Relationship between β-Endorphin/β-Lipotropin, Hyperglycemia, and Hyperinsulinemia in Obese Male Zucker Rats (42971)

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Abstract. **The relationship between β-endorphin(β-EP)/β-lipotropin(β-LP) and insulin secretion in the basal state and after glucose challenge was studied in obese male Zucker rats and their lean littermates. Baseline plasma β-EP/β-LP concentrations were similar in the two groups of animals. Baseline plasma insulin and serum glucose concentrations were significantly higher in the obese animals. Following glucose challenge, the increase in plasma β-EP/β-LP concentrations was significantly lower in the obese animals than in their lean littermates. Opioid blockade with naloxone failed to alter the baseline hyperinsulinemia and hyperglycemia seen in the obese animals. The data suggest that the hyperinsulinemia in the obese Zucker rat is not due to endogenous hyperendorphinemia as shown in humans with polycystic ovary syndrome. The obese rats showed dissociation between glucose-stimulated plasma levels of β-EP/β-LP and insulin levels which may contribute to the hyperinsulinemia and insulin restance in these animals.**

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Obesity is commonly associated with carbohydrate intolerance, basal hyperinsulinemia, and an abnormal insulin response to a glucose challenge. Elevated levels of endogenous opioids have been found in obese hirsute patients with amenorrhea (1) and studies in humans with the polycystic ovary syndrome, many of whom are obese, have demonstrated a relationship between hyperendorphinemia and hyperinsulinemia (2). The hyperendorphinemia has been implicated as being the primary cause of the hyperinsulinemia since the latter can be significantly decreased by pharmacologic opioid blockade with naloxone (2). Furthermore, studies have shown that β-endorphin is capable of stimulating insulin release from the pancreas (3, 4). Endogenous opioids promote feeding, at least in some animal species (5), and previous studies have shown that elevated concentrations of β-endorphin are seen in the pituitaries of obese rats and mice (6) and in the plasma of obese strains of rats (6, 7). Moreover, overfeeding in obese mice (*ob/ob*) and rats (*fa/fa*) can be abolished by small doses of the narcotic antagonist, naloxone (6, 7). Elevated levels of endogenous opioids could account for obesity seen in congenitally obese animals if, as in humans with polycystic ovary syndrome, it was responsible for hyperinsulinemia because of the lipogenic actions of insulin. In light of these previous studies we examined the relationship between circulating concentrations of β-endorphin (β-EP)/β-lipotropin (β-LP) and insulin concentrations in congenitally obese animals such as the obese (*fa/fa*) strain of the Zucker rat in the basal state and after an intravenous glucose challenge.

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Materials and Methods

Animals. Twelve obese male Zucker (*fa/fa*) rats and an equal number of their lean littermates were studied. The two sets of rats were of the same age (5 weeks) and were housed in the same environment. The animals were provided by the Department of Nutrition, University of California at San Diego, California, and were given unlimited access to rat chow (Ralston Purina Co., St. Louis, MO) and water. The rats were significantly different in weight ($P < 0.01$). The obese rats ranged in weight from 170 to 358 g (mean ± SD, 258.2 ± 21.4). The lean rats ranged in weight from 140 to 185 g (142.5 ± 13.4).

Each rat was weighed and anesthesized with diethyl ether. The femoral vein was exposed as previously

described (9) and the vessel was catheterized using a 22-gauge catheter (Quik-Cath; Travenol Laboratories Inc., Deerfield, IL). Blood was withdrawn through the catheter for measurement of serum glucose, β-EP/β-LP, and insulin concentrations at time 0 min. One and one-tenth g of glucose/kg body weight (based on 75 g in a 70-kg man) was then given iv and blood was removed at 5, 10, 15, 20, 30, 60, 90, 120, and in some animals at 150 min for measurement of glucose and β-EP/β-LP as before. Volume was replaced by administration of heparinized normal saline in an amount equal to 2.5 times the blood removed. In an additional group of lean (n = 11) and obese rats (n = 10), 0.4 mg of naloxone was given 15 min before the glucose challenge which was given at time 0 min. Serum glucose concentrations were measured and hormone determinations were performed before naloxone administration and after the glucose challenge as noted above.

