REGULATION OF THE TRYPTOPHAN-NICOTINIC ACID-DPN PATHWAY IN THE RAT

Conrad Wagner

Research Laboratory, VA Hospital and Department of Biochemistry, Vanderbilt University, Nashville, Tennessee

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A precursor-product relationship between tryptophan and nicotinic acid has been inferred in higher animals as well as in some lower organisms from nutritional studies in which tryptophan can replace the requirement for nicotinic acid. In higher animals the presence of nicotinic acid in the diet diminishes the animal's requirement for tryptophan (Salmon, 1954). This has been referred to as the sparing effect of nicotinic acid. One implication of this sparing effect which has not been previously appreciated is that the presence of nicotinic acid in the diet somehow regulates the conversion of tryptophan to nicotinic acid. If no regulatory mechanism exists, the presence of nicotinic acid in the diet should not diminish the amount of tryptophan which is being converted via this pathway. Control of this pathway should be of utmost importance to the organism since the starting material, tryptophan, is itself an essential nutrient, and frequently is present in limiting quantities in the diet.

Recently Nishizuka and Hayaishi (1963) have elucidated details of the metabolic pathway between tryptophan, nicotinic acid, and DPN

in the rat. This pathway is outlined in Figure 1.

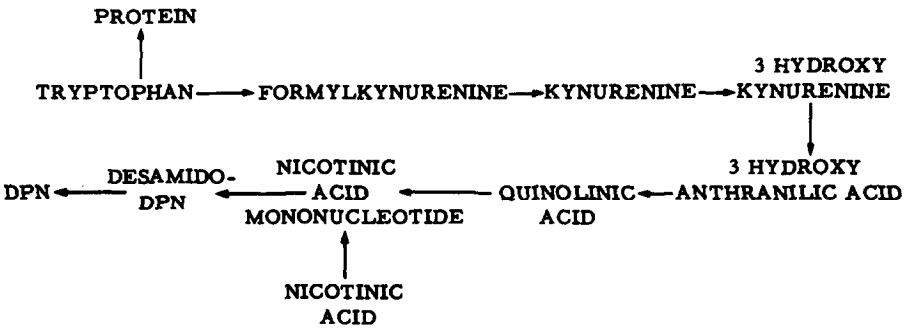


Figure 1

Thus, DPN may be considered the end product of tryptophan metabolism and free nicotinic acid is not an intermediate although the pathways from both tryptophan and nicotinic acid to DPN have common intermediates at the nucleotide level.

With the foregoing points in mind we have investigated the molecular basis for the sparing effect of nicotinic acid in the rat. DPN and the intermediates in the pathway from nicotinic acid to DPN have been found to exert a feedback type of inhibition upon the first enzyme in the pathway from tryptophan to DPN, i.e., tryptophan pyrrolase.

Materials and Methods: Livers from adult male Sprague-Dawley rats were used as a source of the enzyme. Four hours prior to sacrifice the activity of tryptophan pyrrolase was increased by the intraperitoneal injection of 100 mg. of L-tryptophan/100 g. body weight. (Greengard and Feigelson (1961). The enzyme was prepared according to the method of Greengard and Feigelson (1962) and a purification of 120-fold was achieved.

Tryptophan pyrrolase activity was measured spectrophotometrically by the increase in absorbance at 321 mμ. due to formation of formylkynurenine. Assays were carried out at 25°.

All chemicals were obtained from the Sigma Chemical Co. with the exception of nicotinic acid mononucleotide and desamido-DPN which were prepared according to the procedures of Lamborg, et al. (1958), and Kaplan and Stolzenbach (1959).

Results and Discussion: With the exception of formylkynurenine, the immediate product of tryptophan pyrrolase activity, each of the compounds intermediate between tryptophan and DPN and between nicotinic acid and DPN, was tested for its ability to inhibit the purified enzyme. In addition, DPN, nicotinic acid, and several other related compounds were tested for inhibition of tryptophan pyrrolase activity.

The results are presented in Table I. Although nicotinic acid itself is not inhibitory, the derivatives of nicotinic acid which are common to both pathways of DPN synthesis, nicotinic acid mononucleotide, desamido-DPN, and DPN itself, are potent inhibitors of the enzyme. An interesting observation is the very strong inhibition by the phenolic intermediates, 3-hydroxyanthranilic acid and 3-hydroxykynurenine. The physiological significance of this is uncertain since these compounds are not involved in the conversion of nicotinic acid to DPN and the irreversibility of several steps from tryptophan to nicotinic acid mononucleotide would preclude accumulation of these compounds solely on the basis of reversal of the pathway. Frieden, et al, (1961) have reported

Table I

INHIBITION OF TRYPTOPHAN PYRROLASE

The assay mixture contained 80 μmoles Tris-chloride buffer, pH 7.4; 2.5×10^{-3} μmoles hematin; 0.6 μmoles L-tryptophan; and 0.1 mg. of protein plus inhibitor in a total volume of 1.5 ml. The activity is expressed as per cent of the activity in absence of inhibitor.

