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by [Beving H](#), [Malmgren R](#), [Olsson P](#), [Tornling G](#), [Unge G](#)

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Increased uptake of serotonin in platelets from car painters occupationally exposed to mixtures of solvents and organic isocyanates

by Håkan Beving, PhD,¹ Rigmor Malmgren, DrMedSc,¹ Per Olsson, MD,¹
Göran Tornling, MD,² Gunnar Unge, MD²

BEVING H, MALMGREN R, OLSSON P, TORNLING G, UNGE G. Increased uptake of serotonin in platelets from car painters occupationally exposed to mixtures of solvents and organic isocyanates. *Scand j work environ health* 9 (1983) 253–258. Twelve car painters occupationally exposed to mixtures of solvents and organic isocyanates were investigated concerning serotonin uptake in platelets. The data from the exposed workers were compared to data from a reference group consisting of 50 nonexposed volunteers. The mean platelet count in whole blood of the exposed workers was markedly lower than the corresponding value of the reference group. Three workers had values below the lower limit of the 95 % tolerance interval of the referents. In both groups the serotonin transport strictly obeyed Michaelis-Menten's simple saturation kinetics. Nine of the twelve exposed workers had uptake rates which were significantly higher ($p = 0.05$) than those of the referents. The obtained data suggest that the present method may be used to reveal early organ damage, prior to the appearance of clinical symptoms, due to intermittent exposure to organic solvents and isocyanates.

Key terms: 5-hydroxytryptamine, organic solvents, uptake kinetics.

Platelets have proved to be suitable model cells for presynaptic neurons (18, 26). Among the several striking resemblances are possession of an excitable cell membrane sensitive to environmental changes (1, 15, 18) and the specific uptake and storage of serotonin (10, 12, 27).

We have recently developed a technique for making kinetic measurements of serotonin transport in human platelets. This method permitted characterization of a transport disorder in platelets from patients with endogenous asthma (14).

Organic solvents are known to exert toxic effects, particularly on the central nervous system and blood-forming organs (7, 20, 29). Car painters are exposed to a mixture of organic solvents and chemicals

(5). The garage routine involves, in addition to color mixing and spray-painting, grease removal and grinding, filling with isocyanate-containing putty and hardeners, and frequent cleaning of various tools and the worker's own hands. The main solvents used are, in order of use, methyl-n-butyl-ketone, white spirit (17–22 % aromatics, methyl benzenes included), toluene, methylene chloride, and sometimes trichloroethane and trichloroethylene (5).

As mentioned, the workers are exposed to isocyanates, which are one of the components in hardeners. The isocyanates are toxic to mucosa membranes, especially conjunctiva and the respiratory tract (3, 22, 28). Long-term exposure to low concentrations of organic isocyanates may cause asthma-like reactions (3, 16, 17). The isocyanates react in vitro with free amino groups and other sites on protein molecules. The reaction products may be similar to derivatives formed in vivo which cause immunogenic and/or allergenic symptoms in exposed workers (28). With hydrolysis hexamethylene diisocyanate,

¹ Department of Experimental Surgery, Karolinska Institute, Stockholm, Sweden.

² Department of Thoracic Medicine, Karolinska Hospital, Stockholm, Sweden.

Reprint requests to: Prof P Olsson, Department of Experimental Surgery, Thoracic Clinics, Karolinska Hospital, S-104 01 Stockholm, Sweden.

which was present in low amounts in the hardeners used by the workers in the present study, yields hexamethylenediamine, a toxic substance, the fumes of which have been shown to cause respiratory difficulties in exposed rats (6).

Since isocyanates occasionally lead to a specific form of severe asthma and patients with endogenous asthma show serotonin uptake kinetics which do not adhere to Michaelis-Menten's saturation kinetics (14), we found it of interest to investigate whether concomitant exposure to solvents and organic isocyanates in any way influences serotonin transport in platelets from occupationally exposed car painters.

Material and methods

Material

The ACD solution used in the analyses was 1.37 g of citric acid, 2.5 g of sodium citrate, and 2.0 g of anhydrous glucose per 100 ml of distilled water. The pH of the solution was 4.5 (Karolinska Pharmacy, Stockholm). 5-Hydroxy-(2-¹⁴C)tryptamine creatinine sulfate (¹⁴C 5-HT), specific activity 58 mCi/mmol (2.15 GBq/mmol, Radiochemical Centre, Amersham, United Kingdom) was used in the incubation of the platelet-rich plasma. The ethanol added by the manufacturer was removed by freedrying, and the residue was dissolved in 0.9 % saline. Soluene 100® (protein solubilizer) and Instagel I® (scintillation liquid) (Packard Instrument, Co, Downers Grove, IL, United States) were also used in the analyses.

For the platelet-rich plasma, blood samples from a cubital vein were taken with a 1.4-mm wide stainless steel cannula and distributed into seven polystyrene centrifuge tubes containing citrate solution (ACD 1 ml). Nine milliliters of blood was added to each tube. Immediate centrifugation at 190 × g for 15 min at room temperature and decanting of the plasma followed.