Radioimmunoassay of β-EP/β-LP. β-EP and β-LP measurements were made on blood collected in polypropylene tubes containing EDTA and assayed using a modification of the radioimmunoassay procedure described by Carr *et al.* (10). In order to eliminate nonspecific interfering factors in the radioimmunoassay, the plasma samples were routinely extracted using silica (Prepak 500; Waters Associates, Milford, MA) before radioimmunoassay. In a typical assay, 1 ml of plasma was mixed with 75 mg of silica and vortexed for 10 min at room temperature. After centrifugation the supernatant was discarded. The peptide adsorbed to silica was washed twice with distilled water and eluted with 1 ml of acidified aqueous acetone (50% acetone v/v, 49% water v/v, and 1% of 1 N hydrochloric acid). The elution was repeated with an additional 1 ml of acidified acetone. The pooled eluate was dried under nitrogen, and the dried eluate was reconstituted with 0.5 ml of assay buffer (0.1 M sodium phosphate (pH 7.4), 0.05 M sodium chloride, 0.01 M EDTA, 2% aprotinin (Trysalol) (v/v), and 0.05% crystalline bovine serum albumin). After reconstitution and centrifugation, 0.2 ml of clear supernatant was mixed with 0.2 ml of antiendorphin (rabbit) and incubated at 4°C. After 20 hr of incubation, 0.1 ml of ^{125}I-β-EP was added and the incubation continued for 20 hr at 4°C. At the end of the incubation, 0.1 ml of 4% normal rabbit serum was added as carrier protein. The bound/free separation was achieved using a second antibody (goat anti-rabbit γ-globulin) accelerated by polypropylene glycol. The radioimmunoassay described above is sensitive to 1.6 pg/tube. Interassay variation is 16% and intraassay variation is 9.2%.

The antiendorphin used in this assay has equimolar cross-reactivity with β-EP and β-LP and has <1% cross-reactivity with leu-enkephalin, met-enkephalin, α-endorphin, γ-endorphin, and adrenocorticotropin. Total β-EP/β-LP was assayed rather than individually after separation because of the limited amount of plasma available. In the separation procedure, the extracted samples are assayed after fractionation on a calibrated Sephadex G-25 column (0.5 × 40 cm). The column is eluted with assay buffer and the fractions corresponding to β-EP and β-LP are pooled separately and assayed according to the above procedure. For column chromatography studies 3–5 ml of samples are extracted and reconstituted in a 0.5-ml volume and loaded onto the column for separation. After separation each fraction is pooled and concentrated in a Speed Vac Concentrator (Savant) and assayed by radioimmunoassay. Extraction efficiency for β-EP is 71% and for β-LP 68%. Values that we report for samples that go through the column are corrected for loss in the column (10%). The rest of the samples are corrected only for extraction efficiency (approximately 70%).

Radioimmunoassay of Insulin. The concentration of insulin in rat plasma was measured by radioimmunoassay using ^{125}I-porcine insulin and antiporcine insulin antibody (11). Rat insulin has 100% cross-reactivity in this system and the rat insulin values are expressed based on the porcine insulin standard. Plasma samples from both groups (lean and obese) diluted parallel with the standard. In a typical assay, 0.1 ml of rat plasma is mixed with 0.1 ml of insulin antibody (raised in guinea pigs) and 0.3 ml of ^{125}I-insulin and the mixture is incubated at 25°C for 4 hr. At the end of the incubation period, the bound/free separation was achieved using rabbit anti-guinea pig γ-globulin and polyethylene glycol. The anti-insulin antibody has no cross-reactivity with glucagon, C-peptide, adrenocorticotropic hormone, vasoactive intestinal peptide, and gastrin. The intraassay variation is 4.7% and the interassay variation is 8.1%.

Statistical Analysis. This was performed using Student's t test for grouped data and the Mann-Whitney and Wilcoxon tests.

Results

The results are summarized below and in Tables I and II. All values are mean ± SD.

Basal Glucose, Insulin, and β-EP/β-LP Concentrations. Basal glucose concentrations were significantly higher in the obese rats than in their lean littermates (192.8 ± 30.7 mg/dl vs 120.9 ± 38.5 mg/dl, $P <$ 0.01). Insulin concentrations in the obese animals paralleled the glucose concentrations and were 18.56 ± 3.73 microunits/ml in the obese animals and 7.64 ± 2.51 microunits/ml in the lean animals ($P < 0.001$). In a separate group of animals, naloxone administration failed to alter basal insulin (19.25 ± 3.95 vs 18.56 ± 3.73 microunits/ml, P = ns) concentrations in the obese animals. β-EP/β-LP concentrations were 68.75 ± 21.70 pg/ml in the obese rats and 86.4 ± 24.11 pg/ml in the lean animals, P = ns). Naloxone treatment failed to

Table I. Baseline Concentrations of Serum Glucose and Plasma Concentrations of Insulin and β-Endorphin/β-Lipotropin in Obese and Lean Zucker Rats[a]

	Glucose (mg/dl)	Insulin (microunits/ml)	β-endorphin/β-lipotropin (pg/ml)	
Obese	192.80 ± 30.7	18.56 ± 3.73	68.75 ± 21.70	53.75 ± 21.40*
	$P < 0.01$	$P < 0.001$	P = NS	
Lean	120.90 ± 38.50	7.64 ± 2.51	86.40 ± 24.11	81.00 ± 15.64*

[a] Postnaloxone values of β-endorphin/β-lipoprotein are represented by *. Values are mean ± SD.

