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INHIBITION OF PINEAL HYDROXYINDOLE-*O*-METHYL TRANSFERASE BY PYRIDOXAL-5′-PHOSPHATE

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Abstract—Pyridoxal-5′-phosphate (PLP) *in vitro* was found to inhibit strongly the conversion of *N*-acetylserotonin to melatonin by hindering the pineal hydroxyindole-*O*-methyl transferase (HIOMT) activity. This inhibition was competitive with respect to the methyl donor *S*-adenosylmethionine but non-competitive as regards the substrate *N*-acetylserotonin. Noradrenaline, which had no effect on HIOMT activity, almost completely abolished the inhibitory effect of PLP on that enzyme.

The terminal reaction in the biosynthesis of melatonin, considered to be a specific pineal hormone, involves transfer of a methyl group from *S*-adenosylmethionine to the 5-hydroxy group *N*-acetyl-serotonin. This reaction is effected by hydroxyindole-*O*-methyl transferase (HIOMT), which was believed to be an enzyme of the pineal gland only [1] but has now been traced in homogenates of Harderian gland and retina of rats [2]. Changes in HIOMT activity were evoked by external or internal factors which could be responsible for controlling synthesis of melatonin [1].

Several groups of methyl transferase inhibitors have been characterized. In the transmethylation reaction of acetylserotonin with *S*-adenosylmethionine to produce melatonin, *S*-adenosylhomocysteine (SAH), the secondary product formed, was found to be a strong inhibitor of the primary product melatonin, thus inhibiting HIOMT activity [3, 4]. Compounds structurally similar to SAH such as cysteic acid, homocysteic acid, GSH and GSSG were also found to inhibit HIOMT, although at much higher concentrations. However, adenosine, methionine, homocysteine and homocystine had no effect [4]. Melatonin was reported by Weiss [5] to act as an HIOMT inhibitor but was found to be inactive, even in very high concentrations, by others [4]. Neuroleptic drugs such as haloperidol and to a lesser extent fluphenazine were reported to inhibit pineal HIOMT activity [6], as were the biogenic amines noradrenaline, serotonin and histamine [5, 7], but only in high concentrations of 10^{-3} M and above.

Conversely, psychomimetic agents such as dimethyltryptamine, methoxybufotenin, mescaline, LSD and amphetamine [8] and, when in low concentrations, the salts of the intermediates in the citric acid cycle were found to be good activators of HIOMT activity *in vitro* [4].

The present paper describes inhibition of HIOMT *in vitro* by the naturally occurring vitamin pyridoxal-5′-phosphate (PLP), which was found to be strongly inhibitory in the methoxylation of noradrenaline by the enzyme catechol-*O*-methyl transferase [9].

MATERIALS AND METHODS

Pyridoxal-5′-phosphate and *N*-acetylserotonin were obtained from Sigma Chemical Co., and [^{14}C]methyl-*S*-adenosylmethionine (sp. act. 50 m Ci/m-mole) from Schwartz/Mann, Orangeburg, N.Y. Noradrenaline HCl was generously donated by Teva Ltd., Jerusalem.

Male rats of the Hebrew University "Sabra" strain weighing 160–180 g each and which had been kept in alternate light and darkness (overhead fluorescent 40 W "daylight" tubes switched on at 7 a. m. and off at 7 p. m. by automatic timer) were decapitated between 10 a. m. and 12 noon. Their pineal glands were quickly dissected and placed in phosphate buffer, pH 7·9 (0°), until homogenization. Pineals were homogenized in batches of four to five, and five batches (i.e. 20–25 glands) were pooled for each experiment. Homogenization was carried out in phosphate buffer, pH 7·9, allowing 500 μl for each pineal. Two hundred μl of the crude homogenate was incubated for 1 hr with various concentrations of *N*-acetylserotonin ($5 \times 10^{-4} - 1 \times 10^{-5}$ M), [^{14}C]methyl-*S*-adenosylmethionine ($2 \times 10^{-5} - 4 \times 10^{-7}$ M), pyridoxal-5′ phosphate (5×10^{-4}–10^{-6} M) and noradrenaline HCl (10^{-3} to $2{\cdot}5 \times 10^{-6}$ M) in a final volume of 300 μl according to the method of Axelrod *et al.* [10]. All dilutions were made in triplicate.

RESULTS

PLP at concentration of 5×10^{-6} M was associated with 50 per cent inhibition of HIOMT; over the range of $2{\cdot}5 \times 10^{-5}$ M more than 90 per cent inhibition occurred (Table 1).

