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Hydrocortisone induction of tyrosine aminotransferase activity in genetically obese and diabetic mice—Effects of a multiple dosage schedule*

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THE ACTIVITY of liver L-tyrosine: 2-oxoglutarate aminotransferase, TA (EC 2.6.1.5) increases several-fold after the administration of hydrocortisone.[1] This enzyme system is one of the first to be induced by the hormonal action of glucocorticoids.[2] The rapid induction effect is attributed primarily to the rapid turnover rate of the enzyme protein.[3] When hydrocortisone is administered as a single intraperitoneal injection, the enzyme activity increases to a maximum in several hours and then declines rapidly to basal levels. Multiple injections of hydrocortisone maintain the induced enzyme activity for longer periods of time. In the multiple injection dosage schedule, smaller amounts of hydrocortisone result in the same or higher levels of induced TA activity.[4] The multiple injection schedule, with the total dose subdivided into small doses, is a more effective method of inducing and maintaining TA activity compared to a single large dose.

The present report deals with the hydrocortisone induction of liver TA activity in genetically obese and diabetic mice of the C57BL/6J-*ob* and C57BL/Ks-*db* strains using a multiple injection dosage schedule. Previous studies indicated that hydrocortisone was less effective in the induction of liver TA activity in C57BL/6J-*ob* (genetically obese) mice than in the normal littermates.[5] These results demonstrate that the induction of liver TA activity in the obese mice was approximately the same by either the single or multiple dosage schedule, indicating possibly an insensitivity of this enzyme system in the mutants to glucocorticoids. Other possible explanations are proposed. Similar results of

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TABLE 1. EFFECTS OF SINGLE AND MULTIPLE DOSES OF HYDROCORTISONE ON TYROSINE AMINOTRANSFERASE ACTIVITY IN DBA/2J, C57BL/6J AND C57BL/6J-ob MICE*

	Enzyme activity								
	DBA/2J			C57BL/6J			C57BL/6J-*ob*		
	Units	Mean response		Units	Mean response		Units	Mean response	
Injection schedule		(Δ increase)	(%)		(Δ increase)	(%)		(Δ increase)	(%)
Control (no treatment)	8·4 ± 0·5		100	7·5 ± 0·6		100	3·9 ± 0·2		100
Multiple saline	16·5 ± 0·7	8·1	204	10·8 ± 1·2	3·3	144			
Single hydrocortisone (1×)	21·9 ± 3·9	13·5	261	11·5 ± 0·7	4·0	153	8·5 ± 2·2	4·6	218
Single hydrocortisone (2×)	32·8 ± 4·8	24·4	391	20·0 ± 2·4	12·5	272			
Multiple hydrocortisone (1×)	33·8 ± 3·1	25·4	403	29·2 ± 1·5	21·7	389	10·4 ± 2·2	6·5	266

* Mice 8–10 weeks of age received intraperitoneal injections of hydrocortisone monosodium succinate (Solu-Cortef) in a total dose of either $5{\cdot}0 \times 10^{-5}$ moles (1×) or $1{\cdot}0 \times 10^{-4}$ (2×) per kg of body weight. In the single dose schedule, the total dose was administered at 0900 hr. In the multiple dosage schedule, the total dose was subdivided equally among several injections and was administered at hourly intervals starting at 0900 hr. Physiological saline, in volumes equivalent to the hydrocortisone dose, was administered intraperitoneally in the multiple dosage schedule. Control values refer to enzyme activity at 0900 hr. The DBA/2J and C57BL/6J mice were killed at 1500 hr. The C57BL/6J-*ob* mice were killed at 1330. The results are expressed as the mean ± the standard error for five mice in each experimental condition. The data are also expressed as mean response by average change (Δ) in enzyme activity and per cent increase.[4]

decreased glucocorticoid sensitivity of the TA induction system were demonstrated in the diabetic mice of two age groups. The younger group of mice were more resistant to exogenously administered glucocorticoid. In comparisons between normal mice of two inbred strains, hydrocortisone produced a greater enzyme induction effect in the DBA/2J mice relative to the C57BL/6J mice.

Inbred strains and mutant mice* were purchased from the Production Department of The Jackson Laboratory, Bar Harbor, Me. Mice were fed *ad lib.* a pelleted standard mouse diet manufactured by the Emory Morse Company of Guilford, Conn., containing 19 per cent protein and 6 per cent fat.

Tyrosine aminotransferase activity was assayed in 12,000 *g* liver supernatant fluid by the enol borate method of Lin and Knox,[1] adapted to an endpoint determination in which the concentration of *p*-hydroxyphenylpyruvate was calculated from the absorption differences at 310 mμ between the arsenate–borate mixture and the arsenate blank. The methods of liver enzyme preparation and enzyme assay are described in detail in another report.[5] Enzyme units are expressed as micromoles of *p*-hydroxyphenylpyruvate accumulated per gram of liver supernatant protein per minute at 25°.

