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EFFECT OF HYPERPHYSIOLOGICAL LEVELS OF STEROID HORMONES ON NICOTINAMIDE DEAMIDASE AND NAD SYNTHESIS IN MOUSE LIVER TISSUE*

L. S. DIETRICH

Department of Biochemistry, University of Miami School of Medicine, Miami, Fl., U.S.A.

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Abstract—The daily administration of a hyperphysiological, nontoxic level (10 mg/kg) of hexestrol to mice, for 6 consecutive days, progressively lowered the capacity of hepatic tissue to synthesize NAD upon nicotinamide challenge. The degree of inhibition of NAD synthesis was correlated with a decrease in the nicotinamide deamidase activity of the tissue.

Hepatic nicotinamide deamidase activity was also lowered by equimolar amounts of dienestrol, diethylstilbestrol, estradiol-17β, estrone, estriol, cortisone, and hydrocortisone.

All the compounds that decreased the hepatic nicotinamide deamidase also lowered the capacity of hepatic tissue to synthesize NAD upon nicotinamide challenge.

HORMONAL control of steady-state levels of pyridine nucleotides has been demonstrated by Greengard *et al.*,[1] who showed in hypophysectomized animals a prolongation in the elevation of hepatic NAD, produced by the administration of nicotinamide. Subsequent studies by the same author[2] demonstrated that the enzyme nicotinamide deamidase[3] apparently is under the control of pituitary.

The present study demonstrates that at hyperphysiological levels certain steroid hormones inhibit nicotinamide deamidase activity and NAD synthesis initiated by nicotinamide administration, whereas other steroid derivatives are devoid of activity.

METHODS

Black mice (C_{57}) and CD_8F_1 (BALB/C♀ × DBA/8♂ hybrid) mice, 3 to 4 months of age, were employed throughout. The animals were maintained in a room having constant temperature and constant humidity and fed a diet of Purina chow and water, *ad libitum*. The hormone derivatives were dissolved in sesame oil or were suspended in saline. Sesame oil or saline was administered to control animals. All hormone derivatives were administered i.m. at the level indicated for six consecutive days. On the seventh day the animals receiving nicotinamide (500 mg/kg) were injected i.p. The animals were sacrificed 4 hr later by decapitation, the livers immediately removed, and the NAD extracted and assayed by the procedure of Jedeikin and Weinhouse.[4]

Nicotinamide deamidase assays were carried out on the tissue homogenized on 0·44 M sucrose, by the procedure of Petrack *et al.*[3]

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RESULTS

The effect of hexestrol administration on NAD accumulation following the administration of nicotinamide is shown in Fig. 1. Groups of mice were killed at the indicated times after a single injection of either nicotinamide or isotonic sodium chloride solution, and concentration of NAD in each liver was determined. Each point on the curve represents the mean of 6–10 male mice. As can be seen from the figure, the

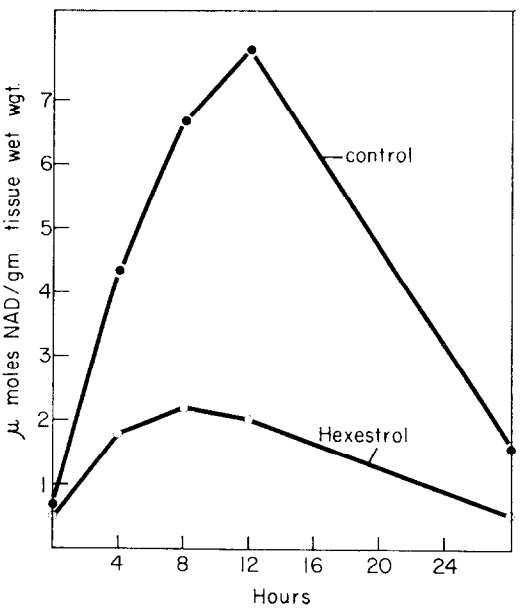

FIG. 1. Effect of hexestrol on the ability of mouse liver tissue to form NAD after nicotinamide administration. Male C_{57} black mice received hexestrol (10 mg/kg) in sesame oil for six consecutive days. Control animals received an equivalent amount of sesame oil. Animals were sacrificed at time intervals indicated after having received nicotinamide at a level of 500 mg/kg. Each point represent the mean values ± standard error from six to ten individual mice; ●, control; ○, hexestrol-treated.

