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ALTERATIONS IN THE BINDING CHARACTERISTICS OF GLUCOCORTICOID RECEPTORS FROM OBESE ZUCKER RATS

B. DOUGLAS WHITE* and ROY J. MARTIN
University of Georgia, Department of Foods and Nutrition, Dawson Hall, Athens, GA 30602, U.S.A.

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Summary—Obese Zucker rats appear to lack a circadian rhythm of serum corticosterone and maintain relatively high concentrations throughout the 24-h day. The binding characteristics of glucocorticoid receptors in lean and obese Zucker rats were examined in three tissues suggested to be involved in the feedback inhibition of corticosterone: the anterior pituitary, hypothalamus and hippocampus. Hepatic glucocorticoid receptors were also examined to determine if receptor alterations exist in a peripheral tissue. The dissociation constant (K_d) of glucocorticoid receptors in the anterior pituitary of obese rats was 50% greater than the K_d of receptors derived from lean rats. This suggests a decrease in the affinity of these receptors and could indicate a reduced feedback inhibition of corticosterone at the anterior pituitary. Hepatic glucocorticoid receptors of obese rats also showed an increase (150%) in the K_d of binding and a reduction (40%) in the number of receptors. No difference was observed in the K_d or maximal binding of receptors from the hypothalamus or hippocampus of lean and obese rats. It appears that glucocorticoid receptor alterations exist in obese Zucker rats and that these alterations may affect the drive of the pituitary–adrenal axis and possibly the expression of obesity.

INTRODUCTION

The obese Zucker rat is a genetic model of juvenile-onset obesity. The obese trait is passed to successive generations by an autosomal recessive mode of inheritance, which suggests that the obesity may be caused by a single gene defect. Corticosterone has been implicated as having an important role in the expression of obesity in Zucker rats. Many of the abnormalities associated with the obese syndrome are attenuated or reversed by adrenalectomy, while corticosterone replacement negates the effect of adrenalectomy [1–3]. It has been suggested that the primary lesion(s) in the obese Zucker rat may be sensitive to circulating levels of corticosterone [4].

Obese rats appear to lack a circadian rhythm of serum corticosterone [5, 6] and maintain relatively high concentrations of serum corticosterone throughout the 24-h day. This results in a significant elevation in the morning corticosterone concentration in obese rats as compared to lean rats. We have previously shown that the metabolic clearance rate of corticosterone is elevated in obese rats on a per rat basis, suggesting that the adrenal output of corticosterone may actually be greater than expected by observing the serum corticosterone concentration [7]. Examination of the *in vitro* corticosterone output of isolated adrenalcortical cells from lean and obese rats has shown no difference in the sensitivity or maximal responsiveness to adrenocorticotropin (ACTH) on a per protein or DNA basis [8]. This suggests that the elevated corticosterone output in obese rats may be due to a defect at the level of the anterior pituitary, hypothalamus or higher brain center, rather than at the level of the adrenal gland. This is supported by the finding of elevated serum ACTH concentrations in obese male Zucker rats as compared to their lean counterparts [8].

The amount of ACTH secreted is determined by the balance between positive (corticotropic-releasing hormone, vasopressin) and negative (glucocorticoids) influences on the corticotrophs of the anterior pituitary. In this study, we examined the glucocorticoid receptor binding characteristics in three tissues suggested to be involved in the feedback inhibition of corticosterone: the anterior pituitary, hypothalamus and hippocampus. Alterations in the binding characteristics of these receptors in obese rats may indicate an alteration in corticosterone feedback inhibition. Additionally, the binding characteristics of glucocorticoid receptors in the liver were examined to determine if receptor alterations exist in a peripheral tissue.

The affinity of glucocorticoid receptors for [^{3}H]-dexamethasone was reduced in the anterior pituitary of obese rats, which may indicate a decrease in feedback inhibition at that level. Additionally, the affinity and number of glucocorticoid receptors in the

*To whom correspondence should be addressed.

liver was reduced in obese rats. The binding characteristics of glucocorticoid receptors in the hypothalamus and hippocampus were similar in lean and obese rats.

EXPERIMENTAL

Animals

Ten lean (Fa/?) and 8 obese male Zucker rats (approx. 15 weeks old) were used in this experiment. Lean rats had an average body weight of 391 ± 10 g, while obese weighed an average of 597 ± 17 g. Rats were obtained from our colony at the University of Georgia. They were reared under constant environmental conditions (22–24°C ; 7:00 a.m. lights on—7:00 p.m. lights off) with *ad libitum* access to Purina lab chow and water.

