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The Absence of Tryptophan Hydroxylase Activity in Blood Platelets (39702)

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Introduction. Blood platelets accumulate, store, and can release the biogenic amine neurotransmitters serotonin, norepinephrine, and dopamine (1–5). These properties have led to a consideration of the use of the blood platelet as a model of a neuron (1–5). Platelets have an avid uptake mechanism for serotonin (6–9) and it is thought that platelet serotonin is largely derived from serotonin entering the plasma from the intestinal mucosa and other tissues (10).

It has also been reported (11) that platelets contain tryptophan hydroxylase, one of the enzymes required for serotonin synthesis from tryptophan. It therefore appeared that in addition to active uptake and storage of serotonin, platelets may also have the capacity to synthesize this amine from tryptophan.

Since the synthesis of serotonin by platelets is an important point in the understanding of these organelles, we decided to investigate this problem with the current more sensitive and specific assay systems for tryptophan hydroxylation.

Methods. Platelet-rich plasma (PRP) was prepared by centrifugation of heparinized whole blood obtained from human volunteers or male Sprague–Dawley rats at 200*g* for 15 min at 4°. The resulting PRP was again centrifuged at 200*g* for 15 min at 4° to remove any contaminating leukocytes. Examination of this final PRP by light microscopy (12) revealed little if any contamination by leukocytes. Platelets were sedimented at 2500*g* for 15 min at 4° and washed twice in 50 m*M* Tris–Cl, pH 7.4, 100 m*M* NaCl, 2 m*M* dithiothreitol by resuspension-centrifugation. The final platelet pellet was resuspended in a small volume of 50 m*M* Tris–Cl, pH 7.4, and 2 m*M* dithiothreitol, sonicated essentially as described by Wolf and Shulman (13), and centrifuged at 27,000*g* for 15 min at 4°. The resulting supernatant fractions were retained for assay. Rat pineal glands and brain regions were homogenized in 50 m*M* Tris–Cl, pH 7.4, and 2 m*M* dithiothreitol and similarly centrifuged.

Tryptophan hydroxylase activity was determined in tissue extracts by the method of Friedman *et al.* (14) as modified by Baumgarten *et al.* (15) with volume alterations to give a more sensitive microassay. A 0.05-ml portion of platelet extracts or 0.02 ml of pineal, tegmentum, or striatum extracts was incubated in a final volume of 0.08 ml containing 12 μmole of Tris–Cl, pH 7.4, 0.4 μmole of dithiothreitol, and 17 nmole of 6-methyltetrahydropterin (Calbiochem) at 37° for 5 min. The reactions were initiated by the addition of 0.02 ml of 2 m*M* tryptophan. After 40 min the reactions were stopped by the addition of 0.02 ml of 6 *N* perchloric acid and centrifuged to remove the precipitated protein. A 0.1-ml portion of supernatant was mixed with 0.03 ml of concentrated hydrochloric acid. The fluorescence emission of the solution was measured at 540 nm with an excitation wavelength of 290 nm and compared with 5-hydroxytryptophan standards. The activity of tryptophan hydroxylase was measured as the difference in activity with and without 6-methyltetrahydropterin cofactor in the reaction mixture.

The tryptophan hydroxlase activity of cultured rat pineal gland and human and rat platelet-rich plasma was determined essentially by the procedure of Hakanson and Hoffman (16). Briefly, rat pineals were cultured in 60 × 15-mm culture dishes containing 2.5 ml of BGJ_b–Fitton–Jackson medium (Grand Island Biological Co.) supple-

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mented with 100 μg/ml of ascorbic acid and 2 mM glutamine. The pineal cultures and platelet-rich plasma were incubated with 4 μCi/ml of L-tryptophan (side chain-3-^{14}C; 57 mCi/mmole; New England Nuclear Corp.) in the presence of either 10^{-4} M NSD 1055 (3-hydroxybenzylhydrazine; Smith Nephew Research, Ltd.), an aromatic acid decarboxylase inhibitor, or 10^{-4} M pargyline (Abbott Laboratories), a monoamine oxidase inhibitor, for 2 hr at 37° under an atmosphere of 95% oxygen-5% carbon dioxide. At the end of the incubation period the platelets were sedimented and washed in 50 mM Tris-Cl, pH 7.4, 100 mM NaCl, 2 mM dithiothreitol at 2500g for 15 min at 4°. The pineal glands were blotted free of medium. The pineals and platelet pellets were extracted with 2 ml of acetone-1 N HCl (95:5) containing 50 μg each of nonradioactive tryptophan, 5-hydroxytryptophan, and serotonin. The extracts were pipetted free of denatured pineal or platelet protein and evaporated under nitrogen. The dried residues were dissolved in a minimal volume (approximately 25 μl) of acetone-1 N HCl (95:5) and applied to 2-mm-thick 20 × 20-cm silica gel thin-layer plates (Merck, Darmstadt) and developed in the ascending direction with 14% sodium chloride (16). The plates were scraped in 5-mm sections into scintillation vials and counted with 10 ml of Instabray (Yorktown Research).

Results. The tryptophan hydroxylase activities of platelet extracts were compared to the activities found in the rat pineal and tegmental region of the brain (high tryptophan hydroxylase activity) and the striatal region of the brain (relatively low tryptophan hydroxylase activity). As seen in Table I, the apparent tryptophan hydroxylase activity of both rat and human platelets is approximately 0.1–0.45% of the activity found in the rat pineal and 0.17–0.75% of the rat tegmentum activity, and was not significantly higher than the blank. The apparent enzyme content is also considerably less than the activity found in the striatum. The tryptophan hydroxylase activities of platelets reported here are 1/50 to 1/100 of the activities reported previously (11).