Noradrenaline had no effect on HIOMT activity. However, the inhibitory effect of PLP on that enzyme system was almost completely abolished by noradrenaline at concentrations of M^{-3} and M^{-4} (Table 2). Lower concentrations of noradrenaline only partially abolished the inhibition of HIOMT.

When studying the kinetics of the inhibition by PLP, various concentrations were tested with different concentrations of either *N*-acetylserotonin or *S*-

Table 1. Inhibition of pineal HIOMT by pyridoxal-5'-phosphate (PLP)*

PLP conen (M)	HIOMT activity†	% Inhibition
	74·0	
5×10^{-3}	3·7	95
$2{\cdot}5 \times 10^{-3}$	4·3	94
10^{-3}	15·0	80
5×10^{-4}	37·5	50

* Incubation was for 1 hr at 37° and each tube contained: homogenate rat pineal (0·4 pineal), *N*-acetylserotonin (50 μg), *S*-adenosylmethionine (1 nmole, 50 n Ci) and pyridoxal-5'-phosphate in 300 μl phosphate buffer, pH 7·9.

† Picomoles of melatonin[^{14}C] formed/pineal hr.

adenosylmethionine. The results and their corresponding Lineweaver Burk plots are given in Figs. 1 and 2.

Double reciprocal plots indicated that the inhibition of HIOMT by PLP was non competitive with respect to *N*-acetylserotonin and competitive as regards *S*-adenosylmethionine. The apparent K_m values were 1×10^{-4} M for *N*-acetylserotonin and $0{\cdot}5 \times 10^{-5}$ M for *S*-adenosylmethionine, and the K_i value was $0{\cdot}59 \times 10^{-5}$ M for PLP with *S*-adenosyl-methionine.

DISCUSSION

PLP *in vitro* was found to inhibit strongly the conversion of *N*-acetylserotonin to melatonin by hindering pineal HIOMT activity. This inhibition, noncompetitive with respect to the substrate *N*-acetylserotonin, was found to be competitive as regards the methyl donor *S*-adenosylmethionine. In *N*-methylating processes of indolethylamine [11] and phenylethanolamine [12], the enzyme *N*-methyltransferase also was found to be competitively inhibited by *S*-adenosylhomocysteine with respect to the methyl donor *S*-adenosylmethionine. However, a structural similarity exists between *S*-adenosylhomocysteine and *S*-adenosylmethionine, whereas no such similarity is present between PLP and adenosylmethionine. On the other hand, Black [9] reported that PLP competitively inhibits the methoxylation of noradrenaline by catechol-*O*-methyl transferase (COMT) with *S*-adenosylmethionine as methyl donor. The mechanisms of inhibition has not been defined and, although a competition between PLP and noradrenaline for enzyme binding sites was suggested, a competition of PLP with *S*-adenosyl-methionine could be equally consistent. Our findings would appear to give support to this latter postulation. It is quite possible that the mechanism in both cases, ours with HIOMT and Black's with COMT, is the same, i.e. competition between PLP and *S*-adenosylmethionine for enzyme binding sites. PLP may well be a competitive transmethylation inhibitor not only of the *O*-methylation of catecholamines and indolamines but also of *N*-methylations of biogenic amines.

The fact that noradrenaline almost competely abolished the inhibitory effect of PLP is not surprising, since noradrenaline has been found to form a complex with PLP [13], thus canceling the action of PLP by direct interaction with it.

The apparent Michaelis Menten constant for *S*-adenosylmethionine is of the same order of magnitude as that reported by Weiss [5] and Cardinali and Wurtman [2]. The apparent K_m value for *N*-acetylserotonin (1×10^{-4} M) was, however, 20 times higher than that found by others ($0{\cdot}4 \times 10^{-5}$ M) [2]. This may be explained by the fact that our experiments were performed with crude rat pineal extract which contains serotonin, *N*-acetylserotonin and possibly other substrates of HIOMT, whereas Cardinali and Wurtman [2] worked with dialysates free from indolamines. Our reaction mixtures actually contained more substrates for HIOMT than are shown in Figs. 1 and 2, and caused the significantly higher K_m value compared to that reported by Cardinali and Wurtman [2].

It is interesting that PLP, the coenzyme of the decarboxylases and an essential factor in the enzyme reaction converting 5-hydroxytryptophan to serotonin, becomes an inhibitor for another enzyme activity (HIOMT) in the metabolic pathway of serotonin to melatonin. The concentration of PLP found by us to cause 50 per cent inhibition of pineal HIOMT activity (5×10^{-6} M ≃ 1·24 ng/μl) is in the same order of magnitude as the concentration recorded in rat brain tissue, i.e. 1·7 to 1·9 ng/mg [14]. This suggests that PLP in the pineal gland may play a role in regulation of the activity of HIOMT and perhaps of other methyltransferases in the brain. Indeed, substances causing a decrease in brain PLP were found to increase the HIOMT activity of the pineal gland and *vice versa* [6, 8, 14].

