

Defective Serotonin (5-HT) Transport Mechanism in Platelets from Patients with Endogenous and Allergic Asthma

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The kinetics of the 5-HT-uptake by platelets in platelet-rich plasma (PRP) and PRP diluted with autologous platelet-poor plasma (PRP/PPP) were determined in normal subjects, patients with asymptomatic exogenous asthma and those with symptomatic endogenous asthma.

In normal subjects the 5-HT-uptake by platelets strictly obeyed Michaelis-Menten's kinetics. In patients with asymptomatic exogenous asthma, the active 5-HT-transport was moderately distorted in PRP and severely altered in PRP/PPP. Patients with symptomatic endogenous asthma exhibited already in PRP a dramatic transport disturbance.

The alteration of the active 5-HT-transport was characterized by increasing uptake inhibition by rising 5-HT concentrations in plasma. This phenomenon could be due to changes in the platelet environment, defects in the platelet membrane or intrinsic platelet disorders. It is concluded that asthmatics have a genetically or later acquired platelet disorder, which is more clearly observed in patients with symptomatic endogenous asthma than in those with asymptomatic exogenous asthma.

Key words: endogenous and exogenous asthmatics; platelets; serotonin transport.

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CLINICAL ASPECTS

The 5-HT (serotonin) uptake by platelets from patients with asthma was studied. In patients with symptomatic endogenous asthma, the 5-HT-uptake by platelets was severely impaired, and slightly disturbed in platelets from patients with asymptomatic exogenous asthma.

Serum and whole blood from asthmatic patients has proved to contain higher amounts of 5-HT than that of normal subjects, and elevated levels of 5-HT-metabolites such as kynurenine has been demonstrated in such patients. Furthermore, tryptophan load on asthmatics leads to abnormally high urinary excretion of xanthurenic acid.

As tryptophan is the main precursor in 5-HT synthesis, a reduced tryptophan intake might thus be of importance.

It is reported that patients with allergic asthma have higher blood levels of 5-HT than normal persons (3, 9) and excrete more 5-HT metabolites in urine (21). Asthmatic patients also seem to be more sensitive to administration of 5-HT than healthy subjects (4). Since the circulating platelet is involved in the uptake, secretion, and transport of 5-HT (15), we have previously found reason to study platelets from

asthmatic patients. It was demonstrated that such platelets have a reduced capacity to accumulate 5-HT (11) and a disturbed active 5-HT uptake mechanism (12).

The experimental conditions, under which the 5-HT binding and uptake by platelets should be performed, have been further elaborated, as has the mathematical treatment of data for detecting abnormalities in kinetic pat-

terns (10). In the present investigation, these prerequisites are considered in studies on the 5-HT binding and uptake by platelets from patients with symptomatic endogenous asthma and those with allergic asthma, but who were asymptomatic at the time of examination.

MATERIAL

ACD solution: citric acid 1.37 g, sodium citrate 2.5 g, anhydrous glucose 2.0 g/100 ml distilled water, pH 4.5. ^{14}C -5-HT: 5-hydroxytryptamine $3^1\text{-}^{14}\text{C}$ -creatinine sulphate, specific activity 58 mCi/mmol (Radiochemical Centre, Amersham, U.K.). The ethanol added by the manufacturer was removed by freeze-drying. Soluene 100® and Instagel I® (Packard Instrument Co., Downers Grove, Ill., U.S.A.).

Platelet-rich plasma (PRP): Blood was sampled from a cubital vein with a 1.4 mm wide stainless steel cannula and anti-coagulated with one part ACD solution to nine parts of blood. Immediate centrifugation at $190 \times g$ for 15 min at room temperature and decanting of plasma were performed.

Platelet-poor plasma (PPP) was obtained by centrifugation of PRP at $2,500 \times g$ for 20 min at room temperature. The platelet count was $<1 \times 10^9/\text{l}$.

