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TRANSPORT OF TAURINE BY NORMAL HUMAN BLOOD PLATELETS

LISA AHTEE, D.J. BOULLIN[1] & M.K. PAASONEN

Department of Pharmacology, University of Helsinki, Siltavuorenpenger 10, SF00170, Helsinki, Finland

1 Because normal human blood platelets contain higher concentrations of taurine than any other amino acid, and have a platelet : plasma concentration gradient exceeding 400 : 1, we isolated the cells *in vitro* and incubated them with radioactively labelled taurine in order to investigate the existence of a metabolically-dependent accumulation process.

2 Platelets incubated with taurine (1 to 100 nmol/ml) in autologous plasma or Krebs solution accumulated [^{14}C]-taurine against the concentration gradient.

3 The transport process was saturable at high concentrations, showed a requirement for sodium ions, and was temperature-dependent.

4 The kinetics of transport fulfilled the criteria of Michaelis & Menten for saturable enzyme/substrate interactions, but the kinetic constants were influenced by the incubation medium.

5 The metabolic inhibitors 2,4-dinitrophenol and iodoacetic acid in combination inhibited taurine transport in Krebs solution, but stimulated transport in autologous plasma. The latter result suggested the involvement of a sodium-dependent ATPase in taurine transport.

6 We conclude that platelets actively transport taurine *in vitro* under experimental conditions closely resembling those likely to occur *in vivo*, and that this taurine transport process may be involved in the maintenance of the platelet : plasma concentration gradient.

Introduction

Blood platelets are known to contain all the essential amino acids. In addition taurine is present in a concentration six times greater than that of any other amino acid (see Maupin, 1969). From Maupin's data it may be calculated that there is a platelet : plasma concentration gradient for taurine of approximately 440 : 1 with a plasma concentration of 48 nmol/ml.

There are many mechanisms whereby cells may maintain their constituents at concentrations much greater than those of plasma or extracellular fluid. These include intracellular binding and energy-dependent transport processes. As platelets are known to accumulate a variety of amino acids by several different mechanisms, including energy-dependent transport (Boullin & Green, 1972), we considered it worthwhile to see whether platelets possess a transport process for taurine uptake.

Some of the results of our experiments have been presented in preliminary form (Boullin, Ahtee, Airaksinen & Paasonen, 1973).

[1] Present address: MRC Unit & University Department of Clinical Pharmacology, Radcliffe Informary, Oxford, OX2 6HE.

Methods

Blood was collected by venipuncture from normal adult volunteers of either sex into 0.1 volumes of 1% disodium edetate (EDTA) and then centrifuged at 150 *g* for 15 min to obtain platelet rich plasma (PRP). In experiments in which platelets were resuspended in normal or low Na^+ Krebs solution, aliquots of PRP were centrifuged at 18,000 *g* for 5 min in order to prepare a platelet pellet. The cells were subsequently resuspended in the saline medium by agitation on a vortex mixer.

Platelet counting

Platelets were counted visually by the method of Feissley (1961) and the volume of cells per ml of PRP was determined with thrombocytocrits as described by Boullin & O'Brien (1969).

Incubation experiments

Platelets were incubated in autologous plasma or Krebs solution of the following composition (mM): NaCl, 117; KCl, 4.9; NaH_2PO_4, 1.0; $NaHCO_3$, 25.0; KH_2PO_4, 2.2; $MgSO_4$, 1.2; EDTA, 3.0; glucose, 11.1.

In the experiments to study the Na^+ depend-

ency of amino acid transport processes, the following low Na^+ Krebs solution was used (mM): choline chloride, 143; KCl, 4.9; KH_2PO_4, 2.2; $MgSO_4$, 1.2; EDTA, 3.0; glucose, 11.1. The Na^+ content of this solution was 3.0 mM.

Incubations were carried out in a metabolic shaker at 37°C, or in ice at 0°C. The medium was gassed with 5% CO_2 in O_2 before the experiment.