Moreover, a suggestion has been made that schizophrenia may be associated with abnormal methyla-

Table 2. Suppressive effect of noradrenaline on inhibition of pineal HIOMT by pyridoxal-5'-phosphate (PLP)*

PLP conen (M)	Noradrenaline conen (M)					
		10^{-3}	10^{-4}	5×10^{-5}	10^{-5}	5×10^{-6}	$2{\cdot}5 \times 10^{-6}$
	62·7	53·2	61·0	65·0	63·0		1·0
5×10^{-4}				14·1	5·1	3·5	
10^{-4}	4·7	53·8	58·4	40·4	10·9	4·6	4·0
5×10^{-5}	3·5	52·0	61·5	48·9	27·0		
$2{\cdot}5 \times 10^{-5}$	3·9		62·0	64·0	43·6		
10^{-5}	14·4	53·0	63·0	63·0	50·5	37·2	13·2
5×10^{-6}	31·3			71·0	62·0		

* Results represent picomole of melatonin [^{14}C] formed pineal hr. For conditions of incubation see Table 1.

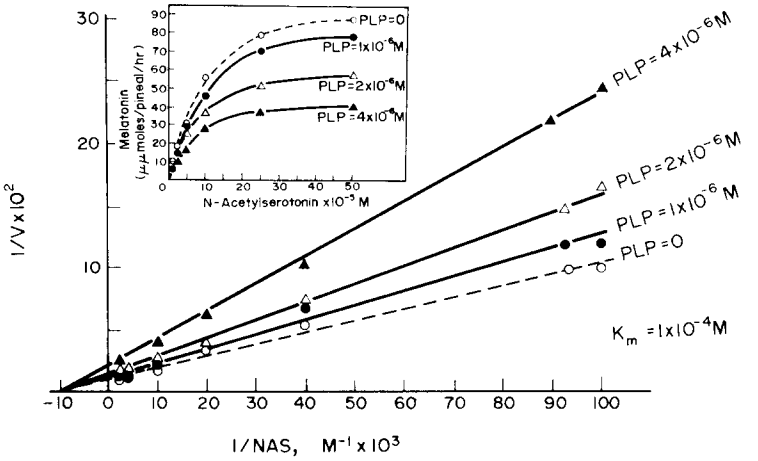

Fig. 1. Inhibition of HIOMT by pyridoxal-5'-phosphate (PLP). Double reciprocal plot with *N*-acetylserotonin (NAS) as the variable substrate. Insert graph represents a straight plot of HIOMT activity as a function of NAS concentration in the presence and absence of PLP.

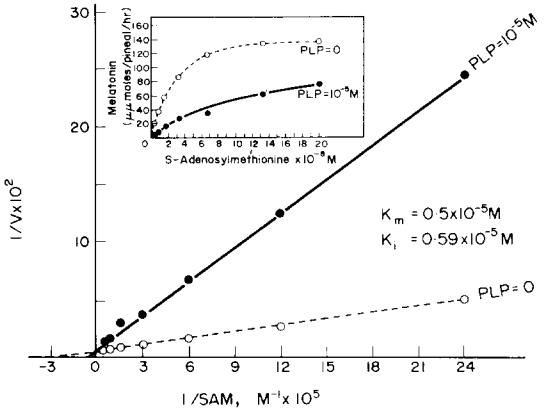

Fig. 2. Inhibition of HIOMT by PLP. Double reciprocal plot with *S*-adenosylmethionine (SAM) as the variable substrate. Insert graph represents a straight plot of HIOMT activity as a function of SAM concentration in the presence and absence of PLP.

tion by pineal HIOMT [15]. Haloperidol, used in the treatment of schizophrenia, inhibits this methylation process [15] and has been shown to concentrate more in the pineal gland than in any other organ [16]. Phenothiazines, other neuroleptic drugs, were found to increase brain PLP *in vitro* and decrease pineal HIOMT activity [6,14]. On the other hand, psychotomimetics shown to produce psychoses in normal subjects bring about a decrease in PLP concentration in the rat brain and an increase in the melatonin-forming capacity of the pineal gland *in vitro* [8, 14]. However, *in vivo*, the process of pineal *O*-methylation might require methyltetrahydrofolic acid as methyl donor, while *S*-adenosylmethionine could serve for *N*-methylations [17]. Hence it could be that pineal HIOMT *in vivo* may not be affected by extraneous doses of PLP.

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