EXPERIMENTAL PROCEDURE

The experiments were performed between 45 and 60 min after blood sampling on PRP and PRP diluted 1:2 with autologous PPP. The dilution was made immediately prior to incubation with ^{14}C -5-HT. 0.9 ml PRP (or PRP/PPP) was prewarmed for 5 min at 37°C and then incubated with 10 μl ^{14}C -5-HT saline solution at increasing concentrations for 60 sec at 37°C . The final concentrations of ^{14}C -5-HT were 0.17, 0.43, 0.86, 1.29, 1.72 μM , respectively. Each concentration was run in triplicate samples. The reaction was interrupted with 0.1 ml 15% formaldehyde -4.5% EDTA solution and the samples were placed in an ice-bath.

0.2 ml PRP was transferred to a counting vial containing 1 ml Soluene/isopropanol (1:2), and left for digestion for 30 min at room temperature. Thereafter, 15 ml Instagel I were added to the vial and the ^{14}C -activity of the sample was determined in a liquid scintillation spectrometer (Tricarb 2425, Packard Co.). The remaining PRP (0.8 ml) was centrifuged at $20,000 \times g$ for 30 min at 4°C . The supernatant, i.e. the platelet-free plasma (PFP) was carefully decanted and 0.2 ml of the supernatant was transferred to a counting vial, whereafter 15 ml Instagel I were added and the ^{14}C -activity determined. The platelet-bound ^{14}C -5-HT was calculated by subtracting the amount of ^{14}C -5-HT from that in PRP.

The platelet count in whole blood and plasma samples was determined according to Kristenson and with the additional use of a phase contrast microscope (8).

Simultaneously with the blood sampling for plasma preparation, blood was also collected for Phadebas RAST® (Pharmacia Diagnostics, Uppsala, Sweden) analysis according to Ceska et al. (5), firstly to confirm reagenic pollen allergy and secondly to exclude reagenic milk, egg, fish and wheat allergy (titres > 2 , diagnostic). Serum levels of total IgE were determined with PRIST-technique (7).

The skin prick test (SPT) was made with histamine hydrochloride, 18 immunochemically and biologically characterized allergens (23) and evaluated according to the Standardization Committee of the Northern Association of Allergy (1).

Calculations

In order to establish the degree of linearity between the 5-HT concentrations in the medium (c , μM) and the degree of 5-HT uptake (v , $\text{pM}/10^6 \text{ pts}/\text{min}$), analysis according to Scatchard was applied ($v/c = K_n - K_v$) (19). The apparent Michaelis-Menten's constants, K_m and V_{max} , were calculated according to Lineweaver-Burk and Eadie-Hofstee.

In order to obtain a mathematical appraisal of the homogeneity of the 5-HT uptake, the

index of heterogeneity (α) according to the Sips's distribution equation was applied to the Scatchard plot (20). A low correlation coefficient in the Scatchard plot (r (S)) and $\alpha \ll 1$ will denote a heterogeneous 5-HT uptake mechanism without addition of significant passive diffusion. In the Scatchard plot the curve will be "left bending" (10).

Blood donors

Ten patients (age range 22–58 years) with exogenous seasonal asthma were investigated. Manifest exogenous atopic allergic asthma was considered when the sum score for history of positive symptomatology (max score 3), SPT (max score 3) and positive RAST titres (max score 3) was at least 5 (2). At the time of blood sampling, all patients had been free from all drugs, including oral contraceptives, for at least 10 days. The investigation was made during the winter when they were asymptomatic (Table 1).

Eight patients with endogenous asthma (age range 14–56 years) were investigated at the same time as the exogenously asthmatic patients. The definition endogenous asthma included non-seasonal obstructive airway disease of reversible type in non-smokers with no evidence of atopic illness. History of recurrent nasal polyps, deterioration during respiratory infections and after intake of acetylsalicylic acid

or tryptophan/serotonin rich food served as positive criteria. The IgE levels should be normal and SPT and RAST titres negative. All patients had asthmatic symptoms at the time of the investigation. Five of them required aerosol β_2 -stimulants in conventional dosage. Other drugs were criteria for exclusion. The remaining three patients had been free of medication for at least 10 days (Table 2).

