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GABA Uptake by Human Blood Platelets: Effects of Lithium and Rubidium Chlorides

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The methodologic and kinetic characteristics of GABA uptake by platelets were determined in blood platelets of volunteer donors. Extrapolation of a reciprocal plot indicates two uptake systems: a high affinity and a passive diffusion mechanism. When LiCl and RbCl were added *in vitro*, they had no effect on platelet GABA uptake. Our data suggest that platelet GABA and 5-HT uptake are carried out by different systems.

Blood platelets are able to accumulate several neurotransmitters such as 5-hydroxytryptamine (5-HT), noradrenaline (NA) and dopamine (DA). Platelet uptake and storage of these monoamines have been proposed as a useful model for similar processes in brain nerve endings (19-21).

A less clear aspect is to know whether the blood platelets are able to transport inhibitory neurotransmitters such as γ-aminobutyric acid (GABA) because there is no clear evidence in respect to this problem (1, 5).

Since GABA is the most important inhibitory neurotransmitter in the central nervous system (CNS), the knowledge of the existence of a GABA transport system in platelets might provide a useful model in the study of interactions between inhibitory and excitatory neurotransmitters due to the fact that platelets is a more available and a less complicated system than the brain.

Because a dysfunction in the GABA system has been implicated in affective disorders (7, 15) in which lithium has well established therapeutic effects (13) we have also examined the effects *in vitro* of lithium and rubidium salts on GABA platelet uptake, an aspect completely unknown at present.

Materials and Methods

Blood was obtained by venipuncture from normal volunter donnors of both sexes between the age of 26 and 51. Blood was taken in a polyethylene syringe with

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ACD-A (2) as an anticoagulant. Polyethylene pipettes and polyethylene tubes were used throughout. Platelet rich plasma (PRP) was obtained by centrifugation at 300 x*g* during 8 minutes at 15° C. Subsequent platelet isolation was made in discontinous dextran gradients (P_D) according to GRAF *et al* (10). Platelets were counted by a contrast phase microscopy in triplicate, using 10 μl aliquots of suspended platelets diluted in 2 ml of 1% amonium oxalate. Then, platelet preparations were resuspended in tyrode-tris buffer and mixed. Platelet suspensions did not present aggregation when they were observed under contrast phase microscopy.

GABA uptake. 100 μl of platelet samples containing at least 0.25×10^5 platelets/mm^3 and no more than 3×10^5 platelets/mm^3, were incubated in triplicate, with concentrations of [^{3}H]-GABA or ^{14}C -GABA between 10^{-9} and 10^{-7} M and between 10^{-7} and 10^{-4} M, respectively. Samples were incubated in polypropylene tubes, and platelet contact with glassware was strictly avoided. Radioactive substrates were added to each sample after preincubation for 10 minutes. Incubations were carried out between 1 and 30 minutes in a Heto Denmark bath at 37° C or in an ice bath at 4° C for samples and blanks respectively. The uptake process was rapidly stopped by addition of 10% formaldehyde and rapid cooling in ice. Platelets were then quickly isolated by filtration in a vacuum filter pump (Millipore) with different kinds of filters after addition of 1 ml of ice cold saline solution. Filters were washed several (1-5) times and then transferred to scintillation vials to which 10 ml of counting fluid (biofluor NEN) was added. Platelet radioactivity was determined by liquid scintillation spectrometry using a Packard Tri-Carb Counter model 2425 and cpm were determined with internal standardization by the sample channels ratio method. When the effects of lithium and rubidium chlorides were studied, LiCl and RbCl replaced equimolar quantities of NaCl in the suspension medium. This replacement was performed in order to avoid any change in osmolarity of incubation medium.

