Plasma Corticosterone Concentrations in Diabetic (*db*) Mice*

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Summary. Plasma corticosterone concentrations in diabetic (*db*) mice maintained on two different inbred backgrounds were found to be elevated significantly when compared to normal controls. These data are similar to that reported with the phenotypically similar obese (*ob*) mouse. These results suggest that the hyperadrenocorticism is not related to the primary gene action of either gene but rather is a consequence of the development of the obesity-diabetes syndromes.

Key words: Corticosterone, diabetes, obesity, mutation.

The role of the adrenal gland in the aetiology of the obesity of the genetically obese (*ob*) mouse has been extensively studied (see review by Bray & York, ref. 1). Adrenal cortical hypertrophy [2, 3], increased glucocorticoid production in vitro [4] and increased plasma corticosterone concentrations [5, 6, 7] have all been described. Bilateral adrenalectomy has been reported to decrease the rate of accumulation of body weight, reduce blood sugar concentrations, improve glucose tolerance and reduce insulin resistance [8].

Another obese mutant, diabetes (*db*), is phenotypically identical to the obese mouse when both mutants are maintained on the same inbred background [9, 10]. The original studies on the diabetes (*db*) mutant were done on inbred mice of the C57BL/KsJ strain of origin (BL/Ks) rather than on mice of the C57BL/6J (BL/6) strain in which the obese (*ob*) mu-

tation originally was maintained. On these genetically different backgrounds both mutations caused distinctly different disease syndromes. However, the role of the adrenal gland and corticosterone production on the obesity-diabetes state has not been studied in the diabetes mutant on the BL/Ks background. When it was established that the diabetes and obesity genes presented in an identical clinical fashion when both were maintained in the same inbred background, we wondered whether the abnormal adrenal function observed in BL/6 obese mice was the result of the obese (*ob*) gene itself or represented the modifying effects of the BL/6 inbred background. Both BL/6-*ob/ob* and BL/Ks-*db/db* mice are available at this laboratory, making possible studies on the effect of background on adrenal function in both strains of diabetic (*db*) mice for comparison with the previous studies undertaken with obese (*ob*) mice.

Materials and Methods

The inbred and mutant mice used in this study were produced in our research colony and were housed in stainless steel pens on pine shavings. Food and tap water were provided ad libitum. The pelleted food was a standard mouse chow, containing 11 per cent of protein and 6 per cent of fat, manufactured by the Emory Morse Company of Guilford, Connecticut. One week prior to assay the mice were separated and placed individually in pens so as to avoid any stress of crowding. Twenty-four hours prior to the assay the mice were removed for acclimatization to the room where they would be bled. All mice studied were between 12 and 13 weeks of age and were all bled on the same day, between 13:00 and 14:00 h, for corticosterone determinations. Prior to the separation of the mice into separate cages, 6 mice of each diabetic group were bled for blood sugar and plasma immunoreactive insulin (IRI) determinations to assess

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Table 1. Plasma corticosterone concentrations in normal and diabetic mice maintained on two inbred strains

Strain	Plasma corticosterone	Blood glucose	Plasma IRI
	$\mu\text{g}/100\text{ ml}$	$\text{mg}/100\text{ ml}$	$\mu\text{U}/\text{ml}$
BL/6-+/+	3.06 ± 0.32^a	N.D.	N.D.
BL/6-db/db	9.40 ± 0.81	228 ± 3.2	903 ± 7.8
BL/Ks-+/+	2.61 ± 0.52	N.D.	N.D.
BL/Ks-db/db	10.74 ± 0.88	390 ± 3.7	107 ± 2.7

^a Figures represent value $\pm$ standard error of the mean. Corticosterone determinations were made on 11 male mice of each genotype and strain whereas blood sugar and plasma IRI were determined only on 6 mice from each group. The normal values, not determined (N.D.) in this study, for blood sugar and insulin for both strains are 140 mg/100 ml and 80 μU per ml respectively

the extent of development of diabetes. Blood was obtained from the orbital sinus (glucose and IRI determinations) and by cutting the jugular vein (corticosterone). Blood sugar and plasma immunoreactive insulin (IRI) were determined as previously described [11]. Corticosterone was measured fluorimetrically by a technique used in this laboratory that has been specifically modified for the small volumes of plasma available from the mouse [12].

The experimental groups consisted of 11 normal and 11 diabetic mice each of both the BL/6 and BL/Ks strains.

Results

The results are shown in Table 1. As expected, the BL/Ks diabetic mice had typically higher blood sugar and lower plasma IRI concentrations than the BL/6 diabetic mice. These data are in keeping with our previous results [9, 10], with the BL/Ks stock having the more severe diabetes and the BL/6 stock having a few diabetic symptoms but a marked obesity.

Plasma corticosterone concentrations for both mutants on the two different backgrounds are also seen in Table 1. No differences are apparent between normal mice of either strain while both mutants on either strain had about 3 to 4 times the corticosterone concentration found in normal plasma. The values obtained, 9.40 $\mu\text{g}/100\text{ ml}$ (BL/6-db/db) and 10.74 (BL/Ks-db/db), did not differ significantly from each other. The low corticosterone concentrations observed in normal mice are similar to those values determined by others [6, 7] and are typical for unstressed normal mice.

Discussion

These data suggest that the adrenal abnormalities reported in obese mice are probably neither effects of the background genome on the disease expression nor

do they reflect the primary gene action of either obese or diabetes, since one would not expect two different genes on separate chromosomes to cause the same primary metabolic abnormality. These data suggest that the hyperadrenol-corticism observed in both these mutants on either genetic background is a consequence of the developing obesity-diabetes syndrome rather than being of primary aetiological significance in the development of the disease. However, adrenal dysfunction may well contribute significantly to the magnitude of the insulin resistance and obesity. Similar conclusions regarding hyperadrenalcorticism in obese mice have been put forward by Naeser [5] and others (see ref. 1).

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