Stabilization of Rat Liver Tyrosine Aminotransferase *in vivo* by Pyridoxine Administration

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Administration of pyridoxine stabilizes rat liver tyrosine aminotransferase *in vivo*, whereas administration of cortisol, cyclic AMP, glucagon, insulin, tryptophan or tyrosine does not. The results of these and other experiments with pyridoxine are discussed in relation to the mechanisms of action of this vitamin on the activity of the enzyme.

Holten *et al.* (1967) reported that, whereas pyridoxine derivatives added *in vitro* protect rat liver tyrosine-2-oxoglutarate aminotransferase (EC 2.6.1.2) against degradation, the enhancement of activity caused by the administration of pyridoxine is not associated with stabilization but involves an increased synthesis of the enzyme. Lee *et al.* (1977) also found that the rate of degradation of the enzyme is not influenced by pyridoxine deficiency. These findings were therefore taken to suggest that tyrosine aminotransferase is not stabilized *in vivo* by its pyridoxal 5'-phosphate cofactor.

During an investigation (Badawy *et al.*, 1980) of the effects of acute ethanol administration of the activity of hepatic tyrosine aminotransferase, we considered that it might be useful to determine the half-life of the enzyme after administration of a number of compounds, including pyridoxine. We found that only the latter agent stabilizes the enzyme, and the present paper describes and discusses the results of these experiments.

Materials and Methods

Locally bred male Wistar rats (150–200g), maintained on cube diet 41B (Oxoid, Basingstoke, Hants., U.K.) and water, were killed at 15:00h by stunning and cervical dislocation. Tyrosine aminotransferase activity was determined in liver homogenates in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added (40 μ M) pyridoxal 5'-phosphate by a modification (Badawy *et al.*, 1980) of the method of Diamondstone (1966).

Chemicals were purchased from Sigma (London) Chemical Co. and BDH Chemicals Ltd. (both of

Poole, Dorset, U.K.) and were of the purest commercially available grades. Various compounds were injected intraperitoneally as solutions in 0.9% NaCl (2–20 ml/kg) or in dimethylformamide (1 ml/kg), and control animals received equal volumes of the appropriate solvent. Specific experimental designs are described with the relevant results. Statistical analysis of results was performed by using Student's *t* test.

Results and Discussion

The half-life of rat liver tyrosine aminotransferase was determined by monitoring the rate of decline in enzyme activity after administration of the protein-synthesis inhibitor cycloheximide. The results in Fig. 1 show that the half-lives of the holoenzyme and total enzyme in control (0.9%-NaCl-treated) rats were 2.03 and 2.09 h respectively and that pyridoxine administration (400 mg/kg, at 4 h before cycloheximide) prolonged the half-lives of both forms of the enzyme by 2.3-fold and 2.7-fold respectively. It should be pointed out here that 4 h after administration of the above dose of pyridoxine hydrochloride the holoenzyme and total enzyme activities were enhanced by 3.17-fold and 2.94-fold respectively.

No stabilization of the enzyme was observed after administration of a number of hormones and amino acids. The doses of these compounds, and the half-lives (in h) of both holoenzyme and total enzyme observed on their administration, were as follows: cortisol (dose 20 mg/kg; half-lives 2.06 and 2.09); cyclic AMP (50 mg/kg; 1.92 and 2.15); glucagon (10 mg/kg; 2.07 and 2.24); insulin (2 i.u./kg; 1.80 and 2.10); tryptophan (400 mg/kg; 2.01 and 1.77); tyrosine (400 mg/kg; 1.99 and 2.19). The basal enzyme activities at 4 h after adminis-

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Fig. 1. Effect of pyridoxine administration on the degradation of rat liver tyrosine aminotransferase