Procedures

The rats were anesthetized with ether and bilaterally adrenalectomized via a dorsal approach within 24 h of sacrifice. Rats were sacrificed by decapitation between 9:00 and 11:00 a.m. To determine the completeness of adrenalectomy, trunk blood was collected and serum isolated for corticosterone analysis. Serum was stored at −80°C until corticosterone determination. Brains were removed and the anterior pituitary, hypothalamic block and hippocampus isolated according to previously described procedures [9]. Like brain tissues from two rats of the same phenotype were pooled to form 4 and 5 independent groups (obese and lean, respectively). Liver tissue was excised from each animal.

Brain tissues were homogenized with a glass tissue homogenizer in 6 ml of Tris buffer (4°C). (5 mM Tris, 1 mM EDTA, 1 mM dithiothreitol, 10 mM sodium molybdate, and 10% glycerol, pH 7.4). Two grams of liver tissue was homogenized in 12 ml of Tris buffer. Tissue homogenates were centrifuged at 100,000 *g* for 1 h at 4°C to obtain the crude cytosolic receptor fraction. The lipid layer was aspirated from liver samples. Supernatants were aliquoted for glucocorticoid receptor and protein determinations and stored at −80°C.

Cytosolic glucocorticoid receptor assays were performed by a modification of the methods described by Saplosky *et al.* [10]. Total binding was determined by the addition of 100 μl of cytosolic receptor fraction to a mixture of 100 μl of [1, 2, 4, 6, 7-^{3}H]-dexamethasone (Amersham, Arlington, Ill.) and 100 μl of Tris buffer. Seven concentrations of tritiated dexamethasone were used. The final dexamethasone concentrations ranged from 0.1 nM to 30.0 nM. Non-specific binding was determined in parallel tubes by replacing the Tris buffer with 1000-fold excess cold dexamethasone. Total binding and non-specific binding tubes were run in triplicate and incubated at 4°C overnight. Bound hormone was collected by LH-20 columns and the radioactivity quantitated on a scintillation counter. Specific binding was determined by the difference between total binding and non-specific binding. A Scatchard analysis was initially run on each sample to determine its linearity. The maximal binding (B_{max}) and the dissociation constant (K_d) of each sample was determined with the computer program LIGAND [11]. B_{max}s were corrected for the amount of protein present in the sample.

Serum corticosterone concentrations were determined by a radioimmunoassay kit (ICN; Carson, Calif.). Protein concentrations were determined by the Bradford assay (Bio-Rad Laboratories; Richmond, Calif.).

Differences between the receptor binding parameters and serum corticosterone concentration of lean and obese rats were determined by Student's *t*-tests. Differences were deemed statistically significant at the 0.05 level.

RESULTS

The average serum corticosterone concentration for the adrenalectomized animals was 1.1 ± 0.1 ng/ml and 3.1 ± 1.7 ng/ml for lean and obese rats, respectively. These values were not statistically different. Serum corticosterone concentrations for intact lean and obese rats at this time of day have been measured to be around 20 ng/ml and 120 ng/ml (lean and obese, respectively) [5]. Adrenalectomy was considered complete for all the rats.

A representative binding curve (A) and Scatchard plot (B) for the anterior pituitary, hypothalamus, hippocampus, and liver are shown in Fig. 1(a–d), respectively. The averaged correlation coefficient of the Scatchard plots are shown ± SE. Values of the correlation coefficients ranged from 0.96 for the anterior pituitary to 0.99 for the hippocampus.

The maximal binding and dissociation constant (K_d) for the four tissue types are listed in Table 1. The K_d of glucocorticoid receptors in the anterior pituitary from obese Zucker rats was approximately 50% higher than the K_d of receptors derived from lean rats. Likewise, glucocorticoid receptors in the liver from obese rats had a 150% greater K_d than did those from lean rats. No difference in K_d between lean and obese rats was observed in the hypothalamus or hippocampus. In the liver, obese rats showed 40% fewer glucocorticoid binding sites as compared to lean rats. No difference in maximal binding was found in the anterior pituitary, hypothalamus or hippocampus of lean and obese rats.

DISCUSSION

In the present study, alterations were observed in the binding characteristics of glucocorticoid receptors from obese Zucker rats. We believe this to be the first demonstration of a difference in glucocorticoid receptor binding in lean and obese Zucker rats.