Fifty volunteers, 32 females and 18 males (age range 25–51 years) served as controls and were investigated mainly during the same period as the patients. Meticulous interviews were made with regard to general health, diet and medication. Intake of drugs, including oral contraceptives, was not accepted for 10 days prior to blood sampling.

RESULTS

The platelet-count in whole blood from the controls ranged from 183 to 255 $\times 10^9/l$ and in PRP from 380 to 562 $\times 10^9/l$. There was no difference between men and women and no relationship to age. The patients did not differ from the controls in these respects.

The 5-HT uptake by the platelets in undiluted PRP from the control subjects always displaced a saturable transport mechanism (Fig. 1). The 95% tolerance intervals for the Scat-

Table 1
Patients with asymptomatic exogenous asthma

Patient No.	Age/Sex	Age of asthma debut	Serum IgE Normal 0-120 k IU/l	History (score)	SPT (score)	RAST (score)
1	28 M	20	100	3	2	2
2	24 F	20	543	3	3	1
3	37 M	28	136	3	2	1
4	29 M	6	122	3	3	3
5	45 M	43	1233	3	3	3
6	30 F	14	77	3	3	3
7	24 F	20	91	3	2	3
8	58 F	56	47	3	1	1
9	34 M	1	53	3	3	3
10	38 M	13	34	3	3	2

Table 2

Patients with symptomatic endogenous asthma. Serum IgE normal (< 120 k IU/l), all SPT and RAST tests negative

Patient	Age/Sex	Age of asthma debut	Nasal polyps	Triggers				β -2-stimulant aerosols
				Resp. inf.	Milk	Wine/Beer	Aspirin	
1	44 M	40	+		+			+
2	32 F	26		+	+	+	+	+
3	50 F	45	+	+		+	+	+
4	66 F	54	+	+	+	+	+	+
5	61 F	58		+	+	+		+
6	24 M	19				+	+	
7	51 F	43	+	+			+	+
8	49 M	4		+	+	+	+	

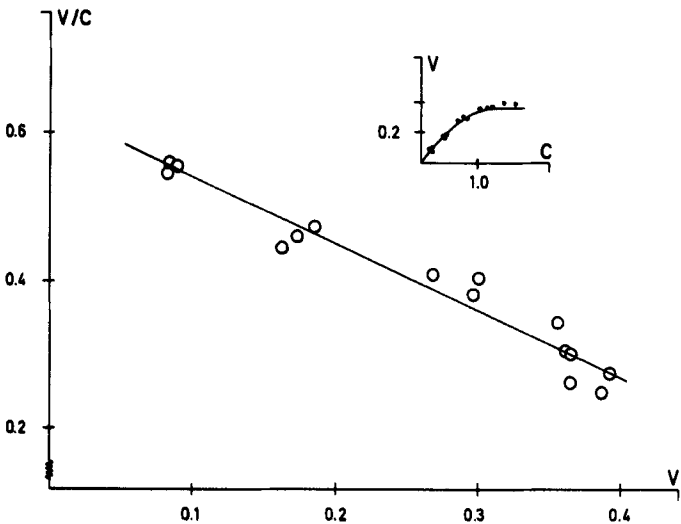


Fig. 1. Scatchard plot of the 5-HT uptake in undiluted control PRP.

Table 3

95 per cent tolerance intervals and mean values for the kinetic parameters of the 5-HT uptake by platelets in undiluted PRP from 50 control donors

	r (S)	α	K_m (μ M)	V_{max} (pmol/ 10^6 pts)
	-0.785; -0.973	0.904; 1.002	0.44; 1.96 ^a	0.52; 2.42
			0.38; 1.85 ^b	0.58; 2.26
Mean	-0.910	0.966	1.20 ^a	1.47
			1.11 ^b	1.38

^a Derived according to Lineweaver-Burke.