The protein concentration of the liver supernatant fluid was determined by the biuret method[6] with 0·1% potassium iodide added to the reagent. Bovine serum albumin was utilized as the protein standard for the biuret determinations.

Hydrocortisone sodium succinate (Solu-Cortef) was purchased from the Upjohn Company, Kalamazoo, Mich.

The data presented in Table 1 show the effects of single and multiple injections of hydrocortisone on TA activity in DBA/2J, C57BL/6J, and C57BL/6J-*ob* mice. Hydrocortisone was more effective as an inducer of TA activity in the DBA/2J mice, suggesting a pharmacogenetic strain variance in the effectiveness of glucocorticoids to cause liver enzyme induction. A single injection of hydrocortisone (1X) to DBA/2J and C57BL/6J mice caused the TA activity to increase approximately 13·5 and 4·0 units, respectively, after 6 hr. A single injection of hydrocortisone (2X) caused the enzyme activity to increase approximately 24·4 and 12·5 units. When DBA/2J mice were treated with hydrocortisone in a multiple dosage schedule (1X), TA activity increased about 25·4 units above the control value and about 11·9 units above the single dosage schedule (1X). The multiple dosage schedule (1X) in the DBA/2J mice was about twice as effective as the single dose schedule (1X) and approximately equivalent to the response of the single dose schedule (2X). The maximum extent of enzyme induction appears to be more readily achieved in the DBA/2J mice, since the single hydrocortisone (2X) and multiple hydrocortisone (1X) produced about the same level of enzyme activity in 8 hr.

When C57BL/6J mice were treated with hydrocortisone in a multiple dosage schedule (1X), TA activity increased about 21·7 units above the control value and about 17·7 units above the value for the single dose schedule (1X). The multiple dosage schedule (1X) in the C57BL/6J mice was about five times more effective than the single dosage schedule (1X) and nearly twice as effective as the single dosage schedule (2X).

In speculation about causes for the strain differences in enzyme induction, one possible explanation is that the turnover rate of the inducer (glucocorticoid) is greater in the C57BL/6J mice, due to a higher rate of metabolism or removal from the body. As the concentration of the inducer decreases, the induced enzyme activity decreases. Glucocorticoid action is required to maintain the induced TA activity.[4] Another possible explanation is that there are kinetic differences between the two strains in rates of enzyme synthesis or degradation.[7]

The results presented in Table 1 also show the effects of single and multiple injections of hydrocortisone on liver TA activity in the obese C57BL/6J-*ob* mice. After a single dose of hydrocortisone, the enzyme activity increased about 2·2-fold above the control in 6·5 hr compared to 2·7-fold in the multiple dosage schedule. In normal C57BL/6J mice, the multiple dosage schedule was approximately 5·0 times more effective than the single dosage regimen. Solu-Cortef was less effective as an inducer of liver TA activity in obese mutant mice regardless of the dosage schedule. Liver TA activity in C57BL/6J-*ob* mice can be induced to higher levels by the use of a potent synthetic glucocorticoid such as flumethasone.[5]

The demonstration of a decreased sensitivity of a metabolic control mechanism in obese mice to exogenously administered glucocorticoid raises the question whether this system, and possibly other metabolic controls, are less responsive to glucocorticoids secreted endogenously. The basis for decreased glucocorticoid sensitivity of the liver TA induction system in obese mice may be due to

* The principles of laboratory animal care as promulgated by the National Society of Medical Research are observed in this laboratory.

several factors including steroid transport mechanisms, the regulation of steroid metabolism, or the binding of steroids to tissue specific "receptors". It is of interest that abnormal production of glucocorticoids has been implicated as a possible causal factor in Cushing's syndrome,[8] a disease associated with obesity and metabolic abnormalities characteristic of prediabetic or diabetic states. The specificity of the glucocorticoid control in obese mice could be tested with other hormones that modify TA activity such as epinephrine, glucagon, insulin and growth hormone[4]

The results presented in Table 2 show the effects of hydrocortisone in a multiple dosage schedule on liver TA activity in the diabetic C57BL/Ks-*db* strain. In the normal mice, ages 8 to 10 weeks, multiple injections of hydrocortisone increased the TA activity about 4·7-fold in 8 hr as compared to approximately 1·9-fold in the diabetic mice of the same age group. In the normal mice, 20 weeks of age, multiple injections of hydrocortisone increased the TA activity about 4·3-fold in 8 hr as compared to approximately 2·5-fold in the diabetic mice of the same age group. The multiple dosage schedule had a greater effect in the 20-week-old diabetic mice, resulting in smaller differences between the normal and