Results

Methodologic considerations. The evaluation of certain parameters of platelet GABA uptake was studied on both platelet rich plasma —since it provides a me-

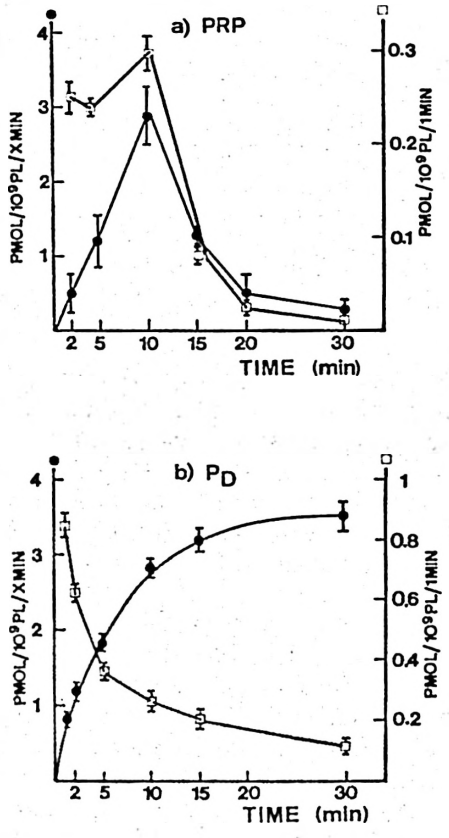

Fig. 1. *Time course of GABA uptake.*
Human platelets in tyrode-tris buffer at pH = 7.4 were incubated with 10^{-7} M [^{3}H]-GABA at 37° C at indicated times. a) PRP, b) P_D. Values represent the means ± SD of three experiments, each preformed in triplicate. (□) Values expressed as pmol/10^9 plts/min. (●) Values expressed as pmol/10^9 plts/min

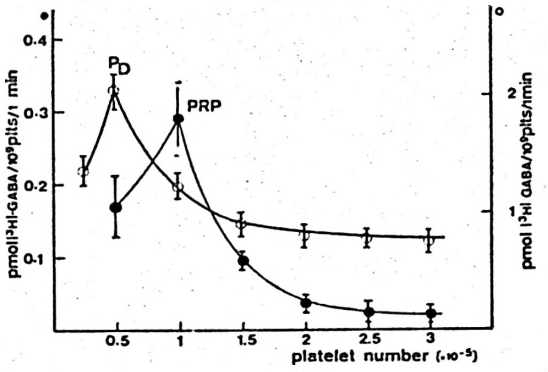

Fig. 2. *Effect of platelet number on [³H]-GABA uptake by PRP (●) and P_D (○).*
Points represent the mean ± SD of three experiments, each preformed in triplicate. Experimental conditions: Concentration of [³H]-GABA = 10^{-7} M; the remaining conditions are those indicated in Table I.

dium which is as close as possible to the physiological— and on dextran isolated platelets in order to avoid the possible effects of anticoagulant and plasma proteins.

Analysis of [³H]-GABA (10^{-7} M) at incubation times between 1 and 30 minutes revealed a saturable GABA uptake in P_D (fig. 1b) while in PRP the uptake/time relation was increased during the first 10 minutes and then decreased (fig. 1a). This decrease suggests that the integrity of the platelets in PRP is lost after 10-12 minutes of incubation.

The GABA platelet uptake was also examined as a function of the platelet number. Concentrations from 0.5 to 3×10^5 platelets/mm³ were incubated with 10^{-7} M [³H]-GABA. The maximum transport was obtained with 10^5 platelets/mm³ for PRP and with 0.5×10^5 platelets/mm³ for P_D (fig. 2).

Table I. *Optimal methodologic conditions of assay of GABA uptake by human blood platelets.*
A) PRP = Platelet rich plasma. B) Dextran isolated platelets.

