UP TAKE OF SEROTONIN IN BLOOD PLATELETS:
DEPENDENCE ON SODIUM AND CHLORIDE, AND INHIBITION
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Received 13 March 1969

1. Introduction

Blood platelets from most species, including man, have the ability to take up serotonin (5-hydroxytryptamine, 5HT) from the surrounding medium, by two different mechanisms: one so-called “active” mechanism, following a Michaelis-Menten type kinetics, and one “passive” mechanism, having the characteristics of simple diffusion [1–3]. The active uptake is inhibited non-competitively by a variety of metabolic inhibitors [2,4,5]. Tricyclic antidepressants, phenothiazines, cocain, and tryptamine have been shown to inhibit the uptake competitively [6,7].

Studies of 5HT uptake in platelets are of interest not only with regard to platelet biochemistry and transport phenomena in general, but also with regard to neurochemistry. It is well established that 5HT plays an important role in the central nervous system, probably as a transmitter. Furthermore, the mechanisms for uptake and storage of 5HT in neurones and platelets seem to be analogous, if not identical. Since the metabolism of neurones is difficult to study, the easily available blood platelets can be regarded as a convenient model with regard to 5HT uptake and storage.

The purpose of the present report is to demonstrate that the active uptake of 5HT in human blood platelets in vitro requires the presence of sodium as well as chloride, and that choline (Ch+), which is generally considered to be an inert cation, exerts a competitive inhibitory effect on the uptake of 5HT.

2. Methods

Blood was drawn from the cubital vein of healthy donors and mixed in cellulose nitrate tubes with one tenth the volume of EDTA, 1.5% in 0.5% NaCl. Platelet-rich plasma (PRP) was obtained by centrifugation (250 X g) for 20 min at room temperature. Ali-quots of 1 ml PRP were transferred (with a plastic pipette) to thin-walled polypropylene tubes, mixed with 1 ml cold isotonic saline and centrifuged for 15 min (2500 X g) at 4°. The supernatant was discarded, the test tubes allowed to drain, and the remaining supernatant carefully wiped away. The platelet pellet was resuspended (using a “Whirlimixer”, Scientific Industries Internat., Inc.) in 2 ml cold incubation medium, containing phosphate buffer, and NaCl, KCl, or choline chloride (ChCl) in varying concentrations. The final medium was isotonic or slightly hypertonic (shown not to interfere significantly with the rate of 5HT uptake). Incubation with 14C-5HT (5-hydroxytryptamine-3-14C creatinine sulphate from The Radiochemical Center, Amersham, specific activity adjusted to 10 μc/mole X 10−6, dissolved in 10−3 M HCl) was carried out for 5 to 8 min in a water shaking bath at 37°, after temperature equilibration for 9 min. The 5HT was added in a volume not exceeding 25 μl. The incubation was interrupted by transferring the test tubes to ice water, and the platelets were isolated by centrifugation (2500 X g) for 25 min at 4°. After draining, 1 ml of distilled water was added to the pellet, and the 5HT was completely released by freeze-
ing overnight at $-20^\circ$, followed by thawing and centrifugation. One half ml of the supernatant was mixed with Bray's solution [8] and the radioactivity measured in a Packard Tri-Carb liquid scintillation counter. Blank values, to be subtracted from the total measured radioactivity, were obtained by adding $^{14}$C-5HT after the test tubes had been transferred to ice water, just prior to centrifugation. ("Washing" was not found suitable, since it was found to give loss of intracellular 5HT). As internal standards were used samples to which $^{14}$C-5HT was added after the final separation.

The reliability of this method has been calculated in duplicate experiments. Standard error (based on differences within pairs) were found to be $\pm 2\%$ of pair means. Platelets were counted in a phase contrast microscope. Platelet recovery by resuspension was found to be fairly constant at 85%.

3. Results

3.1. Sodium dependence

In one series the platelets were incubated with sodium chloride and sodium phosphate buffer at 6 different pH values, while in a duplicate series sodium was replaced by potassium. It is seen from fig. 1 that there is no net uptake without Na$^+$ present. The uptake is also markedly dependent on pH, which is in accordance with the findings of Stacey [6].

3.2. Chloride dependence and inhibitory effect of choline

Platelets were incubated with sodium and potassium phosphate buffer plus increasing amounts of NaCl or ChCl. The total osmolality of the medium varied from about 0.29 to 0.39 M. It is seen from fig. 2 that there is no net uptake without Cl$^-$ present. The 5HT uptake increases up to a NaCl concentration of about $5 \times 10^{-2}$ M. With increasing concentrations of ChCl, on the other hand, the serotonin uptake levels off at a ChCl concentration of about $2 \times 10^{-2}$ M, whereafter it decreases at higher concentrations, and gives a maximal 5HT uptake which is only about 50% of that obtained with NaCl. Independent experiments have shown that the lower uptake with ChCl than with NaCl can only partially be explained by a stimulatory effect of Na$^+$ (which was already present in near optimal concentration as part of the buffer). Thus, Ch$^+$ seemed to have an inhibitory effect on the uptake.

To study the kinetics of this inhibition, platelets...
were incubated in phosphate buffer with increasing concentrations of \(^{14}C\)-SHT at different levels of [ChCl]. The chloride concentration and total osmolality were kept constant by adding NaCl in proper amounts to the different samples. The reciprocal plots of the net uptakes (fig. 3) show that the inhibitory effect of Ch\(^+\) is of a pure competitive type. When the slopes are plotted against [Ch\(^+\)] (insertion in fig. 3), the inhibition is thus seen to be linear, with an inhibitor constant \(K_i = 2 \times 10^{-2}\) M.

4. Discussion

Sodium dependence is a property shared by many membrane transport mechanisms. Recently it has been shown that SHT uptake by synaptosomes prepared from rat brain is also sodium-dependent [9]. Prada and collaborators have found that sodium is essential for maintaining normal ultrastructure and SHT binding capacity in rabbit blood platelets [10], which might suggest that the sodium dependence demonstrated in the present study is an unspecific phenomenon. However, the present author has found that, within the time intervals used in this study, preincubation without Na\(^+\) has little effect on subsequent uptake rates, and that the loss of \(^{14}C\)-SHT from platelets was practically unaffected by [Na\(^+\)] in the medium. Thus sodium dependence seems to be a specific property of the uptake mechanism per se. Corresponding results have been found with regard to chloride [11], showing that chloride dependence also is a specific property of the uptake mechanism. However, chloride can partly be replaced by some unphysiologic, small diffusible anions like Br\(^-\), NO\(_2\) and NO\(_3\).

Since the uptake of SHT is competitively inhibited by choline, and choline itself is taken up in certain neurones by an active, sodium-dependent process [12], a common uptake mechanism for SHT and choline must be considered. However, the author has been unable to demonstrate active uptake of \(^{14}C\)-choline chloride in human blood platelets [11].

Choline adds to the group of compounds known to inhibit competitively the uptake of SHT in blood platelets. The fact that the competitive inhibitors have widely different chemical structures, suggests that the binding of SHT to a hypothetical carrier in the plasma membrane is rather unspecific. In contrast, the storage of SHT within platelets, in the so-called dense osmiophilic granules [13], seems to be dependent on a specific mechanism [14].

The inhibition of SHT uptake by choline is of interest in connection with the common use of choline as an inert substitute for sodium in experiments on membrane transport. The possibility that choline may interfere with the transport mechanism under study has to be taken into consideration.

Acknowledgements

This work was supported in part by a grant from Norsk Medicinaldepot. I am very grateful to Dr. Elling Kvamme and his coworkers for useful suggestions and inspiring discussions during the study.
References