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A Comparison of Pituitary Cells from Lean and Obese Zucker Rats[1] (41518)

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Abstract. Pituitary cells were isolated from lean and obese Zucker rats and compared with the use of two approaches. The first approach was to evaluate the capacity of an equal number of pituitary cells to stimulate growth in hypophysectomized rats. The rats implanted with lean pituitary cells grew at a similar rate as rats implanted with obese pituitary cells. Body composition was also determined to be similar in those rats receiving lean and obese rat pituitary cells. The second approach was to evaluate the *in vitro* secretory capacity of rat pituitary cells when placed in a superfusion system. Basal and stimulated growth hormone release was the same for lean and obese rat pituitary cells. These data support the hypothesis that the low level of serum growth hormone found in the Zucker obese rat is the result of a hypothalamic disorder and not a pituitary cell defect.

Protein deposition in the obese Zucker rat (fa/fa) is low when compared with that in its lean littermate (1–3). This is even more evident when the obese rat is pair-fed with the lean rat (4, 5). In addition to the poor lean body growth, skeletal growth is markedly depressed (6). We have proposed that the poor lean body growth of the Zucker obese rat is the result of low serum growth hormone (7, 8) and serum somatomedin activity (9). These hormones are essential for maximum postnatal cell division and protein synthesis (10).

The purpose of this study was to compare pituitary cells from lean and obese Zucker rats. Two approaches were utilized. The first measured the *in vivo* capacity for stimulating growth of hypophysectomized rats. The second approach was to measure *in vitro* growth hormone release.

Methods and Materials. The procedure for transplanting pituitary cells was previously described (11). Hypophysectomized rats (6 weeks of age) were obtained from Zinc-Miller, Allison Park, Pennsylvania. The animals were housed in shoe box cages in a room maintained at 27°. The lights were adjusted to a 12-hr light/dark cycle. All rats received a high carbohydrate diet *ad libitum*. The drinking water was supplemented with 5% glucose plus electrolytes in the following proportions: NaCl, 0.20% KCl, 0.008%; $MgCl_2$, 0.001%; and $CaCl_2$, 0.0035%. Those hypophysectomized rats gaining more than 1 g per day were not used in the implantation study. On the day of implantation, donor animals, consisting of both lean and obese Zucker rats, were killed. Their pituitaries were removed aseptically and minced. The pituitary mince was treated with 0.1% trypsin in Eagle's minimal essential medium and 0.1% bovine serum albumin. After 2 hr of trypsin digestion the suspension was centrifuged and resuspended in Medium 199 to determine cell yield. The pituitary cells were centrifuged again and resuspended in mock cerebral spinal fluid (MCSF) to a concentration of 1×10^6 cells per 10 μl. Previously it was reported that pituitary transplants into the left cerebral ventricles results in a stimulation of growth in hypophysectomized rats (11). In the same study it was established that growth was proportional to the number of cells transplanted, up to 3×10^6 cells. The number of cells transplanted in this study was in the linear range of response. The cells were stereotaxically implanted directly into the left cerebral ventricles of the hypophysectomized rats. The rats implanted with pituitary cells grew for approximately 4 weeks. The rats

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TABLE I. EFFECT OF PITUITARY CELLS FROM LEAN AND OBESE RATS ON GROWTH OF HYPOPHYSECTOMIZED RATS

Parameters[a]	Hypophysectomized control (4)	Lean pituitary cells (5)	Obese pituitary cells (6)
Body weight gain (g)	4.9 ± 0.8[b]	33.8 ± 1.7[c]	33.0 ± 3.5[c]
Body composition (%)			
Dry matter	30.9 ± 1.0[b]	34.2 ± 0.1[c]	35.4 ± 0.6[c]
Protein	19.6 ± 0.2	19.9 ± 0.5	20.1 ± 0.3
Lipid	7.8 ± 0.9[b]	10.8 ± 0.4[c]	11.9 ± 0.9[c]
Ash	3.4 ± 0.2	3.5 ± 0.1	3.4 ± 0.1

[a] Values represent mean ± SEM.
[b,c] Values with different superscripts are significantly different ($P < 0.05$), Duncan's multiple range test.

were exsanguinated, and carcasses were saved for determination of body composition (12).