Correlation coefficients of the Scatchard plots were fairly linear [Fig. 1(a–d)]. These data are consistent

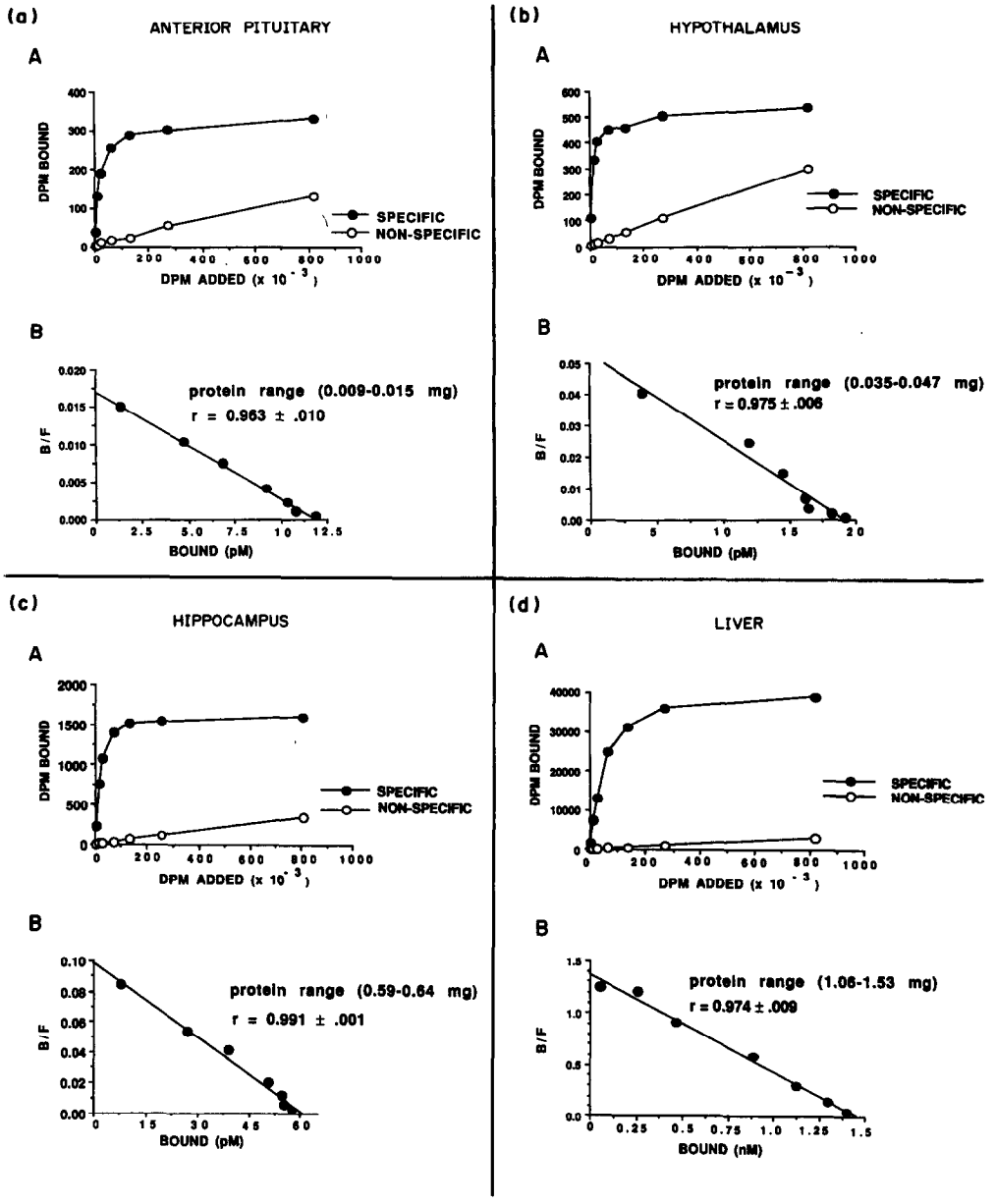

Fig. 1. (a) Representative binding curve (A) and Scatchard plot (B) for the anterior pituitary. The protein range of the cytosolic receptor fraction is shown. The average correlation coefficient ± SE of all Scatchard plots of anterior pituitary tissue is shown. (b) Representative binding curve (A) and Scatchard plot (B) for the hypothalamus. The protein range of the cytosolic receptor fraction is shown. The average correlation coefficient ± SE of all Scatchard plots of hypothalamic tissue is shown. (c) Representative binding curve (A) and Scatchard plot (B) for the hippocampus. The protein range of the cytosolic fraction is shown. The average correlation coefficient ± SE of all Scatchard plots of hippocampal tissue is shown. (d) Representative binding curve (A) and Scatchard plot (B) for the liver. The protein range of the cytosolic receptor fraction is shown. The average correlation coefficient ± SE of all Scatchard plots of hepatic tissue is shown.

with a single class of glucocorticoid receptor with a homogeneous affinity for the ligand.

