RELEASE OF INHIBITION OF RAT LIVER NICOTINAMIDE DEAMIDASE BY HYPOPHYSECTOMY.

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Earlier studies in this laboratory suggested that liver NAD synthesis may be subject to hormonal control (2). These studies demonstrated, for instance, that hypophysectomy increases both the magnitude and duration of the elevation in rat liver NAD which follows administration of nicotinamide. In the preceding report (4), it was suggested that the conversion of nicotinamide to nicotinic acid, catalyzed by nicotinamide deamidase, might be the rate-limiting step in the biosynthesis of NAD from nicotinamide. In the present investigation a study of the possible hormonal control of this step has been carried out.

The nicotinamide deamidase activity of livers of rats which had been hypophysectomized 4 to 8 weeks previously was compared with that of normal rats of the same age. Activity was measured at a nicotinamide concentration of 5 mM. This is the approximate concentration occurring in liver during maximal NAD synthesis in vivo following nicotinamide injection (1). Activity is nearly proportional to substrate at this concentration and, therefore, an effect of hypophysectomy on either the Km or the Vm of the enzyme is likely to be apparent, whereas at saturating concentrations of substrate only effects on Vm are measured.

As shown in Table I, in each of five experiments, the activity of liver homogenates from hypophysectomized rats was considerably elevated compared to the activity of the normal control rats. The higher nicotinamide deamidase activity of

the hypophysectomized rats presumably contributes significantly to the higher level of NAD reached in hypophysectomized animals in vivo following nicotinamide administration.

Table I

Comparison of nicotinamide deamidase activity of liver homogenates from normal and hypophysectomized rats.

Incubation tubes (0.5 ml final volume) contained 25 μmoles of triethanolamine (TEA) buffer, pH 8.8, 2.5 μmoles of nicotinamide-7-C^{14} and liver homogenate (50 mg wet weight) from either normal or hypophysectomized animals. Incubations and determinations of the nicotinic acid formed were carried out as described in the preceding communication (4). The data for deamidase activity represent mean values in mμmoles/gm wet weight/hr. ± S.E. for the number of animals indicated in parentheses. N, Normal rats; H, Hypophysectomized rats.

Exp.	Deamidase Activity		Ratio
	N	H	H/N
1	91.9 ± 3.3 (8)	247.0 ± 14.8 (9)	2.69
2	83.6 ± 2.2 (7)	280.2 ± 22.4 (7)	3.46
3	74.0 ± 16.2 (3)	222.0 ± 19.2 (3)	3.00
4	66.3 ± 3.3 (3)	269.0 ± 13.1 (3)	4.06
5	46.8 ± 3.5 (9)	126.7 ± 8.1 (9)	2.70

By comparing the effect of substrate concentration on the rate of the nicotinamide deamidase reaction in liver homogenates from normal and hypophysectomized rats, it has been found that the increase in deamidase activity as a result of hypophysectomy is due to a decrease in Km. Results of a typical experiment are shown in Figure 1, in which 1/V is plotted against 1/S according to the method of Lineweaver and Burk (3). The Km of the liver homogenate from the hypophysectomized rat was 256 mM, about one-fourth the Km of 975 mM found with the liver homogenate from the normal rat. The differences in deamidase activity and Km between normal and hypophysectomized rats are not caused by differences in dietary intake.*

The lower Km of the deamidase from hypophysectomized rats compared to that from normal rats, taken together with the

*Unpublished experiments.

observation reported in the preceding communication (4) that normal rat liver contains inhibitory material which markedly increases the Km of the partially purified enzyme, suggests that the extent of inhibition of the deamidase decreased as a result of hypophysectomy.