^b Derived according to Eadie-Hofstee.

Table 4

Kinetic parameters of the 5-HT-uptake by platelets in undiluted PRP from patients with asymptomatic exogenous and symptomatic endogenous asthma

Patients	Exogenous asthma				Endogenous asthma			
	r (S)	K_m	V_{max}	α	r (S)	K_m	V_{max}	α
1	-0.934	1.56 ^c 1.49 ^d	2.50 ^c 2.46 ^d	0.985	-0.770 ^b	—	—	0.897
2	-0.906	0.93 0.83	1.28 1.20	0.971	-0.582 ^a	—	—	0.786
3	-0.763 ^b	0.76 0.51	1.07 0.88	0.898	-0.777 ^b	—	—	0.916
4	-0.812	0.81 0.60	1.11 0.96	-0.908	-0.819	0.75 0.57	0.86 0.76	0.916
5	-0.899	1.15 0.95	1.16 1.03	0.960	-0.884	1.20 1.01	1.26 1.14	0.961
6	-0.948	0.73 0.71	0.83 0.80	0.984	-0.641 ^a	—	—	0.892
7	-0.756 ^b	0.70 0.62	0.95 0.92	0.909	-0.614 ^a	—	—	0.767
8	-0.862	1.13 1.05	1.08 1.05	0.966	-0.521 ^a	—	—	0.750
9	-0.741 ^b	0.86 0.75	0.68 0.64	0.910				
10	-0.652 ^b	1.23 0.76	1.65 0.99	0.903				
Mean		1.05	1.32	0.939				0.861
SD		0.30	0.60	0.036				0.080
Statistics	$P < 10^{-3}$ vs Table 3 ^e				$P < 10^{-6}$ vs Table 3 ^e			$P < 0.01$ vs Table 3 ^e

^a No significant correlation.

^b Values outside the lower limit of the 95 % tolerance limit for controls.

^c Derived according to Lineweaver-Burk.

^d Derived according to Eadie-Hofstee.

^e Fisher's exact test for 2×2 table.

^f Approximate Student's t-test.

chard analysis correlation coefficient and the index of heterogeneity were narrow as compared to those of K_m and V_{max} , although the latter were identical when derived from the Lineweaver-Burk and Eadie-Hofstee analyses (Table 3).

The 5-HT uptake by platelets in undiluted PRP from the patients with asymptomatic exogenous asthma was disturbed insofar that four out of the 10 patients displayed a Scatchard analysis correlation below the 95 % tolerance interval limit of the normals. The index of heterogeneity, however, remained within

normal limits (Table 4, Fig. 2). The K_m and V_{max} fell within the normal range when calculated according to Lineweaver-Burk. However, the K_m and V_{max} values derived according to Eadie-Hofstee deviated proportionally and increasingly from those obtained from Lineweaver-Burk by decreasing correlation in the Scatchard analysis (Fig. 3). Thus values of K_m and V_{max} were not considered relevant when the correlation coefficient of the Scatchard analysis fell outside the 95 % tolerance interval of the normals. In the following, K_m and V_{max} were derived according to Lineweaver-Burk

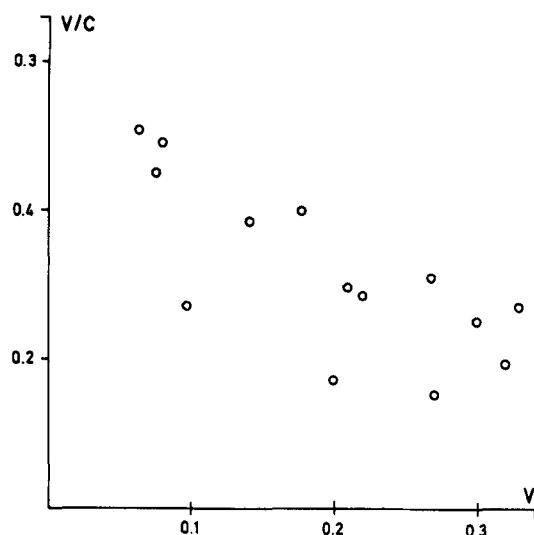


Fig. 2. Scatchard plot of the 5-HT uptake in undiluted PRP from a patient with asymptomatic exogenous asthma (Case No. 7).