The capacity for *in vitro* release of growth hormone from cells of lean and obese rats was compared with the use of pituitary cells prepared in the same manner as above. A superfusion technique described by Mulder and Smelik (13) was used to determine basal release of growth hormone and response to cyclic AMP. Growth hormone release stabilized after 15 min of perfusion and was constant for at least 4 hr. In preliminary tests growth hormone release was proportional to the concentration of cyclic AMP from 0.1 to 10 m*M*. The flow rate of the column was 0.5 ml/min, with 5×10^6 pituitary cells per column. Growth hormone assays were performed with the double-antibody technique and materials supplied by the Rat Pituitary Hormone Distribution Program, National Institute of Health. The isolated pituitary cells from lean and obese rats contained similar concentrations of immunoreactive growth hormone. No attempt was made to determine total pituitary cell number per rat because of the potential loss in cellular yield caused by the trypsin digestions; however, recovery of cells from pituitaries averages 70% with viability of >95% (15). Cells from the Zucker rat pituitaries also had viabilities >95% as determined by trypan blue exclusion.

Results. The ability of pituitary cells from lean and obese Zucker rats to stimulate growth of hypophysectomized rats was evaluated (Table I). Pituitary cells from both lean and obese rats stimulated growth when compared to control hypophysectomized rats receiving the vehicle. There was no significant difference in the capacity of lean and obese pituitary cells to stimulate growth. By analysis of body composition it was determined that the composition was the same for rats receiving the two types of pituitary cells.

The *in vitro* capacity for growth hormone release was compared for lean and obese rat pituitary cells (Table II). The basal release of growth hormone (per 5×10^6 cells) was the same for both lean and obese rats. When cyclic AMP was added to the perfusion system, growth hormone secretion was increased twofold for both cell types. There was no apparent difference between lean and obese rats' pituitary cells in the capacity to respond to cyclic AMP.

Discussion. Obese rats incorporate a greater proportion of dietary amino acids into body lipids and metabolize a greater portion of dietary amino acids to carbon dioxide (3). Our studies showed that when protein intake is equalized, protein gain is twice as efficient in the lean rat as in the obese rat (2). These

TABLE II. PITUITARY CELL RELEASE OF GROWTH HORMONE AND STIMULATION BY CYCLIC AMP

	Lean (ng/ml)	Obese (ng/ml)
Study I		
Baseline	920 ± 35[a]	890 ± 60
1 m*M* cAMP	2109 ± 107	2050 ± 124
Stimulation	229%	230%
Study II		
Baseline	850 ± 67	906 ± 80
1 m*M* cAMP	1785 ± 96	1875 ± 133
Stimulation	210%	206%

[a] Values represent the mean ± SEM. Three observations per study.

shifts in amino acid utilization are probably caused by an alteration in growth hormone secretion. We have previously shown that the obese rat has lower serum immunoreactive growth hormone in an ontogeny study (7) and in a diurnal study (8). That these low growth hormone levels were physiologically important was substantiated when it was established that the Zucker obese rat had lower serum somatomedin activity (9) and shorter tibias (6) than control lean rats.

In this study we have established that the defective growth hormone status is probably not dependent on a specific defect in the secretory capacity of pituitary cells or the growth hormone released from these cells. Hypophysectomized rats implanted with lean or obese rat pituitary cells grow at the same rate and gain the same proportion of lipid and protein. Further evaluation of growth hormone release demonstrated that cells from lean and obese rats respond to stimulation to the same extent. By process of elimination, it would appear that the defective growth hormone status of the Zucker rat is caused by an alteration in hypothalamic function.

Hypothalamic control of growth hormone secretion is probably mediated through the action of catecholamines (14). It has been shown that catecholamine deficits occur in the hypothalamic nuclei of the obese Zucker rat (16). These deficits may play a causal role in altering hypothalamic function and growth hormone secretion. Studies of catecholamine turnover in those central nervous system areas thought to be intimately involved in growth hormone secretion are in progress.

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