Rats received an intraperitoneal injection of either pyridoxine hydrochloride (400 mg/kg) or an equal volume (20 ml/kg) of 0.9% NaCl and were killed 4 h later. Similarly treated rats were given an injection of cycloheximide (10 mg/kg) (i.e. at 4 h after the above injections) and the animals were then killed 1 or 2 h later. The enzyme activity was determined as described in the Materials and Methods section in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added (40 μ M) pyridoxal 5'-phosphate, and the half-life was calculated by plotting the decline in enzyme activities with time at 0, 1 and 2 h after cycloheximide administration. Values are means $\pm$ S.E.M. for each group of four rats. Symbols: ●, holoenzyme activity of 0.9%-NaCl-treated rats; ○, total enzyme activity of 0.9%-NaCl-treated rats; ■, holoenzyme activity of pyridoxine-treated rats; □, total enzyme activity of pyridoxine-treated rats.

tration of cortisol, tryptophan and tyrosine were increased by 324–543%, whereas smaller increases were observed with cyclic AMP (2.4–2.6-fold), glucagon (1.2-fold) and insulin (1.4–1.6-fold). The inability of cortisol, cyclic AMP and glucagon to stabilize tyrosine aminotransferase is consistent with their action on the enzyme activity being primarily on enzyme synthesis, probably by increasing the amount of tyrosine aminotransferase-specific mRNA (Ernest & Feigelson, 1978; Nickol *et al.*, 1978). The mechanisms of actions of insulin, tryptophan and tyrosine on the enzyme activity are, however, less clearly understood, but the present results suggest that these agents do not act by decreasing aminotransferase degradation.

Holten *et al.* (1967) suggested that pyridoxine administration does not stabilize liver tyrosine

aminotransferase when the half-life of the enzyme was determined by a different method. This involved giving two injections of pyridoxine (1 g/kg each) at 2 h intervals to rats that had been pretreated with cortisol (50 mg/kg) 6 h before the first injection. It was then found that the enzyme activity fell in a first-order fashion during the 4 h after the first pyridoxine injection. This is surprising, because Holten *et al.* (1967) also found that the above doses of pyridoxine alone caused about a 4-fold increase in the enzyme activity at 4 h after the first injection. Moreover, by using the above dose regimens in adrenalectomized rats, Greengard & Gordon (1963) and Black (1970) observed increases in the enzyme activity of 1.9–2.8-fold at 2–4 h after the first injection.

We have repeated some of the experiments performed by Holten *et al.* (1967) by giving rats a smaller (200 mg/kg) single dose of pyridoxine hydrochloride or an equal volume of 0.9% NaCl at 6 h after pretreatment with either cortisol (20 mg/kg) or an equal volume of the solvent dimethylformamide (Table 1). In cortisol-pretreated rats, the enzyme activities fell during the 2 h period after administration of 0.9% NaCl, and the half-lives of the holoenzyme and total enzyme were calculated to be 1.78 and 1.68 h respectively. By contrast, administration of pyridoxine caused, at 1 h, small (5–11%, $P > 0.10$) increases in enzyme activities, whereas at 2 h the holoenzyme activity was further increased (in relation to the value at 1 h) by 28% ($P < 0.02$). Although the increases caused by pyridoxine administration to cortisol-pretreated rats could be attributed to pyridoxine itself (as the results of experiments in which pyridoxine was administered to dimethylformamide-pretreated rats suggest), it should be pointed out that, in relation to the effects of cortisol alone, pyridoxine treatment did in fact cause much larger increases in the enzyme activities. Since pyridoxine did not do so alone, it may therefore be suggested that the cortisol-induced enzyme is stabilized by the presence of pyridoxine.