Radioactive materials

[^{14}C-1,2]-taurine, specific activity 59.3 mCi/mmol and [^{3}H]-methoxy-inulin, specific activity 218 μCi/mg were both obtained from New England Nuclear Corp., Boston, Mass, U.S.A. [^{14}C]-carboxylic acid inulin, specific activity 9.8 mCi/mmol was obtained from the Radiochemical Centre, Amersham. Taurine was dissolved in distilled water to give a stock concentration of 10 mM which was subsequently diluted as required. Inulin was similarly diluted to 200 μCi/ml. In all experiments the radioactive solutions were added to the platelet incubation mixtures in a volume of 10 μl.

Separation of platelets

At the end of the incubation the platelets were separated from the incubation medium by high-speed centrifugation as described above. The cells were then disrupted by ultrasonic impulses (20,000 kHz) provided by a Branson 'Sonic Power' sonifier, model S25 (Branson Instruments Inc., Danbury, Conn., U.S.A.) fitted with an exponential titanium microtip. This instrument was used at low power setting (no. 2) and tuned to deliver a current of approximately 2.4 A. The platelet pellet comprising cells from 1 ml PRP was disrupted by short bursts of impulses for an aggregate time not exceeding 10 s in a volume of 1 ml distilled water.

Counting of radioactivity

Aliquots (0.1-5 ml) of the platelet pellet lysate and the platelet incubation medium were counted by liquid scintillation spectrometry using 10 ml of a scintillation phosphor of the following composition: toluene, 1.2 l; ethylene glycolmonoethylether, 0.8 l; naphthalene, 160 g; 'Omnifluor' (NEN Chemicals GmbH, Frankfurt am Main, West Germany), 8.0 g. The liquid scintillation spectrometer was a DECEM-NTL 314, manufactured by Wallac OY, Turku, Finland. Counts were corrected for quenching and counting efficiency.

Metabolism of [^{14}C]-taurine by platelets

In 3 subjects, platelets from 10 ml of plasma were incubated with [^{14}C]-taurine (10 nmol/ml) in Krebs solution for 2 hours. The platelets were then separated from the incubation medium by high speed centrifugation and the platelet pellet disrupted ultrasonically, as described above. The disrupted cells were placed upon a column of ZEO Karb 325 (BDH). The effluent was passed over a strongly basic cation exchange resin (Dowex 1 x-10) whereby taurine was separated from other amino acids and identified as described by Agrawal, Davison & Kaczmarek (1971).

Measurement of endogenous platelet taurine

Blood from four adult females was obtained, and platelet pellets prepared from 6 ml PRP as described above. After removal of traces of plasma the pellets were stored at −40°C for one week before analysis of the taurine content. At the time of analysis, the pellets were thawed and disrupted by ultrasonic impulses in 1 ml distilled water as described above. Aliquots of the platelet lysate were deproteinized with 20% sulphosalicyclic acid and assayed by quantitative ion exchange chromatography with a JEOL-5AH automatic amino acid analyser (Japan Electron Optics, U.K., Ltd).

Results

Accumulation of taurine

Platelets rapidly accumulated taurine during incubation in plasma at 37°C. Uptake was detected 5 min after addition of radioactivity, the concentration gradient platelet : incubation medium, $C_i : C_o$, exceeding unity in all experiments at this time. Uptake continued throughout the 2 h incubation period. When the plasma concentration of taurine was low, 1 nmol/ml, accumulation was approximately linear, but at the higher concentrations of 10 and 100 nmol/ml the platelet content of [^{14}C]-taurine approached equilibrium after 2 h incubations (Figure 1).

Uptake of [^{14}C]-taurine also occurred when platelets were removed from plasma by centrifugation and resuspended in Krebs solution. Although the pattern of uptake was qualitatively similar, it was approximately double that in plasma for a given concentration in the incubation medium (C_o). This is reflected in the greater concentration ratios $C_i : C_o$ calculated from the radioactive measurements (Figure 2).

