to adrenalin was not reduced under these circumstances, as it was in the control adrenalectomized animals, this suggests that differences observed in initial lipolysis and in the response of the adipose tissue to adrenalin in the groups of adrenalectomized animals were not due to the intracellular part of the mechanism of lipolysis but, evidently, reflected changes in the membrane component of that system. The response of the adrenalectomized hypertensive rats to adrenalin was as it were "facilitated" compared with the control. A response of the adipose tissue of similar character in an experiment of this sort was found during the study of [^{14}C]-glucose transport under the in-

fluence of insulin [3]. Together with the known data on changes in the function of the plasma membranes in certain cellular tissues of the internal milieu [8, 9], the results now obtained point to the presence of functional changes in the plasma membranes of the fat cells as a special manifestation of a widely distributed phenomenon in these forms of chronic hypertension [1, 2].

Consequently, the increased corticosteroid secretion, reflected in the experiments described above in a general form as hypertrophy of the adrenal cortex in the hypertensive rats, can evidently be regarded as a compensating factor stabilizing the disturbance of the membrane function of the cells in the adipose and other tissues of the internal milieu.

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DYNAMICS OF SOME INDICES OF BLOOD CLOTTING AFTER INTRAPERITONEAL INJECTION OF THROMBIN INTO NONINBRED ALBINO RATS

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The possibility of giving thrombin by intraperitoneal injection as a test of the function of the blood clotting system (the in vivo thrombin test) was demonstrated in experiments on noninbred albino rats.

KEY WORDS: thrombin test; intraperitoneal injection of thrombin.

The in vivo thrombin test [2, 3, 7] involves intravenous injection of thrombin into animals, but this does not always satisfy the experimenter's needs, for intravenous injection may result in severe trauma to the animal and such trauma may itself give rise to changes in the blood clotting system. Intraperitoneal injection of thrombin is interesting from this standpoint [13]. In the investigation described below the results of the study of changes in some indices of blood coagulation after intraperitoneal injection of thrombin were examined.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats of both sexes weighing 160-180 g. Thrombin from Kaunas Bacteriological Preparations Factory was injected intraperitoneally in a dose of 25 units in 0.5 ml of 0.14 M sodium chloride solution, after 2 ml blood had been taken from each animal for control determinations. Later, in the various groups (each of 12 animals), blood was taken for testing after 30 min and 2, 3.5, and 24 h, and stabilized with sodium citrate (1 : 4). The recalcification time in the samples was determined by the method of Bergerhof and Roka in Baluda's modification [9], Quick's test by Tugolukov's method [9], plasma thromboplastin activity as in [10], the total fibrinogen concentration by coagulation [11] and nephelometric methods, fibrinogen B as in [12], and fibrinolytic activity as in [10].

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