There is controversy surrounding the mechanism(s) of the pyridoxine-induced enhancement of liver tyrosine aminotransferase activity. There is no doubt, however, that the vitamin acts primarily by increasing the availability of pyridoxal 5'-phosphate to the apoenzyme. This is suggested by the reported increase in the cofactor saturation of the apoenzyme that occurs both at 4 h (Badawy, 1972) and earlier (Greengard & Gordon, 1963; the present Table 1) after pyridoxine administration, and by the ability of noradrenaline, which binds pyridoxal 5'-phosphate, to block the pyridoxine enhancement of the enzyme activity (Black, 1970). That noradrenaline does so by a mechanism not involving inhibition of enzyme synthesis is suggested by its inability to prevent the dexamethasone induction of the enzyme activity

Table 1. *Effects of administration of cortisol, pyridoxine or both on rat liver tyrosine aminotransferase activity*

Rats were given a first injection of either cortisol (20 mg/kg) or an equal volume (1 ml/kg) of dimethylformamide and were killed 6 h later. Similarly treated rats received a second injection (at 6 h) of either pyridoxine hydrochloride (200 mg/kg) or an equal volume (20 ml/kg) of 0.9% NaCl and were killed 1 or 2 h later. The enzyme activity was determined as described in the Materials and Methods section in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added (40 μ M) pyridoxal 5'-phosphate. Values are means $\pm$ S.E.M. for each group of four rats.

First injection	Second injection	Time of death after second injection (h)	4-Hydroxyphenylpyruvate formed (μ mol/h per g wet wt. of liver)	
			Holoenzyme activity	Total enzyme activity
Cortisol	0.9% NaCl	0	178 $\pm$ 10	357 $\pm$ 30
		1	114 $\pm$ 10	219 $\pm$ 22
		2	75 $\pm$ 4	131 $\pm$ 12
Cortisol	Pyridoxine hydrochloride	0	178 $\pm$ 10	357 $\pm$ 30
		1	198 $\pm$ 9	376 $\pm$ 15
		2	254 $\pm$ 13	374 $\pm$ 1
Dimethylformamide	Pyridoxine hydrochloride	0	41 $\pm$ 3	77 $\pm$ 10
		1	61 $\pm$ 3	109 $\pm$ 13
		2	98 $\pm$ 10	118 $\pm$ 9

(Black, 1970). It is also unlikely that pyridoxine increases the saturation of the apoenzyme with pyridoxal 5'-phosphate at early times after administration by a mechanism requiring protein synthesis, because this effect is insensitive to puromycin (Greengard & Gordon, 1963).

Involvement of protein synthesis in the later pyridoxine-induced enhancement of aminotransferase activity is suggested by the ability of puromycin to prevent this enhancement (Greengard & Gordon, 1963). By contrast, the results of experiments with actinomycin D suggest that mRNA synthesis is only partially involved. Thus, in adrenalectomized rats, actinomycin D has been reported either partially to decrease (Holten *et al.*, 1967) or to exert no effect (Greengard & Gordon, 1963) on the pyridoxine enhancement of the enzyme activity. In intact rats, only 50% of the pyridoxine effect is sensitive to actinomycin D (Badawy, 1972). Moreover, the combined injection of cortisol plus pyridoxine increases the enzyme activity in intact rats to a value that is equal to the sum of the cortisol effect plus the actinomycin D-insensitive portion of the pyridoxine effect, and actinomycin D decreases the effect of the above combined injection to the value observed after pyridoxine administration to actinomycin D-pre-treated rats (Badawy, 1972). These findings therefore suggest that pyridoxine enhances liver tyrosine aminotransferase activity by at least two mechanisms: one requiring mRNA synthesis and the other dependent on protein synthesis only. It should be pointed out that the failure of Holten *et al.* (1967) to demonstrate an additive effect on the enzyme activity of the combined injection of cortisol plus pyridoxine may be explained by their use of a large

dose of cortisol (50 mg/kg), which could have saturated the capacity of the liver to synthesize the enzyme.

The stabilization of tyrosine aminotransferase *in vivo* by pyridoxine administration reported in the present paper suggests that the enzyme may be regulated by this vitamin by a third mechanism. It also suggests that the use of experimental pyridoxine deficiency to demonstrate a lack of an increase in the rate of aminotransferase degradation (Lee *et al.*, 1977) may not be a valid test for stabilization by pyridoxal 5'-phosphate *in vivo*. Further work is, however, required to determine the contribution of each of the above three mechanisms to the quantitative regulation of tyrosine aminotransferase.

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