In the anterior pituitary, glucocorticoid receptors from obese Zucker rats had a greater K_d than did receptors derived from lean rats (Table 1). This indicates that glucocorticoid receptors from the anterior pituitary of obese rats had less affinity for the glucocorticoid, [^{3}H]dexamethasone, than did

Table 1. Dissociation constant (K_d) and maximal binding of [^{3}H]dexamethasone in lean obese Zucker rats

	AP		HYP		HIP		Liver	
	L	O	L	O	L	O	L	O
K_d (nM)	0.39 ± 0.05 (5)	0.58 ± 0.06 (4)	0.36 ± 0.02 (5)	0.38 ± 0.03 (4)	0.58 ± 0.02 (3)	0.54 ± 0.02 (2)	1.31 ± 0.12 (9)	3.36 ± 0.15 (5)
	$P < 0.05$		NS		NS		$P < 0.001$	
b_{max} (fmol/mg)	189 ± 27 (5)	253 ± 47 (4)	141 ± 7 (5)	132 ± 4 (4)	314 ± 7 (3)	309 ± 5 (2)	437 ± 30 (9)	273 ± 31 (5)
	NS		NS		NS		$P < 0.05$	

Means are shown ± SE. Number in parentheses represents the number of observations. Differences between lean (L) and obese (O) rats are shown. (NS–not significant). AP—anterior pituitary, HYP—hypothalamus, HIP—hippocampus. Due to the loss of some samples some groups (i.e. hippocampus and liver) are missing observations.

receptors from lean rats. This suggests that glucocorticoid receptors in the anterior pituitary of obese rats may have less affinity for the endogenous glucocorticoid, corticosterone. A decreased affinity for corticosterone could result in attenuated feedback inhibition. At the anterior pituitary, glucocorticoids inhibit ACTH release from corticotrophs [12, 13]. If feedback inhibition is attenuated in obese rats at the level of the anterior pituitary, they might be expected to have an increase release of ACTH. This is consistent with the finding that obese male Zucker rats have elevated serum concentrations of ACTH [8].

Similar to glucocorticoid receptors in the anterior pituitary glucocorticoid receptors in the liver of obese rats showed an elevated K_d when compared to receptors derived from lean rats. The magnitude of the difference was far greater in the liver than in the anterior pituitary (50 vs 150%; anterior pituitary and liver, respectively). These data suggest that hepatic glucocorticoid receptors in obese rats may have less affinity for circulating levels of corticosterone. No difference, however, was observed in the K_d of glucocorticoid receptors from the hypothalamus or hippocampus of lean and obese rats. This indicates that the elevated K_d of glucocorticoid receptors in obese rats has some tissue specificity.

The reason for the greater K_d in obese rats is not known. It is possible that the differences in K_d of glucocorticoid receptors between lean and obese rats were artifactual. If the cytosolic receptor fractions from obese rats contained greater amounts of endogenous corticosterone, then more competition for receptors sites could have occurred at the lower doses of [^{3}H]dexamethasone. This would result in an elevation in the apparent K_d. However, we do not believe this to be the case. Serum corticosterone concentration between adrenalectomized lean and obese rats were very low and not significantly different. Additionally, differences in the K_d between lean and obese rats showed tissue specificity. If differences in serum corticosterone concentrations resulted in greater cytosolic corticosterone concentrations in obese rats, we would expect the K_d to be elevated in all tissues examined.

In addition to the difference in the K_d, obese rats showed a decreased maximal binding of hepatic glucocorticoid receptors. This indicates that the receptor concentration was decreased in the liver of obese rats. Obese Zucker rats have been shown to have elevated concentrations of serum corticosterone, at least during part of the 24-h day [5, 6, 8, 14]. Autoregulation of hepatic glucocorticoid receptors has been previously demonstrated [15–17]. Chronic or acute glucocorticoid treatment can reduce the number of liver glucocorticoid receptors. The reduced number of liver glucocorticoid receptors in obese rats may be a result of elevated circulating levels of corticosterone prior to adrenalectomy.

