Sensitivity of Adrenal Glands to Adrenocorticotropic Hormone in Animals with Alimentary Obesity

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Blood concentration of corticosterone in obese rats did not differ from the control value. *In vitro* synthesis of progesterone and corticosterone in adrenal slices from obese rats was lower compared to control animals, but these differences disappeared after addition of adrenocorticotropic hormone to the incubation medium. In obese rats, blood content of corticosterone in response to administration of adrenocorticotropic hormone *in vivo* increased by 8 times, while in control animals this parameter increased by only 4.5 times.

Key Words: *alimentary obesity; adrenal glands; corticosteroids; adrenocorticotropic hormone*

Obesity is a serious chronic disease associated with accelerated development of other pathologies. The etiology of obesity has a multifactor nature, the body weight and fat distribution in the body are primarily affected by life-style (character of nutrition and physical activity), psychological, and hereditary factors [3,4]. Environmental factors also play an important role [8,10,14].

Hormones and mediators of the hypothalamic—pituitary—adrenal system participate in adaptation of the organism to environmental conditions at all stages of ontogeny [11]. Excessive secretion of glucocorticoid hormones in neuroendocrine disturbances and under stress conditions is the major cause of the formation of insulin-resistance and visceral obesity [1,2,7,9,12,13]. The important role of these hormones in the mechanisms of the formation of insulin-resistance is confirmed in experiments with long-term treatment of experimental animals with hydrocortisone and dexamethasone [5]. At the same time, the contribution of glucocorticoid hormones into the formation of widespread alimentary-constitutional obesity (AO) is poorly studied.

Here we studied the functional state of adrenocortical system and its sensitivity to ACTH in experimental animals with AO developing from the first days of postnatal ontogeny.

MATERIALS AND METHODS

Male rats with AO developing from the first days of postnatal ontogeny and respective control rats were used in the experiments. AO was modeled by reducing the size of litters after birth (to 3 pups) and by adding fat (100 g/kg) to the ration of dams and male pups; this ration was given *ad libitum* throughout the entire experimental period. For preparing the control group, the liters were adjusted to 8 pups, lactating females, pups, and mature male rats received standard vivarium ration. A total of 29 mature male rats aged 4-5 months for the experimental and control groups were obtained.

In 10 rats from each group, diurnal urea was collected individually from each animal using specially designed urea collectors. The urea was centrifuged, its volume was measured. On the next day, the blood from the cervical veins was collected, and the serum was separated. The adrenal

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glands (AG) were removed, placed in 0.9% NaCl, homogenized in 1 ml 0.1 M potassium phosphate buffer (pH 7.4), centrifuged, and the supernatants were collected. The urea, serum, and supernatants were frozen for further analysis of steroid hormones. Other 10 rats from each group were intraperitoneally injected with ACTH in a dose of 1 U/100 g body weight; the blood was collected 30 min after injection.

In 9 animals of each group, AG were collected for subsequent incubation *in vitro*. The slices of AG were incubated for 2 h at 37°C in Krebs-Ringer bicarbonate buffer (pH 7.4) saturated with 95% O_2 and 5% CO_2 in the presence of ACTH (0.5 U per 3 ml incubation medium), pregnenolone (80 μg per 3 ml incubation medium), or ACTH in combination with pregnenolone.

The urine, serum, supernatants, and incubation medium were extracted with ethylacetate (1:10, v/v); the organic phase was collected and evaporated under vacuum in glass tubes. The content of hormones was measured by radioimmuneassay using RIN-V-3H kit (for corticosterone), STERON-P-125I kit (for progesterone), and SB-ALDO-2-125I kit (for aldosterone).

The intergroup differences were evaluated using Mann—Whitney test, the null-hypothesis was considered valid at $p<0.05$.

RESULTS

The content of progesterone, corticosterone precursor, in AG of rats with AO did not differ from the control value (Table 1). However, the content of corticosterone in AG of experimental rats 2-fold surpassed the control. It should be noted that diurnal excretion of corticosterone and serum concentration of this hormone were similar in these two groups (Table 2). These results suggest that the amount of the hormone stored in AG and not entering the circulation under normal conditions is increased in rats with AO.