Table II. Plasma Concentrations of Insulin and β-Endorphin/β-Lipotropin after Glucose Challenge in Obese and Lean Zucker Rats[a]

	Insulin (microunits/ml)			β-endorphin/β-lipotropin (pg/ml)	
	5 min	20 min	30 min	5 min	20 min (peak)
Obese	27.14 ± 18.12	77.90 ± 28.2	66.60 ± 33.6	56.17 ± 23.17	85.33 ± 27.78
	$P < 0.03$	$P < 0.01$	$P < 0.01$	$P < 0.003$	
Lean	12.64 ± 3.42	39.30 ± 5.90	34.10 ± 7.40	87.10 ± 18.36	101.40 ± 21.74

[a] Values are mean ± SD.

significantly alter basal β-EP/β-LP in either the obese (68.75 ± 21.70 vs 53.75 ± 21.40 pg/ml) or lean (86.4 ± 24.11 vs 81.00 ± 15.64 pg/ml) animals (Table II).

Glucose-Stimulated Insulin and β-EP/β-LP Concentrations. Early phase insulin release (5 min) was significantly different in the obese versus the lean animals (27.14 ± 18.12 vs 12.64 ± 3.42 microunits/ml, $P < 0.03$). Late phase insulin release, as reflected in the 20- and 30-min insulin concentrations, were significantly different in the obese versus the lean rats. Peak insulin concentrations measured at 20 min after glucose injection were 77.90 ± 28.20 microunits/ml in the obese animals and 39.30 ± 5.90 microunits/ml in the lean rats ($P < 0.01$). Corresponding insulin concentrations at 30 min were 66.60 ± 33.60 vs 34.10 ± 7.40 microunits/ml ($P < 0.01$). After glucose loading, β-EP/β-LP concentrations were significantly higher in the lean animals than in the obese animals. Five minutes after glucose loading, β-EP/β-LP levels were 56.17 ± 23.17 pg/ml in the obese rats and 87.10 ± 18.36 pg/ml ($P < 0.003$) in the lean animals, respectively. Corresponding levels at 10 min were 59.60 ± 23.51 and 88.6 ± 13.97 pg/ml, $P < 0.004$. β-EP/β-LP concentrations peaked at approximately 20 min after the glucose challenge (101.40 ± 21.74 in the lean animals and 85.33 ± 27.78 pg/ml) in the obese rats, after which the values progressively declined to baseline values.

Discussion

This study demonstrates that obese Zucker rats have higher basal concentrations of glucose and immunoreactive insulin compared with those of their lean littermates. The pattern of early and late phase insulin release after glucose challenge was similar in both the obese rats and the lean animals, indicating relatively normal β cell reserve in the obese animals. Unlike humans with the polycystic ovary syndrome, the hyperinsulinemia seen in the obese Zucker rats was not associated with elevation of basal β-EP/β-LP concentrations. Moreover, the carbohydrate intolerance and hyperinsulinemia in the obese animals could not be reversed with opioid blockade with naloxone. This is consistent with the report by Feldman *et al.* (12) who showed that naloxone was ineffectual in reversing the hyperglycemia induced by β-endorphin administration in humans with type II diabetes mellitus. These observations point to the conclusion that the peripherally circulating endogenous opioids may not play a prime role in the genesis of the hyperinsulinemia, carbohydrate intolerance, and obesity in the obese Zucker rat. The previously reported decline in feeding following treatment with naloxone in obese mice and rats (6, 8) most likely represents the central effects of opioid blockade and not peripheral effects, particularly as they relate to pancreatic insulin release.

Following glucose challenge, β-EP/β-LP increased in both the obese and lean rats peaking at about the same time (15 min). Although β-EP/β-LP concentrations increased after glucose challenge in both groups of animals, the increment was lower in the obese animals. These findings suggest a possible dissociation between insulin secretion and endorphin release in the obese animals. This may be partly responsible for the persistent hyperglycemia in the obese animals since β-EP has been shown to stimulate insulin release from the endocrine pancreas (3, 13). The diminished β-EP release following glucose challenge, or a reduction in the β-EP to β-LP ratio after glucose challenge, would lead to inappropriately low insulin release relative to the degree of hyperglycemia. There is evidence that the

β-EP to β-LP ratio in some tissues, such as the heart, is influenced by procedures such as castration and testosterone administration (14). It remains to be established whether glucose challenge in the obese male Zucker rat modifies this ratio.

Furthermore, if there is a short-loop negative feedback between pancreatic opioids and basal insulin secretion the relatively lower β-EP release with carbohydrate challenge could, in time, lead to basal hyperinsulinemia, peripheral insulin resistance, and obesity. An increase in opioid-driven feeding behavior as shown by other investigators would serve to exaggerate the insulin resistance, obesity, and carbohydrate intolerance in the animals.

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