NAD levels of livers from hexestrol-treated mice were markedly lower than those of control animals. There was no significant difference in initial values, which were 0·722 ± 0·025 and 0·650 ± 0·030 μmole/g tissue wet weight for the control and hexestrol groups respectively. These values fall into the normal range for mouse liver of this strain and age.

The effect of length of hexestrol treatment on its ability to inhibit NAD formation in mouse liver after the administration of nicotinamide is compared with the nicotinamide deamidase activity of mouse liver in Fig. 2. Each point on the curves represents the mean of 6–10 male mice. The animals were sacrificed 24 hr after the indicated hexestrol injection. During the six-day experimental period, the NAD concentration and the nicotinamide deamidase activity of the liver of the mice receiving sesame oil remained unchanged. The animals receiving a single injection of hexestrol in sesame oil showed only a slight decrease in their ability to form NAD after the administration of nicotinamide. Continued hexestrol administration caused a marked decrease in the ability of mouse liver tissue to form NAD after the administration of nicotinamide. A similar change was observed in the nicotinamide deamidase activity of mice

receiving hexestrol, enzymatic activity decreasing as hexestrol administration was continued.

The effect of various steroid hormones and synthetic estrogens on the NAD levels of mouse liver after nicotinamide challenge is presented in Table 1. The synthetic estrogens hexestrol, dienestrol, and diethylstilbestrol, given at a level of 10 mg/kg

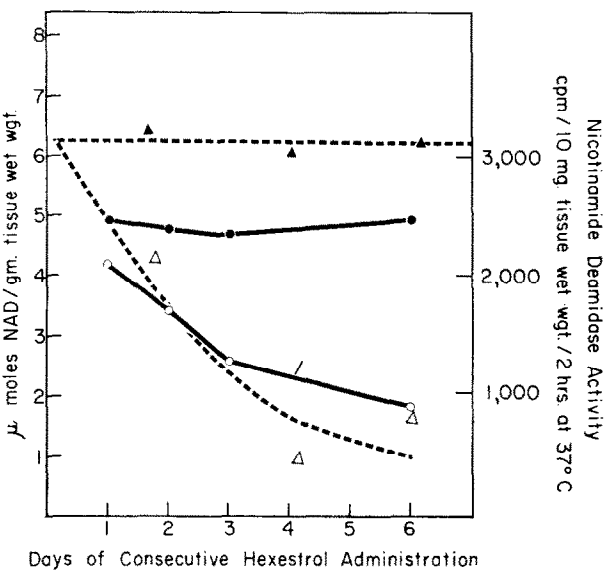

FIG. 2. Effect of hexestrol on the activity of nicotinamide deamidase and the ability of mouse liver tissue to form NAD after nicotinamide challenge. Male C_{57} black mice received hexestrol (10 mg/kg) in sesame oil for the number of consecutive days indicated. Control animals received an equivalent amount of sesame oil. Animals were sacrificed 4 hr after receiving nicotinamide (500 mg/kg) in the case of the NAD studies. Each point represents the mean values from eight to ten individual mice; ●, NAD values from control animals; ○, NAD values from hexestrol-treated animals; ▲, control values for nicotinamide deamidase activity; △, nicotinamide deamidase activity of hexestrol-treated animals.

for six consecutive days markedly lowered the levels of NAD normally produced by nicotinamide administration. An equimolar level of estradiol-17β was also effective under these experimental conditions. Estradiol-17α was without effect, as were progesterone, testosterone, and phloretin. Cortisone acetate at a dosage equivalent to the estrogens (16 mg/kg) was without activity (experiments 1 and 2). At a higher dosage (20 mg/kg), however, cortisone acetate did significantly lower the increase in hepatic NAD levels produced by nicotinamide challenge. Equivalent levels of hydrocortisone (14 mg/kg) under the same conditions significantly suppressed the liver NAD.

The effect of hexestrol administration on the increase in hepatic NAD produced by nicotinamide challenge in female mice is shown in experiment 3, Table 1. As in the male mice of the same strain, a marked suppression of NAD levels was observed.