The number of hepatic cytosolic glucocorticoid receptors has previously been examined in obese and lean Zucker rats [18]. In this study, no difference in receptor number was found between the two phenotypes. This discrepancy with the present data may be due to several procedural differences. The aforementioned study used 6 week-old, female rats, while the present used 15 week-old, male rats. The sex or age of the animal may affect the difference in hepatic glucocorticoid receptors of lean and obese rats. Additionally, in the previous study, the animals were adrenalectomized 2 weeks prior to sacrifice. One of the purposes of adrenalectomy is to promote the translocation of receptors from the nucleus to the cytosol. Thereby, a measure of cytosolic receptors gives an indication of total receptor number. Over extended periods of time, however, adrenalectomy can increase the number of cystolic glucocorticoid receptors by up-regulation as well as receptor translocation. The number of glucocorticoid receptors in the hippocampus has been found to increase over a 13 day period following adrenalectomy [19]. By 2 weeks after adrenalectomy, up-regulation of the glucocorticoid receptors may have occurred. In the previous study, hepatic glucocorticoid receptors of lean and obese rats may have up-regulated to a similar extent, negating a potential difference in the number of hepatic glucocorticoid receptors in the intact rats.

No difference in the receptors' maximal binding was observed in the anterior pituitary, hypothalamus, or hippocampus. This suggests that receptor number changes in obese rats may also be tissue specific. If the receptor number in the liver of obese rats was decreased by elevated circulating concentrations of corticosterone, the finding of no difference in the number of hippocampal glucocorticoid receptors seems somewhat surprising. Corticosterone administration to adrenalectomized rats results in a lower

number of glucocorticoid receptors in the hippocampus [20]. Using a time-frame of adrenalectomy similar to ours, Sapolsky *et al.* [10] observed a decrease in the number of hippocampal glucocorticoid receptors in response to stress and corticosterone administration. These data suggest that the number of glucocorticoid receptors in the hippocampus can be regulated by glucocorticoids. In these previous studies, corticosterone administration did not affect the number of glucocorticoid receptors in the hypothalamus [10, 20] or anterior pituitary [10]. The reason for the lack of difference in the hippocampus in the present study is not known. A species difference between the rats used in the previous studies and the Zucker rats used in the present study may account for this difference. Additionally, the reduction of glucocorticoid receptor numbers in obese rats may be mediated by a factor other than elevated serum corticosterone.

Several studies indicate that obese Zucker rats are more sensitive to the actions of glucocorticoids than are lean rats [1–3]. An increased sensitivity to glucocorticoids has also described for the genetically obese (ob/ob) mouse [21]. These data seem paradoxical to our finding that the number and affinity of hepatic glucocorticoid receptors were reduced in obese rats. A possible explanation for this apparent paradox could lie in the dual nature of the subclasses of glucocorticoid receptors. Two distinct types of glucocorticoid receptor have been found: Type I, corticosterone-preferring receptor (CR) and Type 2, glucocorticoid receptor, which is synonymous with the classic hepatic glucocorticoid receptor (GR). (reviews: [22, 23]). Because of a 10-fold increase in affinity of CR for corticosterone as compared to GR, it has been suggested that the responses to low levels of corticosterone are mediated by CR, while the responses to high or stress levels of corticosterone are mediated by GR [23]. It has also been proposed that corticosterone, through its interaction with these two receptor types, mediates two distinctly opposite metabolic actions [24]. At low concentrations corticosterone promotes weight gain and elevates food efficiency, while at high concentrations corticosterone reduces weight gain and inhibits food efficiency. The use of different agonists suggested that the anabolic phase is mediated by CR and the catabolic phase is mediated by GR. The direction and magnitude of the overall metabolism may be influenced by a balance between the activation of these two receptor types. If the number and/or affinity of glucocorticoid receptors mediating the catabolic processes are reduced in obese Zucker rats, then for a given amount of corticosterone there may be less antagonism against the anabolic processes. The result would be that for a given amount of corticosterone, obese rats would have a greater anabolic response; thus they may appear to be more sensitive to corticosterone.

In summary, glucocorticoid receptors in the anterior pituitary of obese rats showed a decreased affinity to [^{3}H]dexamethasone when compared to their lean counterparts. This suggests a decreased sensitivity to glucocorticoids and may indicate attenuation of feedback inhibition in obese rats at the level of the anterior pituitary. Likewise, glucocorticoid receptors in liver tissue showed an elevated K_d, again suggesting a decreased affinity for glucocorticoids. In addition, the number of hepatic glucocorticoid receptors was found to be reduced in obese rats. No differences were found in K_d or maximal binding in the hypothalamus or hippocampus of lean and obese rats.

The present study demonstrated that alterations may exist in the glucocorticoid receptors of obese Zucker rats. These alterations may increase the drive of the pituitary–adrenal axis through attenuated feedback inhibition of corticosterone and may affect the expression of obesity in Zucker rats through a decrease in glucocorticoid-mediated catabolic processes.

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