TABLE 2. HYDROCORTISONE INDUCTION OF TYROSINE AMINOTRANSFERASE ACTIVITY IN DIABETIC C57BL/Ks-*db* MICE*

Age (wk)	Injection	C57BL/Ks	C57BL/Ks-*db*
8–10	Control (none)	8·5 ± 0·8 (7)	9·6 ± 0·8 (5)
8–10	Hydrocortisone	39·6 ± 2·1 (6)	18·4 ± 2·8 (4)
20	Control (none)	7·0 ± 1·1 (6)	11·4 ± 1·0 (6)
20	Hydrocortisone	30·6 ± 2·2 (6)	27·9 ± 1·6 (4)

* Hydrocortisone monosodium succinate (Solu-Cortef) was administered intraperitoneally at hourly intervals in a multiple dosage schedule in which the total dose of $5{\cdot}0 \times 10^{-5}$ moles was divided equally among eight injections starting at 0900 hr. The results are expressed as the mean ± standard error. Numerals enclosed in parentheses refer to the number of mice.

mutant in the extent of TA induction. By comparison, in the 8- to 10-week-old mice, the multiple dosage schedule increased the enzyme activity 31·0 and 8·8 units above the control level for the normal and diabetic C57BL/Ks mice respectively. The enzyme induction response was greater in the 20-week-old mice, although they were more obese (average body weight, 47·7 g) than in the 8- to 10-week-old mice (average body weight, 41·7 g). The difference in response between the two age groups of diabetic mice suggests the possibility of an age-dependent factor influencing the glucocorticoid sensitivity of the liver TA induction system. The diabetic condition appears to develop in two age-dependent stages.[9] It would be of interest to study the glucocorticoid sensitivity of liver TA in younger diabetic and obese mice.

In summary, multiple small doses of hydrocortisone are more effective than an equivalent, single large dose ($5{\cdot}0 \times 10^{-5}$ moles/kg of body weight) in the 8-hr induction response of mouse liver TA activity. Significant pharmacogenetic variance was demonstrated between the C57BL/6J and DBA/2J inbred strains of mice in the effectiveness of hydrocortisone to cause liver TA induction. In addition, there were differential effects of the multiple dosage schedule on TA induction in the two strains. The multiple dosage relative to the single injection schedule produced about a 5-fold greater response in the C57BL/6J mice and a 2-fold greater effect in the DBA/2J mice. Liver TA activity in obese mutant mice was less responsive to multiple doses of hydrocortisone than in the normal mice. Similar results were demonstrated in the differential response of the diabetic mice of the C57BL/Ks-*db* strain relative to the normal controls.

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Effect of allylisothiocyanate on amino acid incorporation in rat liver microsomes*

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THE SEEDS of various plants of the Brassica family have been shown to be goitrogenic when fed to rats.[1, 2] The active component is considered to be a mixture of esters of isothiocyanic acid in the form of glucosides. These esters, commonly called mustard oils, have a high content of allylisothiocyanate (AITC) which is also goitrogenic.[3]

Leblova-Svobodova and Kostir[4] found that AITC treatment of germinating seedlings increased the content of free amino acids and decreased protein nitrogen, suggesting an inhibition of protein synthesis. Studies by Ahmad *et al.*[5] showed that rats fed AITC had reduced activities of some liver and kidney enzymes. In view of these reports, it appeared to be of interest to determine the effect of AITC on amino acid incorporation into rat liver microsomes.

Methods and materials

Adenosine triphosphate (ATP), guanosine triphosphate (GTP), creatine phosphate and creatine phosphokinase were from Sigma Chemical Company. ^{14}C-1-DL-leucine was obtained from Radiochemical Centre, Amersham, London. Sephadex G-25 Fine was a product of Pharmacia, Uppsala, Sweden.

Two groups of young male albino rats, 40 days old (obtained through the courtesy of the Pakistan SEATO Cholera Research Laboratory) were kept on a standard natural stock diet[6] for 7 days. At this time the rats weighed 70–80 g. The rats of one group were then injected intraperitoneally with 2 mg of AITC in 2 ml of distilled water per 100 g of body weight. The rats of the other group were treated as controls and administered only distilled water. Food consumption of the control rats was restricted to that of the AITC-treated rats. This procedure was continued for 7 days. On day 8, experimental and control rats were sacrificed and the incorporation of C-1-DL-Leucine by liver microsomes determined.

The livers were homogenised individually in 10 ml of ice-cold buffer[7] and cell debris, nuclei and mitochondria were precipitated by centrifugation at 19,000 *g* for 10 min. The supernatant solution was centrifuged at 19,000 *g* for 10 min to remove the last trace of mitochondria.

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