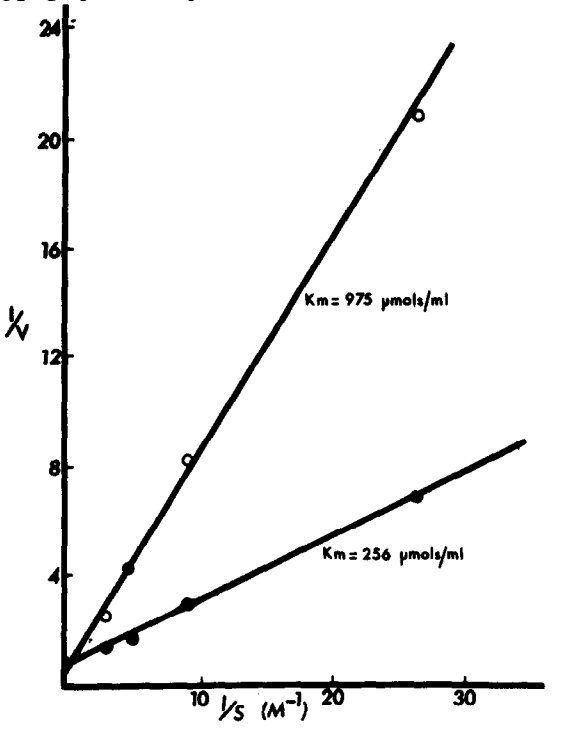

Fig. 1 - Lineweaver-Burk plots of nicotinamide deamidase activity of liver homogenates from normal and hypophysectomized rats.

Incubation tubes (0.5 ml final volume) contained from 18.9 to 209.4 μmoles of nicotinamide-7-C^{14}. Other assay conditions as in Table I. The velocities (V) were expressed as μmoles of nicotinic acid formed per 0.5 ml incubation mixture per 2 hours. Open circles, normal rats; solid circles, hypophysectomized rats.

Further evidence that the extent of inhibition of the deamidase is greater in homogenates from normal than from hypophysectomized animals was obtained in experiments on the stimulatory effect of serum albumin on enzyme activity. Thus, we have found that serum albumin causes a greater percentage increase in the nicotinamide deamidase activity of liver homogenates from normal than from hypophysectomized animals.

Table II

Inhibition of partially purified rat liver nicotinamide deamidase by crude liver homogenates from normal and hypophysectomized rats.

Incubation tubes (0.5 ml final volume) contained a total of 34 μmoles TEA buffer, pH 8.2, 0.75 μmoles nicotinamide-7-C^{14} and either partially purified enzyme (1.9 mg) or crude liver homogenate (50 mg wet weight from either normal or hypophysectomized rats) or a combination of these amounts of partially purified enzyme and crude homogenate. An enzyme preparation carried through the charcoal stage of purification (4) was used as the source of purified nicotinamide deamidase. Incubations and determinations of the nicotinic acid formed were carried out as described in the preceding communication (4). Deamidase activity of the purified enzyme in the presence of the crude homogenate was calculated by subtracting the activity in the tube containing crude homogenate alone from the activity found in the tube containing purified enzyme plus crude homogenate. The deamidase activity calculated in this way is expressed as % of the activity of the purified enzyme incubated in the absence of crude homogenate. In each experiment, the data represent the averages of individual determinations on two normal and two hypophysectomized rats. N, Normal rats; H, Hypophysectomized rats.

	% Residual Activity Source of Inhibitor		Ratio of Residual Activities
Experiment	N	H	H/N
1	15.2	30.0	1.97
2	15.4	28.8	1.87

The decreased extent of inhibition of the deamidase in crude liver homogenates from hypophysectomized animals could be due either to a decreased susceptibility of the enzyme to inhibition or to a decreased concentration of inhibitory material. That a decreased concentration of inhibitory material is responsible, at least in part, for the decreased extent of inhibition observed in the homogenates from hypophysectomized animals is indicated by experiments comparing the ability of crude homogenates from normal and hypophysectomized animals to inhibit the partially purified enzyme from normal liver. Typical results are shown in Table II, in which it can be seen that the residual activity of the purified enzyme was almost twice as great in the presence of liver homogenate from hypophysectomized animals as it was in the presence of an equal

amount of liver homogenate from normal animals. Studies are in progress to determine whether a decrease in the susceptibility of nicotinamide deamidase to inhibition might also have occurred following hypophysectomy. For this purpose, the deamidase is being purified from hypophysectomized animals.

It was proposed (4) that the biosynthesis of NAD from nicotinamide may be controlled by an endogenous inhibitor which regulates the activity of nicotinamide deamidase, the first enzyme in the biosynthetic pathway, by altering the Km of the enzyme. The present work indicates that the reaction catalyzed by nicotinamide deamidase may be subject to hormonal control. Moreover, the data suggest that this hormonal control may be mediated through regulating the Km of the enzyme by an endogenous inhibitor.

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