Blood donors

Twelve men (age range 21–52 years), employed in the car painting industry and exposed daily to solvents and organic isocyanates, were studied. The investigations

were conducted on Tuesday and Wednesday mornings. Fifty volunteers (32 women and 18 men, age range 25–51 years) from the staff of different wards of the Thoracic Clinics, Karolinska Hospital, served as referents and were examined during the same period as the car painters. The volunteers included in this study were participating in a current investigation concerning the platelet serotonin uptake kinetics of healthy people. In the reference data no differences in the kinetic parameters of the men and women have been observed. No intake of any drug was permitted for 10 d prior to the taking of the blood samples. The exposed workers were interviewed regarding abnormal fatigue, irritability, symptoms from the respiratory tract, or allergic manifestations.

Experimental procedure

The experiments were performed between 45 and 60 min after the taking of the blood samples. Platelet-rich plasma (0.9 ml) was prewarmed for 5 min at 37°C and then incubated for 60 s at 37°C with 20 µl of the ¹⁴C 5-HT solution. The final result was five concentrations ranging from 0.1 to 2.0 µmol/l (15). Each concentration was run in triplicate. The reaction was interrupted with 0.1 ml of 15 % formaldehyde – 4.5 % disodium ethylenediamine tetraacetic acid solution, and the samples were placed in an ice bath (4). An aliquot (0.2 ml) of each platelet-rich plasma sample was transferred to a counting vial containing 1 ml of Soluene/isopropanol (1 + 1) and left for digestion for 30 min at room temperature. Thereafter 15 ml of Instagel I/0.5-mol/l hydrochloric acid (9 + 1) was added, and the radioactivity was determined in a liquid scintillation spectrometer (Tricarb 2425, Packard Instrument Co). The remaining platelet-rich plasma was centrifuged for 30 min at 20,000 × g at 4°C. An aliquot (0.2 ml) of the resulting platelet-free plasma (< 1 × 10⁹ platelets/l) from each sample was transferred to a polyethylene counting vial. Fifteen milliliters of the scintillation liquid was added, and the radioactivity was determined. The platelet bound activity (v, µmol/l) was calculated by the subtraction of the amount of ¹⁴C activity in platelet-free plasma from that in platelet-rich plasma, the latter of which represents the total activity (C, µmol/l).

Table 1. Age, platelet number in whole blood and platelet-rich plasma (PRP), and the following kinetic parameters: the correlation coefficient in the Scatchard analysis [$r(S)$], the index of heterogeneity according to Sips (α), the concentration of serotonin needed to achieve half the maximum uptake rate (K_m), and the maximum uptake rate when the carrier is saturated (V_{max}) for 12 solvent-exposed workers, as well as the 95 % tolerance interval for the 50 referents.

Case number	Age (years)	Platelets ($\times 10^9/l$)		Kinetic parameters			
		Blood	PRP	$r(S)$	α	K_m ($\mu\text{mol/l}$)	V_{max} ($\text{pmol} \cdot 10^6 \text{ platelets}^{-1} \cdot \text{min}^{-1}$)
1	24	124	268	-0.856	0.927	1.14	2.51 ^a
2	22	228	412	-0.949	0.991	1.85	3.49 ^a
3	22	145	383	-0.961	0.988	1.18	1.98
4	35	117 ^a	256	-0.860	0.957	1.58	3.52 ^a
5	23	135	274	-0.958	0.983	1.43	3.56 ^a
6	39	171	331	-0.969	0.990	1.65	3.32 ^a
7	38	174	345	-0.884	0.957	1.18	1.79
8	51	125	281	-0.940	0.980	1.07	2.48 ^a
9	23	115 ^a	350	-0.941	0.983	1.60	3.10 ^a
10	20	129	335	-0.967	0.988	1.57	3.11 ^a
11	36	219	496	-0.861	0.935	1.11	1.47
12	29	85 ^a	184 ^a	-0.929	0.983	1.43	4.86 ^a
Mean	.	150	309	.	0.972	1.40	2.93
SD	.	44	110	.	0.022	0.26	0.93
Mean (referents)		220	451	-0.910	0.966	1.20	1.47
95 % tolerance intervals for referents (N = 50)		118 & 332	206 & 696	-0.785 & -0.973	0.904 & 1.002	0.44 & 1.96	0.52 & 2.42

^a Values outside the 95 % tolerance interval.

The platelet count in the whole blood and plasma samples was determined by electronic cell counting (Linson 431 A, LIC Instrument, Sweden).

Calculations

The adherence of the experimental data to Michaelis-Menten's simple saturation kinetics was ascertained by linearization methods according to Scatchard & Sips (23, 25). The kinetic parameters V_{max} ($\text{pmol}/10^6$ platelets/min), ie, the maximum uptake rate when the carrier is saturated, and K_m ($\mu\text{mol/l}$), ie, the concentration of serotonin needed to achieve half the maximum uptake rate, were obtained according to Lineweaver & Burk (9). The combined use of these mathematical methods has earlier been described by us (11, 12). Ninety-five percent tolerance intervals (mean $\pm 2.28 \times \text{SD}$) were calculated for the Scatchard correlation coefficient [$r(S)$], the index of heterogeneity (α) in the Sips analysis, and the K_m and V_{max} . Since

a tolerance interval covers a certain part of a population (95 %) with a certain nominal probability, 5 % values falling outside the limits of the intervals were considered significantly abnormal.

