ACTIVE UPTAKE OF SEROTONIN BY BLOOD PLATELETS OF SCHIZOPHRENIC PATIENTS

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1. Introduction

Platelets have become a popular model for the monoaminergic neurons and especially for studies of serotonin uptake and metabolism [1,2]. Considering that human platelets can be obtained very easily, studies were directed towards comparing storage, metabolism and uptake of serotonin in various neurological and mental illnesses [3,4]. Differences have been reported in serotonin levels and MAO activity between schizophrenics and healthy people [5,6]. This led to the hypothesis that the pharmacodynamics of blood platelet serotonin is abnormal in certain conditions characterized by disturbed brain functions. If platelet serotonin is a reflection of malfunction of indolalkylamine metabolism, such platelet abnormalities may reflect changes that have occurred in serotonin-containing neurons in the central nervous system.

We have shown [7] that the active uptake of serotonin in schizophrenic patients in acute state is much lower (35%) than that of control group. We would like to report here biochemical and pharmacological properties related to this significantly lower uptake. These biochemical parameters may give some indication of the serotonin carrier condition in the platelet membrane.

2. Methods

Blood was drawn from patients or control volunteers with ACD (NIH solution A) as anticoagulant. Blood (8.5 ml) was collected into a plastic tube containing 1.5 ml anticoagulant (trisodium citrate dihydrate (2.2 g), citric acid monohydrate (0.8 g), and glucose monohydrate (2.5 g); diluted to 100 ml with double-distilled water). After 3 consecutive centrifugations at 4°C and removal of the red blood cells, the platelet rich plasma (PRP) was obtained. PRP (475 μl) were preincubated at 37°C for 5 min in Eppendorf plastic tubes and the uptake was initiated by the addition of 25 μl [^{3}H]serotonin solution (2×10^{-6} M). The final serotonin concentration was 1×10^{-7} M. Uptake was studied over a period of 5 min at 37°C, then the tubes were immediately cooled to 2°C and centrifuged in Beckman Microfuge for 2 min. The supernatant was removed and the platelet pellet was washed 3 times with saline containing 1 mM EDTA and counted, using scintillation mixture prepared from: 2,5-diphenyloxazol (8 g); 1,4-di-2-(4-methyl-5-phynyloxazolyl)-benzene (200 mg); toluene (1320 ml); Triton X-100 (660 ml).

Inhibition studies were carried out by preincubation of the PRP with the inhibitor at the appropriate concentration for 5 min and then the serotonin uptake was initiated as described above. Platelets were counted after fixation of 0.1 ml PRP with 2 ml solution: formaldehyde (0.25%); acetone (2.5%); saline (2.5%).

3. Results

Figure 1 shows a time course of serotonin uptake at 1×10^{-7} M. It is clear that the uptake is linear with time for ~6 min then levels off. Accordingly all our studies were done over an incubation period of 5 min. The effect of antischizophrenic drugs on the uptake in vitro was studied using the same drugs

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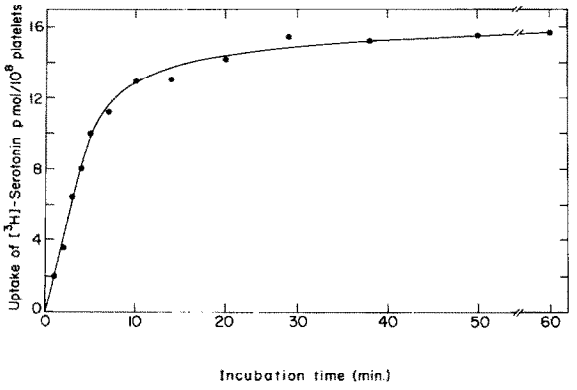

Fig.1. Time course of serotonin uptake by human platelets. Serotonin, 1×10^{-7} M.