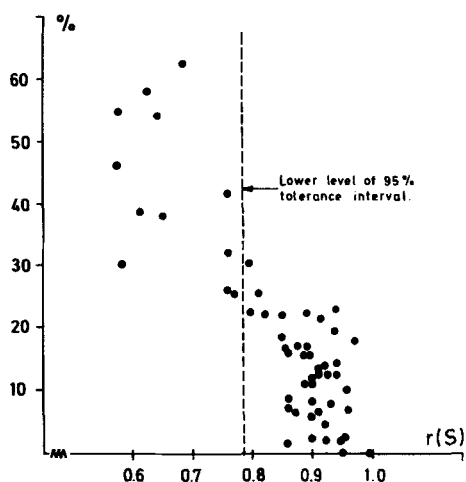


Fig. 3. The degree of discrepancy between K_m values derived according to Lineweaver-Burk and Eadie-Hofstee as percentage – negative or positive – of the Lineweaver-Burk value at various correlation coefficients of the Scatchard analysis. The plot is based on experiments on PRP, PPP-diluted PRP from the controls and exogenously and endogenously asthmatic patients.

A similar plot is obtained for V_{max} values.

only when the correlation coefficient in the Scatchard analysis fell within the 95% tolerance interval.

In the endogenous asthmatic patients, the changes in the 5-HT uptake mechanism

seemed to be more pronounced than in the exogenously asthmatic patients. Only two of eight patients had a normal correlation coefficient in the Scatchard analysis. In four cases there was in fact no correlation between the

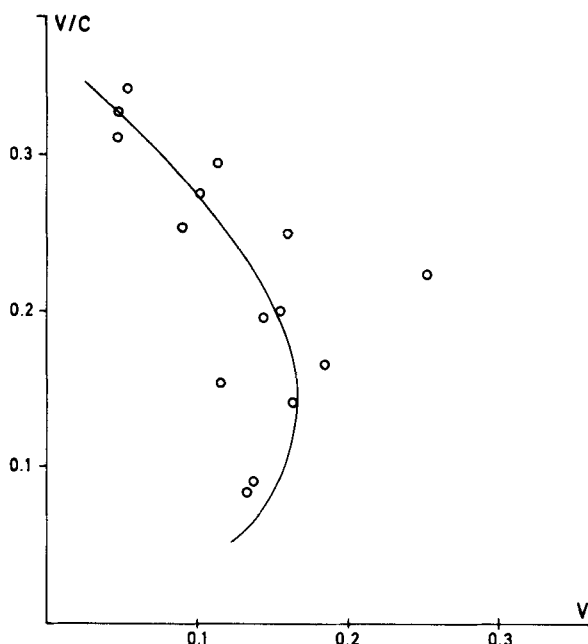


Fig. 4. Scatchard plot of the 5-HT uptake in undiluted PRP from a patient with symptomatic endogenous asthma (Case No. 2). The curve is drawn arbitrarily.

5-HT concentrations in the medium and the uptake velocities. The index of heterogeneity was significantly decreased, indicating a disturbed active 5-HT transport. It was only possible to derive the K_m and V_{max} values in two cases (Table 4, Fig. 4).

The dilution 1:2 of control PRP with autologous PPP changed to a certain extent the platelet 5-HT uptake mechanism as visualized by a small but significant decrease of the index of heterogeneity. In one case, the linearity in the Scatchard plot was absent and in another it fell below the 95% tolerance interval of the controls (Table 5).