The effect of various steroid derivatives and synthetic estrogens on the nicotinamide deamidase activity of mouse liver is presented in Table 2. The synthetic estrogens

diethylstilbestrol, dienestrol, and hexestrol given at 10 mg/kg for six consecutive days markedly suppressed the nicotinamide deamidase activity of mouse liver. A similar effect was observed with equivalent levels of the natural estrogens estradiol-17β, estrone, and estriol. The inactive analogue, estradiol-17α, was without activity, as were the compounds testosterone, progesterone, pregnenolone, phloretin, and

TABLE 1. EFFECT OF VARIOUS STEROID AND SYNTHETIC HORMONES ON THE NAD LEVELS OF MOUSE LIVER TISSUE AFTER NICOTINAMIDE CHALLENGE*

Expt. no.	Hormone	Dose (mg/kg)	NAD (μmoles/wet wt.)	"t" Value†
1	None		4·49 ± 0·21 (10)	
	Hexestrol	10	2·23 ± 0·25 (10)	7·1
	Dienestrol	10	2·59 ± 0·20 (10)	6·5
	Diethylstilbestrol	10	2·49 ± 0·16 (10)	7·7
	Estradiol-17β	10	3·56 ± 0·11 (10)	3·9
	Estradiol-17α	20	4·41 ± 0·26 (9)	< 2·0
	Progesterone	20	4·84 ± 0·30 (7)	< 2·0
	Testosterone	20	4·52 ± 0·23 (8)	< 2·0
	Cortisone acetate	16	4·12 ± 0·08 (8)	< 2·0
	Cortisone acetate	20	3·64 ± 0·19 (8)	3·0
	Phloretin	10	4·50 ± 0·17 (8)	< 2·0
2	None		5·84 ± 0·16 (8)	
	Hydrocortisone	14	5·21 ± 0·14 (8)	2·9
	Cortisone acetate	16	5·31 ± 0·21 (8)	< 2·0
3	None		4·43 ± 0·14 (8)	
	Hexestrol	10	2·23 ± 0·10 (8)	12·9

* The hormonal compounds dissolved in sesame oil except for the cortisone acetate and the hydrocortisone (which were suspended in saline) were administered i.m. for six consecutive days at the dosage indicated. All animals were sacrificed 4 hr after receiving nicotinamide (500 mg/kg). The data are the average of the values obtained from the number of individual animals (indicated in parentheses) ± S.E. Experiments 1 and 2 were carried out in male CD_8 mice, experiment 3 in female CD_8 mice.

† "t" = $[m_1 - m_2]/[\sqrt{(\sigma m_1)^2 + (\sigma m_2)^2}]$.

stilbene. The adrenal hormones desoxycorticosterone and 11-desoxy-17-hydroxycosterone given at an equivalent dosage to the estrogens were without activity. An equivalent level of either cortisone acetate or hydrocortisone significantly lowered the activity of hepatic nicotinamide deamidase.

The administration of hexestrol to female mice of the same strain (experiment 5, Table 2) also produced a marked suppression of hepatic nicotinamide deamidase activity.

Steroid toxicity. Starvation or changes in diet are known to alter the level of many hepatic enzymes. Since the hormone derivatives employed in this study are several magnitudes higher than that needed to produce a hormonal response in target tissues, and in view of the known toxicity of high levels of estrogens, the problem arises of whether or not the effects observed (Tables 1 and 2) are due to general toxicity which altered the food intake of the animals, thereby simulating a starved or semistarved condition. Consequently, body-weight changes and food intake were measured daily in another experiment: groups of 10 mice were employed and control, hexestrol-treated (10 mg/kg), and hydrocortisone-treated (14 mg/kg) animals were compared. The experiment extended one day beyond the six consecutive daily injections. No significant changes were observed in either body weight or food consumption

when the hydrocortisone and control groups were compared. In the case of the hexestrol-treated animals, no significant changes in body weight were observed. The food consumption of the hexestrol-treated animals was slightly lower than that of the control animals during the first three days of therapy but returned to normal for the remaining four days. On the basis of this experiment it is thought that general toxicity produced by these compounds plays a minor role, if any, in producing the observed enzymatic changes.