Platelet material	A) PRP	B) P_D
Blank type	4° C	4° C
Type of filter	Whatman GFC	Whatman GFC
Number of washes	2×5 ml 0.9 % NaCl	2×5 ml 0.9 % NaCl
Incubation time	10 min	1 min
Number of platelets/mm³	10^5	0.5×10^5

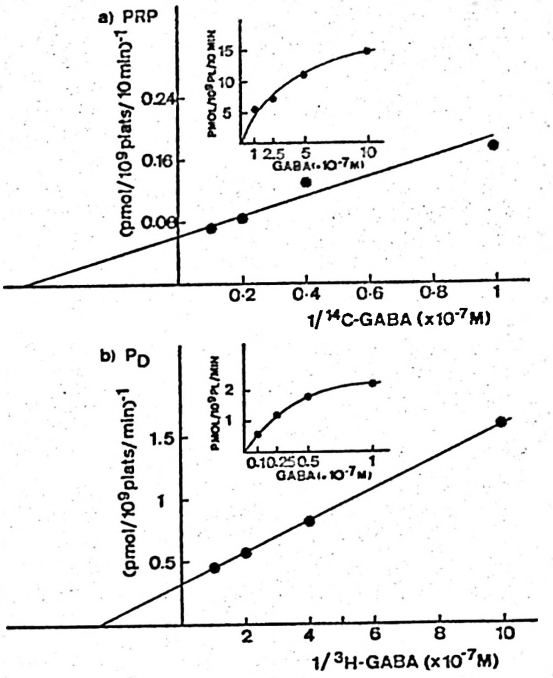

Fig. 3. *Lineweaver-Burk plot showing extrapolated km and Vmax values for high affinity GABA uptake by a) PRP and b) P_D.*
When PRP were assayed at low [¹⁴C]-GABA concentrations between 10^{-7} and 10^{-6} M, we found a km = 2.08 ± 0.57 × 10^{-7} M and a Vmax = 1.61 ± 0.37 pmol [¹⁴C]-GABA/10^9 platelets/10 minutes (fig. 3a). When P_D were assayed at [³H]-GABA concentrations between 10^{-9} and 10^{-7} M, we found a km = 0.37 ± 0.11 × 10^{-7} M and Vmax = 2.85 ± 0.45 pmol [³H]-GABA/10^9 plts / min.

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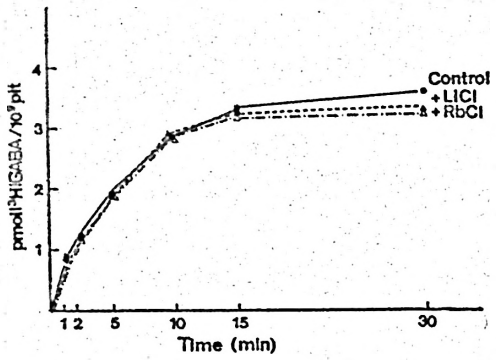

Fig. 4. *LiCl and RbCl effects on time course of [3H]-GABA uptake by dextran isolated human platelets.*

Values represent the mean of three experiments, each preformed in triplicate. The S. D. was less than 3-7% of mean values. The assayed [3H]-GABA concentration was of 10^{-7} M.

Several types of filters (Millipore 0.22 μm, Whatman GFA, GFB and GFC); number of washes (between 1 to 5) and type of blank (uptake at 4° C or stop of uptake by addition of 10% formaldehyde) were checked. The best results were obtained under the following conditions: glass fibre filter Whatman GFC; two washes and blank at 4° C. These conditions were used throughout (table I).

Kinetic studies. [3H]-GABA concentrations were varied between 10^{-9} and 10^{-7} M for P_D and [14C]-GABA between 10^{-7} and 10^{-4} M for PRP. The analysis of these results by the double-reciprocal plot of LINEWEAVER and BURK (14), showed a km of $2.08 \pm 0.57 \times 10^{-7}$ M and $0.37 \pm 0.11 \times 10^{-7}$ M for PRP and P_D respectively and a Vmax of 1.61 ± 0.37 and 2.85 ± 0.45 pmol of GABA/10^9 platelets/10 min and 1 min, respectively for PRP and P_D (figs. 3a and 3b).