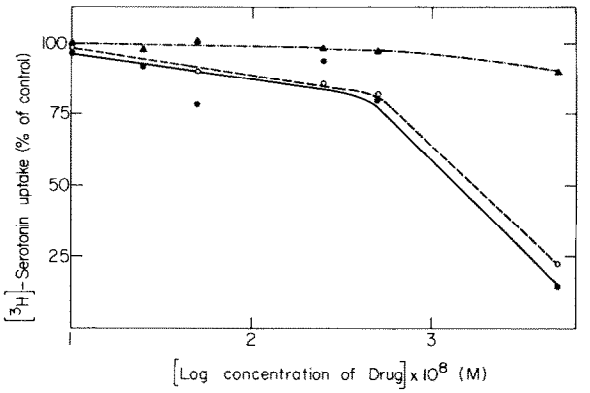

Fig.3. Human platelet serotonin uptake inhibition by halidol (○), Neuleptyl (○), and Melleryl (△), measured over 5 min incubation with serotonin at 1×10^{-7} M.

which were used by the patients in the study. Results shown in fig.2,3 indicate that these drugs have no effect on the active uptake of serotonin at 10^{-6}–10^{-7} M (where the antidepressants imipramine and amitryptaline show 80–99% inhibition). Our most interesting result is shown in fig.4, where the active uptake was studied as a function of concentration. Patients exhibit a saturable active transport with K_m 1.8×10^{-6} M and V_{max} 250 pmol serotonin/10^8 platelets/5 min. Control (healthy human volunteers) also show a saturable process with K_m 1.8×10^{-6} M and V_{max} 350 pmol serotonin/10^8 platelets/5 min.

4. Discussion

The uptake of serotonin by human blood platelets is believed to be mediated by a carrier located in the cell membrane [8,9]. The affinity of the substrate (serotonin) towards the carrier is given by the K_m value while the V_{max} of the process is proportional to the number of carriers or receptors present. Our results indicate that the maximum velocity of serotonin transport into 1 cell is 7000 molecules/s in the control group and only 5000 molecules/s in the patients. It was reported [10] that human platelets

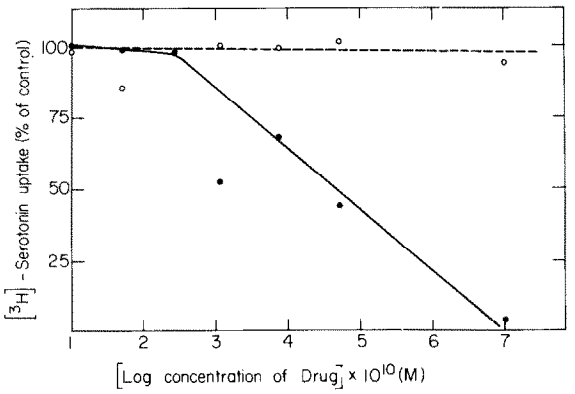

Fig.2. Human platelet serotonin uptake inhibition by chlorpromatine (○) and valium (○) measured over 5 min incubation with serotonin at 1×10^{-7} M.

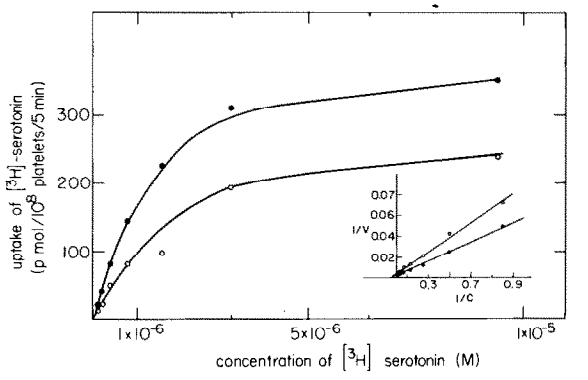

Fig.4. Uptake to serotonin by blood platelets of controls (●) and patients (○) as a function of serotonin concentration over 5 min incubation. Values are average of duplicates of 4 experiments. Insert: Lineweaver-Burk analysis.

contain 2 binding sites/serotonin. The high affinity site is probably associated with shape change while the low affinity site (with a capacity of 4000 sites/cell) is associated with uptake. If we assume that each binding site corresponds to a carrier this means that each carrier can transport 1 molecule of serotonin in 0.57 s. The fact that the V_{max} in patients was much lower than that of controls means that the number of carriers in patients is lower than in the healthy control group. However, the identical K_m values in the two groups indicate that each carrier shows the same affinity towards the serotonin molecule and either carrier (in the schizophrenic patients or the controls) will transport 1 molecule of serotonin into the cell in 0.57 s.

This study gives a molecular and biochemical dimension to the search for the etiology of schizophrenia. The assay of serotonin transport was conducted in a way eliminating metabolic processes and diffusion (by a short incubation time). Thus, the process studied was a membrane phenomenon not affected by the drug taken by patients but dependent rather on the nature and properties of the serotonin carrier. The nature of the lower number of carriers in patients is not yet understood, nor its significance for a probable difference in the brain. Further studies in this direction are now in progress in our laboratory.

Acknowledgement

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