TABLE 2. EFFECT OF VARIOUS STEROID AND SYNTHETIC HORMONES ON THE NICOTINAMIDE DEAMIDASE ACTIVITY OF MOUSE LIVER TISSUE*

Expt. no.	Hormone	Dose (mg/kg)	Enzymatic activity†	"t" value‡
1	None		3,270 ± 264 (8)	
	Hexestrol	10	1,765 ± 310 (8)	3·7
	Dienestrol	10	1,259 ± 269 (8)	5·3
	Diethylstilbestrol	10	1,500 ± 110 (8)	6·2
	Estradiol-17β	10	2,072 ± 115 (8)	4·2
	Estradiol-17α	20	3,020 ± 218 (8)	<2·0
	Testosterone	20	2,810 ± 268 (8)	<2.0
2	Control		2,402 ± 233 (8)	
	Phloretin	10	2,709 ± 136 (8)	<2·0
	Estrone	10	1,096 ± 145 (8)	4·8
	Estriol	11	807 ± 100 (8)	6·3
	Corticosterone	14	1,871 ± 170 (8)	<2·0
	Cortisone acetate	16	1,420 ± 165 (8)	3·4
	Stilbene	10	2,377 ± 212 (8)	<2·0
3	Control		4,550 ± 238 (8)	
	Progesterone	20	3,997 ± 220 (8)	<2·0
	Pregnenolone	12	4,562 ± 148 (8)	<2·0
	Desoxycorticosterone	12	4,595 ± 162 (8)	<2·0
	11-Desoxy-17-hydroxycorticosterone	13	4,555 ± 183 (8)	<2·0
4	Control		4,838 ± 284 (8)	
	Cortisone acetate	16	2,767 ± 120 (8)	6·7
	Hydrocortisone	14	3,356 ± 213 (8)	4·2
5	Control		3,570 ± 264 (8)	
	Hexestrol	10	678 ± 220 (8)	8·4

* The hormonal compounds dissolved in sesame oil, except for the cortisone acetate and the hydrocortisone (which were suspended in saline), were administered i.m. for six consecutive days at the dosage indicated. The data are the average of the values obtained from the number of individual animals indicated in the parentheses ± S.E. All experiments were carried out on male CD_8 mice except in experiment 5 where female CD_8 mice were employed

† Counts per min nicotinic acid formed/10 mg itssue wet weight/2 hr at 37°.

‡ "t" = $[m_1 - m_2]/[\sqrt{(\sigma m_1)^2 + (\sigma m_2)^2}]$.

DISCUSSION

The hyperphysiological levels of hormonal materials employed to elicit a response (10—20 mg/kg) are several magnitudes greater than those needed to produce a hormonal response in target tissue. This raises a serious question as to whether the metabolic alterations observed are due to a direct or indirect hormonal response or to a pharmacological effect of the drug not related to hormonal activity. This is especially important in view of the known toxicity of high levels of steroid hormones and in view of the fact that hepatic enzymatic systems are known to be sensitive to changes in composition of diet and food intake. A study of this problem (see Results)

indicates that, at the dosage employed, general toxicity probably plays a minor role, if any, in producing the observed enzymatic changes.

The present study suggests that various synthetic and natural compounds possessing estrogenic activity as well as the adrenal hormones, cortisone and hydrocortisone, suppress the capacity of hepatic tissue to synthesize NAD after nicotinamide challenge. In the case of the estrogenic substances compared, good correlation between depression of hepatic NAD levels and a lowering of nicotinamide deamidase was observed. A similar correlation employing the cortisol derivatives was not so striking. At dosage levels equivalent to those employed with the estrogenic substances, no significant decrease in NAD levels was observed upon the administration of cortisone, and only a weak response was observed in the case of hydrocortisone. Higher levels of these two compounds did, however, decrease the hepatic NAD levels. Nicotinamide deamidase activity, on the other hand, was markedly lowered in the case of cortisone and hydrocortisone at all levels studied. The significance of these observations is not known, it might indicate that the nicotinamide deamidase reaction is not always the rate-limiting step in hepatic tissue, in the synthesis of NAD from nicotinamide.

It is surprising that corticosterone had no effect on nicotinamide deamidase activity, whereas cortisone and hydrocortisone under the same experimental conditions effected an inhibition, since the effects of these three substances in most biological systems are quite similar. Whether this discrepancy is a reflection of the more rapid absorption and destruction of the corticosterone or nonspecific toxicity on the part of cortisone and hydrocortisone cannot be determined from the data. In the case of the estrogens a better correlation of hormonal activity and inhibition of nicotinamide deamidase is observed. The data indicating that the natural estrogen estradiol 17-β does inhibit hepatic nicotinamide deamidase, although the nonhormonal isomer estradiol-17α has no effect on this liver enzyme, is of particular interest. This type of observation is highly suggestive of some type of endocrine involvement.

Nicotinamide deamidase is apparently under pituitary control, as demonstrated by Greengard *et al.*[2] Whether the effects reported in this communication, that of the compounds tested only those with estrogenic and cortisol activity lower nicotinamide deamidase activity, indicate a direct or indirect hormone effect of these compounds is not known. However, since two types of steroid hormones exhibit the same effect on nicotinamide deamidase activity, one might speculate that these hormonal agents are acting not as estrogens or cortisol hormones, per se, but are acting indirectly by either stimulating or suppressing the activity of other endocrine glands. Such a consideration becomes pertinent when one considers the high level of hormone administered.

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