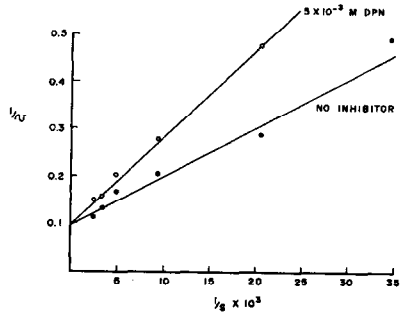
COMPOUND	Per Cent Inhibition			
	5×10^{-3} M	5×10^{-4} M	5×10^{-5} M	5×10^{-6} M
Kynurenine	58	47	36	--
3-OH Kynurenine	--	--	95	67
3-OH Anthranilic Acid	--	100	85	32
Quinolinic Acid	12	10	--	--
Nicotinic Acid	0	--	--	--
*Na MN	83	33	0	--
Desamido-DPN	64	43	22	--
DPN	42	0	--	--
Nicotinamide	0	--	--	--
Anthranilic Acid	0	--	--	--
TPN	12	--	--	--
Nicotinamide Nucleotide	50	0	--	--

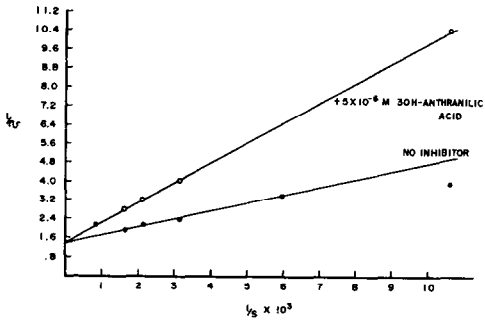
* Nicotinic Acid Mononucleotide

that the phenolic hormones, serotonin and epinephrine are also potent inhibitors of tryptophan pyrrolase.

Figures 2 and 3 show that inhibition of the enzyme by 3-hydroxy-anthranilic acid or by DPN is competitive with tryptophan. This indicates that the inhibitory site is the same as or very close to the catalytic site. There have been several examples of feedback inhibition in which the end product of a pathway competes with the starting material for the first enzyme in the pathway.

This inhibition of tryptophan pyrrolase by intermediates in the pathway from nicotinic acid to DPN and by DPN itself strongly suggests





Figs. 2 and 3. Inhibition of tryptophan pyrrolase by DPN and 3-hydroxyanthranilic acid. Conventional double reciprocal plots of the enzyme activity at different substrate concentration. The reactions were carried out as described under Table I except that tryptophan concentration was varied as indicated and appropriate amounts of DPN or 3-hydroxyanthranilic acid were used.

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that this may be the mechanism by which added nicotinic acid acts to spare tryptophan. Inhibition must occur at the level of this first enzyme in order for conservation of tryptophan to be effective. These compounds are effective inhibitors in the range of 10^{-3} M. Although it is difficult to speculate about localized concentrations within the cell, values in the literature for total liver pyridine nucleotides are in this range (Morrison, et al.) (1963).

Tryptophan also serves as a precursor of DPN in the mold, Neurospora, and the bacterium, Xanthomonas. Studies are now in progress to determine whether similar control mechanisms operate in these organisms.

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REFERENCES

1. Frieden, E., Westmarch, G.W., and Schor, J.M., Arch. Biochem. Biophys., 92, 170 (1961).
2. Greengard, O., and Feigelson, P., J. Biol. Chem., 236, 158 (1961).
3. Greengard, O., and Feigelson, P., J. Biol. Chem., 237, 1903 (1962).
4. Kaplan, N.O., and Stolzenbach, F.E., in S.P. Colowick and N.O. Kaplan (Editors), Methods in Enzymology, Vol. III, Academic Press, Inc., New York, p. 899 (1957).
5. Lamborg, M., Stolzenbach, F.E., and Kaplan, N.O., J. Biol. Chem., 231, 685 (1958).
6. Morrison, M.A., Reynolds, M.S., and Harper, A.E., J. Nutrition, 80, 441 (1963).
7. Nishizuka, Y., and Hayaishi, O., J. Biol. Chem., 238, 3369 (1963).
8. Salmon, W.D., Arch. Biochem. Biophys., 51, 130 (1954).