Results

None of the exposed workers complained of irritability or exhibited symptoms that could be related to massive solvent exposure. They were therefore considered on par with the reference group in regard to general health.

The 95 % tolerance intervals for the platelet count in whole blood, the correlation coefficient in the Scatchard analysis, the index of heterogeneity according to Sips, the K_m , and the V_{max} from the reference group are presented in table 1. The first two mentioned parameters show that the platelets from the reference subjects always displayed a saturable serotonin transport mechanism (fig 1).

Fig 1. Scatchard plot of the serotonin (5-HT) uptake in platelet-rich plasma from one referent.

Fig 2. Michaelis-Menten's plot of the serotonin (5-HT) uptake in platelet-rich plasma from one referent (336 platelets $\times 10^9/l$) (●—●) and case 6 (331 platelets $\times 10^9/l$) (○—○).

The mean value of the platelet count in whole blood from the solvent-exposed workers was markedly lower than the corresponding value of the reference group, and three workers had values outside the lower limit of the tolerance interval of the referents (table 1).

The serotonin uptake by platelets from the subjects exposed to solvents adhered also in all respects to a saturable mode of serotonin transport, ie, both the correlation coefficient in the Scatchard analysis and

the index of heterogeneity according to Sips were within the tolerance intervals of the referents (table 1, fig 1). Nine of the twelve exposed workers had V_{max} values that were however outside the upper limit of the tolerance interval of the referents; this phenomenon is depicted by an elevated Michaelis-Menten's hyperbola in fig 2. The K_m values were all within the normal range.

Discussion

The results of the present investigation show that workers exposed to solvents and organic isocyanates differ as a group from nonexposed referents with regard to the transport of serotonin in platelets. Although the serotonin transport strictly obeyed Michaelis-Menten's simple saturation kinetics, the V_{max} values of the majority of the workers were significantly higher than those of the referents. The platelet count was below the lower limit of the tolerance interval in three of the twelve subjects, and on the average the platelet numbers were lower in the exposed group than in the reference group.

Solvents such as toluene, xylene, and white spirit are present in many paint mixtures. In addition organic isocyanates are often one of the components in the frequently used hardeners. The total daily exposure may consist of synergistic and/or cumulative effects from inhalation and skin adsorption of the different agents (11, 19). It is therefore difficult to discriminate between the impact of the different solvents and isocyanates on the organism.

Humans with evident symptoms of intoxication from benzene, toluene, or xylene show a decreased number of leucocytes and platelets. The reduction is considered to be due to an impaired cell regeneration function of the bone marrow (20, 29). The platelet count of the exposed subjects in the present investigation was lowered as compared to that of the referents. The workers did not, however, complain of the symptoms, like fatigue or headaches, usually related to exposure to organic solvents. We find it unlikely that the subjects, in their modern and well-ventilated environment, should suffer damage to the bone marrow.

The intrinsic metabolic activity of the platelet and the composition of the plasma environment influence the serotonin uptake both in quality and in quantity (8, 15). The normal platelet population shows heterogeneity in both cell size and density. Platelets newly released from the bone marrow are heavy and large, while older platelets are lighter and smaller. The heavy and large cells have a higher metabolic activity and have been shown to have an increased serotonin uptake rate (8). An increased platelet turnover and a subsequent shift towards a younger platelet population should thus result in an increase in $V_{\max}$, a phenomenon which we found in nine of our solvent-exposed subjects. In case of a normal platelet turnover in the solvent-exposed individuals, the rise in $V_{\max}$ would imply that the number of membrane-bound serotonin carrier molecules per platelet have been increased.

It is well known that organic solvents and their metabolites affect the neuronal membrane (22, 24). The platelet is accepted as an appropriate model cell for a pre-synaptic neuron. A similar influence on the platelet membrane of solvent metabolites might therefore be possible and may be an alternative explanation for the aberrant serotonin transport.

Organic isocyanates in the work environment may induce marked irritation in the respiratory tract and on mucous membranes, but the mechanism behind their toxicity is not clear. The investigated workers neither exhibited asthmatic symptoms nor the aberrant serotonin kinetic characteristics for endogenous asthma. Organic isocyanates react in vitro with free amino groups and other sites in proteins (6, 28). Hexamethylenediisocyanate was present in the hardeners used by our workers. In preliminary in vitro experiments we have not found that hexamethylenediamine, a hydrolysis product from hexamethylenediisocyanate in final plasma concentrations of 10^{-8} , 10^{-6} , and 10^{-4} mol/l, alters the serotonin uptake in platelets from healthy volunteers.

Although the mechanisms behind the aberrant serotonin transport in platelets from the investigated workers remain obscure, we are inclined to believe that the solvents play a major role. The present method for determining serotonin transport may indicate very slight and initial

organ damage caused by solvents and solvent metabolites prior to the appearance of clinical symptoms.

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