In *in vitro* system, the biosynthesis of both progesterone and corticosterone by AG slices from rats with AO was reduced (Table 3), which attests to reduced activity of steroidogenesis enzymes. Addition of pregnenolone, an earlier precursor in corticosteroid synthesis compared to progesterone, to the incubation medium eliminated the differences in corticosterone (but not progesterone) production by AG slices from control animals and rats with AO. Addition of ACTH to the incubation medium eliminated all differences in steroid biosynthesis by AG between the two experimental groups. These findings suggest that the stimulating effect of ACTH masks reduced steroid biosynthesis in AG cortex in rats with AO.

TABLE 1. Content of Steroid Hormones in AG and Their Diurnal Urinary Excretion in Control Animals and Rats with AO ($M\pm m$; $n=10$)

Parameter	Group	
	control	experimental
Progesterone, pmol/mg AG tissue	0.56±0.09	0.61±0.06
Corticosterone, pmol/mg AG tissue	13.4±2.2	26.6±4.9*
Progesterone, pmol/day	25.4±1.0	23.8±0.9
Corticosterone, pmol/day	1.44±0.07	1.62±0.07

Note. *$p<0.05$ compared to the control.

No differences were found in aldosterone biosynthesis (mineralcorticoid hormone) by AG slices from rats with AO and controls (Table 3).

The data obtained in *in vitro* system and attesting to increased sensitivity of AG cortex in rats with AO to the stimulating effects of ACTH were also confirmed by measurements of blood corticosterone concentration in animals receiving ACTH i*n vivo* (Table 2).

In rats with AO, the content of corticosterone in response to ACTH injection increased by 8 times, while in controls this parameter increased by only 4.5 times.

Thus, these findings attest to increased sensitivity of cells in AG cortex in rats with AO to the stimulating effects of ACTH. These peculiar changes can be explained as follows: fat-enriched diet starting from the first days of life induced adaptive rearrangement of the hormonal regulation of metabolism in adult animals, and to the greater extent the hypothalamic—pituitary—adrenal system, because functional activity of this system is most sensitive to early postnatal modifying influences [6]. In adult animals with AO, increased reactivity of AG cortex to ACTH can be a factor promoting accumulation and deposition of excessive visceral fat.

TABLE 2. Reaction of AG in Control Animals and Rats with AO to Administration of ACTH *in Vivo* ($M\pm m$; $n=10$)

Group	Serum content of corticosterone, nmol/liter	
	initial value	30 min after ACTH injection
Control	148±16	681±46*
Experimental	107±15	881±49*

Note. *$p<0.01$ compared to initial values.

TABLE 3. Content of Steroid Hormones (nmol/mg Tissue) in Medium after *in Vitro* Incubation of AG Slices from Control Animals and Obese Rats ($M\pm m$; n=9)

Parameter	Additives	Group	
		control	experimental
Progesterone	Without additives	1.08±0.26	0.27±0.05^{+}
	ACTH	1.21±0.24	1.22±0.20*
	Pregnenolone	98.1±4.0*	35.7±5.5*$^{++}$
	ACTH+pregnenolone	118.7±21.2*	126.1±22.3*
Corticosterone	Without additives	83.9±3.5	53.8±7.8^{+}
	ACTH	109.2±15.1	85.7±15.8
	Pregnenolone	123.1±14.4*	125.3±5.2*
	ACTH+pregnenolone	184.2±1.8*	180.2±20.7*
Aldosterone	Without additives	19.7±0.6	14.9±3.6
	ACTH	18.7±2.9	17.1±2.7
	Pregnenolone	31.1±4.1*	20.9±3.7
	ACTH+pregnenolone	29.5±1.7*	26.5±3.7*

Note. *p<0.05 compared to medium without additives; ^{+}p<0.01, ^{++}p<0.001 compared to the control.

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