In control experiments it was found that addition of serotonin to tegmental extracts did not inhibit the hydroxylase activity. This eliminates the possibility that the high endogenous serotonin content of the platelet extract inhibits enzyme activity. Further, the mixing of platelet and tegmental extracts did not enhance or inhibit the tryptophan hydroxylase activity of the tegmental extract. This eliminates the possibility that an endogenous hydroxylase inhibitor is being liberated during the sonic disruption of the platelets. Finally, the sonication conditions for platelets did not affect the tryptophan hydroxylase activity of a similarly treated tegmental extract.

Since platelet extracts did not seem to have tryptophan hydroxylase activity it was decided to see if intact platelets in a platelet-rich plasma could convert labeled tryptophan to serotonin. The rat pineal is capable of converting tryptophan to 5-hydroxytryptophan in culture in the presence of a decarboxylase inhibitor (16; Fig. 1). Furthermore, the total pathway of tryptophan conversion to serotonin may be monitored in the cultured pineal in the presence of the monoamine oxidase inhibitor pargyline (see Fig. 1). This assay system was utilized with platelet-rich plasma. Results in Table II indicate that the cultured rat pineal is capable of hydroxylating tryptophan in the presence of the decarboxylase inhibitor NSD 1055 while platelets cannot. The tryptophan hydroxylase activity of rat and human platelets was found to be 0.35–0.57% of the rat pineal activity which is in reasonable agreement with the relative activities shown in Table I. The tryptophan hydroxylase activity of the cultured rat pineals was found to be similar to those reported by Hakanson and Hoffman (16). In the presence of the mono-

TABLE I. TRYPTOPHAN HYDROXYLASE ACTIVITY OF TISSUE HOMOGENATES.[a]

Source of tissue	Tryptophan hydroxylase activity[b]	
Rat pineal	26.8 ± 3[r]	$n = 3$
Rat tegmentum	16.2 ± 1.9	$n = 4$
Rat striatum	0.6 ± 0.12	$n = 4$
Rat platelets	0.03 ± 0.05	$n = 6$
Human platelets	0.12 ± 0.09	$n = 4$

[a] Tissue and platelet extracts were prepared and assayed for tryptophan hydroxylase as described.

[b] Activity is expressed as the nanomoles of 5-hydroxytryptophan formed per milligram of protein per hour.

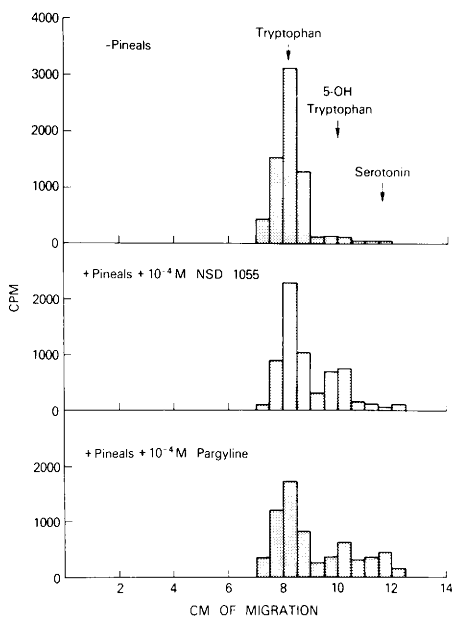

FIG. 1. The synthesis of 5-hydroxytryptophan and serotonin by cultured rat pineals. Pineals were cultured with [^{14}C]tryptophan in the presence of 10^{-4} *M* NSD 1055 or 10^{-4} *M* pargyline as described. Tryptophan metabolites were separated by thin layer chromatography on silica gel layers with 14% sodium chloride (16). The migration of tryptophan, 5-hydroxytryptophan, and serotonin is indicated.

amine oxidase inhibitor pargyline, there was no conversion of labeled tryptophan to 5-hydroxytryptophan or serotonin by platelet-rich plasma (data not shown).

Discussion. From the results presented above it appears that platelets do not have the capacity to synthesize significant amounts of serotonin *de novo* from tryptophan. The reason for the difference between these results and the previous ones from this laboratory (11), indicating the presence of tryptophan hydroxylating capability for platelet extracts, is unexplained. However, the earlier work was done with an assay which at that time was just being developed, and with relatively small amounts of platelet protein. Thus, while the specific activity previously reported (11) was fairly good, it represented only a small amount of apparent enzyme and was probably an artifact of the assay system.

The lack of serotonin synthetic capacity in platelets means that they do not represent a

TABLE II. TRYPTOPHAN HYDROXYLASE ACTIVITY OF INTACT RAT PINEAL GLANDS AND RAT AND HUMAN PLATELETS.[a]

Source of tissue	Tryptophan hydroxylase activity[b]	
Rat pineals	3.68 ± 0.4	$n = 3$
Rat platelets	0.013 ± 0.01	$n = 3$
Human platelets	0.021 ± 0.01	$n = 3$

[a] Tryptophan hydroxylase activity was determined in cultured rat pineal glands or in rat or human platelet-rich plasmas in the presence of 10^{-4} *M* NSD 1055 as described.

[b] Activity is expressed as the percentage of labeled tryptophan converted to 5-hydroxytryptophan per milligram of tissue protein per hour.

complete model of the serotonin neuron, although the serotonin uptake and storage may resemble that found in nerve ending (5). For this latter purpose the platelet may still be considered an adequate model.

Summary. Under conditions that support maximal tryptophan hydroxylating activity in extracts from pineal glands and brain no hydroxylase activity could be detected in platelets. Furthermore, with systems that are adequate to measure serotonin synthesis in cultured pineal glands or whole cells, no serotonin synthetic capacity could be detected in human or rat blood platelets.

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