Effects of lithum and rubidium chlorides on GABA uptake by platelets. When litium and rubidium chlorides were added *in vitro* at concentrations of 0.01 mM (a concentration that provided a marked reduced uptake rate on 5-HT uptake) (18), they did not affect GABA uptake in platelets of normal persons at any assayed incubation times (fig. 4). The effects of both salts were similar and the differences were not statistically significant.

Discussion

At present most authors who have investigated GABA uptake by platelets have demonstrated the existence of a passive diffusion process (1, 5, 22). We have demonstrated, in accordance with ENNS and MCCOY (8), the existence of a high affinity GABA transport by platelets (fig. 3 a and b). This uptake presented an affinity and a Vmax higher in P_D than in PRP, which suggests the possible presence in PRP of a plasma inhibitor of GABA uptake. This inhibitor seems to affect not only the Vmax but also the km. The decrease in GABA uptake observed with increased PRP concentration also supports this observation (fig. 2).

Several transmitter amines, such as dopamine and 5-HT are transported into the cells by a common uptake system according to OMENN and SMITH (17), but according to STAHL and MELTZER (21), both transports are different.

In accordance with our results, when LiCl and RbCl were added *in vitro* at 0.01 mM final concentration, a marked inhibitory effect on 5-HT transport by platelets (% inhibition of 61.54% for LiCl and 51.41% for RbCl) was observed (18), while these salts did not affect GABA transport by platelets. This finding could support the idea that GABA and 5-HT are transported by separate systems, but the confirmation of this hypothesis is under study. The different action of several effectors on GABA and 5-HT uptake are in accordance with ENNS and MCCOY (8), who showed that chlorimipramine and (±)-p-chloro-amphetamine have no apparent effect on GABA uptake while inhibiting > 90% of 5-HT uptake.

The uptake and storage of monoamines

by platelets have been proposed as a model for similar processes in brain nerve endings (19-21) and platelets from subjects with Down's syndrome have been shown to have both a decreased rate of 5-HT (16) and GABA (8) uptake. With respect to GABA we can not affirm this similarity since we observe that the affinity of amino acids for platelet transport systems is higher than in neurones (20 μM) (4, 11), ganglia (10 μM) and cortex (22 μM) (12), while the Vmax (1.61 and 2.85 pmol/10^9 platelets/min $\simeq$ 0.18 and 0.31 pmol/mg of protein) are lower compared with synaptosomes (980 pmol/mg /min) (11), ganglia (2.1 pmol/mg/min) and cortex (115 pmol/mg/min) (12). This result is logical since the quantity of GABA in platelets is very low.

Our results indicate that in platelets there is a transport system which presents a high affinity for GABA but we can not tell yet whether this system is only specific to GABA although the results with LiCl and RbCl seem to indicate that at least 5-HT and GABA transport could be different. There is no evidence about the LiCl and RbCl on the central GABA uptake. It is known that the administration of LiCl to rats decreased glutamate levels and enhanced GABA content (6).

These results seem to demonstrate that platelets are not a suitable model for studying GABA uptake by neurones. However, since some protein synthesis occurs in platelets it is possible that GABA and other amino acids may play a role in the synthesis of proteins and GABA uptake would regulate, in some way, their rate of synthesis. This hypothesis on the possible GABA effect on protein synthesis is supported by data obtained by BAXTER *et al* (3), who found that in brain GABA influences protein synthesis. Mereover, it could be possible that platelets represent a good model in the study of possible implications of GABA in synthesis, metabolism and/or liberation of catecholamines and 5-HT.

Resumen

Se han determinado las características metodológicas y cinéticas de la captación del ácido γ-aminobutírico (GABA) por plaquetas sanguíneas normales. El análisis de Lineweaver-Burk muestra la existencia de dos mecanismos de transporte: un proceso de alta afinidad y una simple difusión pasiva. Los cloruros de litio y rubidio no tienen ningún efecto *in vitro* sobre la captación de GABA por plaquetas. Nuestros datos sugieren dos mecanismos de transporte plaquetar diferentes para el GABA y la 5-hidroxitriptamina (5-HT).

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