Compared to the condition in the diluted control PRP, the disturbance of the 5-HT uptake in diluted PRP from the patients was greater. In eight of the 10 patients with exogenous asthma and in six of the eight with endogenous asthma, there was no linearity between 5-HT concentrations and uptake velocities. Only two patients with exogenous asthma had a Scatchard correlation coefficient permitting derivation of K_m and V_{max} . The index of

heterogeneity in both groups of patients was significantly lower than in the diluted controls (Table 5).

DISCUSSION

In order to ascertain the linearity between substrate concentrations in the medium and the initial uptake velocities, the Eadie-Hofstee analysis was previously applied in a study on the 5-HT uptake by platelets from migrainous and asthmatic patients (12). Such linearity must be established for accurate derivation of the apparent Michaelis-Menten's constants, K_m and V_{max} (16). The Scatchard analysis used here, although it is algebraically interchangeable with the Eadie-Hofstee analysis, permits characterization of the net result of both binding and uptake of 5-HT and of the linearity with the aid of the Sips' analysis (10).

The ^{14}C -5-HT concentrations used were of the same order of magnitude as the 5-HT con-

Table 5

Kinetic parameters for the 5-HT-uptake by platelets in PPP-diluted PRP from controls, patients with asymptomatic exogenous asthma and patients with symptomatic endogenous asthma

Controls	<i>r</i> (S)	<i>K_m</i>	<i>V_{max}</i>	<i>α</i>
1	−0.902	0.90	1.46	0.893
2	−0.953	0.73	1.20	0.973
3	−0.831	0.47	0.52	0.843
4	−0.849	1.17	1.13	0.913
5	−0.862	0.76	1.72	0.955
6	−0.795	0.74	1.10	0.904
7	−0.062 ^a	—	—	0.896
8	−0.702 ^b	—	—	0.853
9	−0.798	0.56	0.88	0.892
10	−0.868	0.82	1.28	0.950
Mean		0.77	1.16	0.911
SD		0.21	0.36	0.044
Exogenous asthma				
1	−0.863	0.78	1.12	0.955
2	−0.206 ^a	—	—	0.487
3	+0.414 ^a	—	—	—
4	−0.819	1.07	1.78	0.940
5	−0.598 ^a	—	—	0.837
6	−0.477 ^a	—	—	0.786
7	−0.505 ^a	—	—	0.763
8	−0.487	—	—	0.759
9	+0.123	—	—	—
10	−0.281 ^a	—	—	0.620
Mean				0.614
SD				0.352
Statistics	<i>P</i> < 0.05 vs controls			<i>P</i> < 0.05 vs controls
Endogenous asthma				
1	−0.689 ^b	—	—	0.833
2	−0.195 ^a	—	—	0.667
3	−0.742 ^b	—	—	0.775
4	−0.342 ^a	—	—	0.491
5	−0.617 ^a	—	—	0.901
6	−0.298 ^a	—	—	0.667
7	+0.924 ^a	—	—	—
8	−0.526 ^a	—	—	0.707
Mean				0.630
SD				0.283
Statistics	<i>P</i> < 0.001 vs controls			<i>P</i> < 0.05 vs controls

a–b, see Table 4.

centrations found in normal human whole blood (17). The 5-HT transport by the platelets in undiluted PRP from healthy persons was described by a strict monophasic curve in the Scatchard plot. Thus, only one active site seems to be involved in the 5-HT transport without addition of significant passive diffusion. When identical experiments were performed at 0°C no diffusion was actually detectable (10). The 95% tolerance intervals for the correlation coefficient in the Scatchard analysis and for the index of heterogeneity in the Sips' analysis were narrow as compared to those of K_m and V_{max} . It may be assumed that 95% tolerance interval denotes the biological variations, and the findings show that the quality of the 5-HT transport by platelets is a more constant feature than the quantity of 5-HT taken up. Hence, a change in the mode of transport is more discriminating than changes in the values of the apparent Michaelis-Menten's constants K_m and V_{max} .

As compared to the controls, the 5-HT transport by platelets was altered in both groups of patients. Although there was always a correlation in the Scatchard plot in undiluted PRP from the patients with asymptomatic exogenous asthma, the correlation coefficient in many cases fell below the normal limits. Under these latter conditions, the derived K_m and V_{max} values would provide meaningless data due to disturbed uptake kinetics. On average, the values of the deviation from linearity and the index of heterogeneity were not of the order of magnitude that permitted conclusions as to whether the active 5-HT transport mechanism was disturbed, or passive diffusion had been added. On dilution of the PRP samples with autologous PRP, however, the 5-HT transport was deteriorated to such an extent that no regular saturable uptake was detectable in the majority of cases.

The patients with symptomatic endogenous asthma exhibited already in undiluted PRP such a severely disturbed 5-HT transport that the PPP dilution could hardly deteriorate it. Compared with these patients, the dysfunction of the 5-HT transport in the patients with asymptomatic exogenous asthma thus seems to indicate a more latent platelet disorder. It re-

mains to be established whether the 5-HT transport in patients with symptomatic exogenous asthma is altered to the same extent as in the endogenous asthmatic patients.

The size of the decrease of the index of heterogeneity mathematically expresses the degree of "left bending" of the curve in the Scatchard plot and demonstrates that the 5-HT transport becomes increasingly inhibited by rising 5-HT concentrations in the medium. The mechanism behind this 5-HT uptake inhibition is obscure, but the factors involved can be sought within the platelet itself, on the platelet membrane and/or in the platelet environment.

It has previously been shown that the normal saturable 5-HT uptake by platelets could be converted to this inhibited type of uptake by the myosin ATP-ase inhibitor *N*-ethylmaleimid and occasionally by dipyridamole (13). The latter is claimed to increase the cAMP level (14). Regarding the platelet environment, it would appear that the disturbance is not related to elevated total plasma IgE. The addition of minute amounts of the supernatant from lysed autologous platelets to fresh PRP has been proven, however, to induce a marked substrate dependent inhibition of the 5-HT uptake (10). Short preincubation of PRP with unlabelled 5-HT in concentrations much below those of the ^{14}C -5-HT used in the test also induced this typical uptake inhibition. Whether this was due to transport disturbance within the platelet or to inhibition of the membrane uptake site is unknown (13). Serum and whole blood from asthmatics patients has proved to contain higher amounts of 5-HT than that of normal subjects (3, 9). This serum 5-HT can originate from platelets or from plasma. Irrespective as to whether the platelets from asthmatics contain more 5-HT than normal platelets, or asthmatics have higher plasma 5-HT concentrations than healthy individuals, the prerequisites for inhibition of the 5-HT uptake may be present.

Centrifugation of normal platelets leads to a stimulation similar to that associated with the platelet release reaction (18). In the event that platelets from asthmatic patients withstand *g*-forces to a lesser degree, thereby increasing the

free 5-HT in the test PRP, the implication would be that they have abnormal membrane characteristics. The effect of PPP-dilution of PRP on the 5-HT uptake, especially in the exogenously asthmatic patients, would tend to support such an assumption.

Proceeding investigations indicate that a reduced tryptophan intake by patients with endogenous asthma normalizes the 5-HT uptake mechanism in undiluted PRP concomitantly with clinical improvement of the asthmatic symptoms. Asthmatic patients may thus have a pathological tryptophan metabolism leading to abnormal variations in free 5-HT levels or to accumulation of other tryptophan metabolites interfering with the 5-HT transport. Elevated levels of serum kynurenine have indeed been demonstrated in such patients (22). Furthermore, the tryptophan load on asthmatic children leads to abnormally high urinary excretion of xanthurenic acid (6). Alternatively or additionally, the asthmatic patients may have a genetical or later in life acquired intrinsic platelet defect that incapacitates the 5-HT uptake.

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