These results suggested that [^{14}C]-taurine was accumulated against the platelet : plasma concentration gradient by an energy requiring process. Accordingly, experiments were done to test this view. Firstly, the effects of low temperature on

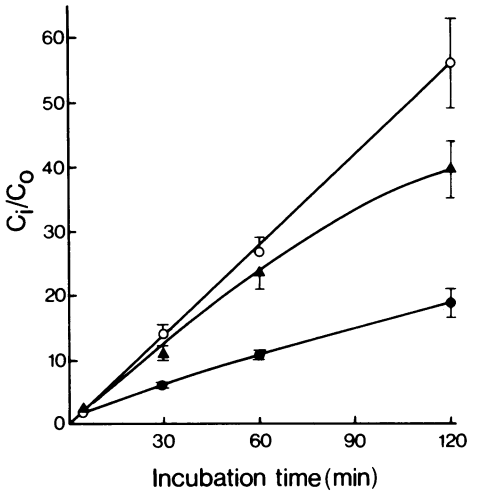

Fig. 1 Time course of accumulation of [^{14}C]-taurine by platelets suspended in plasma containing different concentrations of the labelled amino acid. The values are expressed in terms of the platelet : plasma concentration ratio (C_i : C_o, ordinates) plotted against incubation time at 37° C (min, abscissae). Each value is the mean of 4 to 6 observations. Vertical bars show s.e. mean. (○) 1.0 nmol/ml; (▲) 10.0 nmol/ml; (●) 100 nmol/ml.

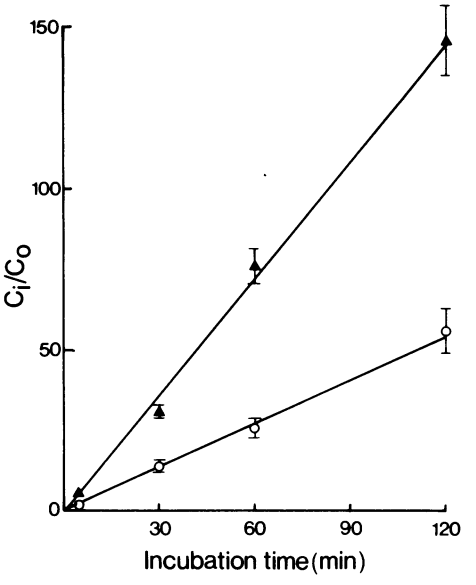

Fig. 2 Comparison of the accumulation of [^{14}C]-taurine by platelets suspended in plasma or Krebs solution with [^{14}]-taurine 1 μmol/ml for 5 to 120 min as described in methods section. Values for C_i : C_o (ordinates) are plotted against incubation time at 37° C (min, abscissae) and are the mean of 4 to 6 observations. Vertical bars indicate s.e. mean. (▲) Krebs solution; (○) Plasma.

taurine accumulation were investigated. When platelets were incubated with taurine at 0° C for 15-120 min, C_i/C_o did not exceed 0.5 irrespective of (C_o) or whether the incubations were in plasma or Krebs solution. Secondly, we studied the actions of the metabolic inhibitors 2,4-dinitrophenol (DNP) and iodoacetic acid on taurine uptake. These substances have been shown to produce varying degrees of inhibition of the accumulation of some amino acids and drugs as demonstrated in earlier work (Boullin & O'Brien, 1969; Boullin & Green, 1972). In the present experiments the results were somewhat unexpected because the effects were dependent upon the medium used for platelet incubations. With Krebs solution, DNP and iodoacetic acid together produced at least 95% inhibition of taurine uptake when added to the medium in a concentration of 100 nmol/ml 30 min before incubation with taurine (1 nmol/ml) for 60 minutes. On the other hand, similar experiments with plasma as the incubation medium produced unexpected results. Both inhibitors enhanced the accumulation of [^{14}C]-taurine (Table 1).

In spite of anomalous results with metabolic inhibitors in plasma, the overall results indicated that taurine was taken up by platelets by an

Table 1 Effects of metabolic inhibitors on taurine transport by platelets suspended in autologous plasma or Krebs solution

Incubation medium	*Change in [^{14}C]-taurine-transport (%)*	
	DNP + IAc	*Low temp.*
Plasma	+60.6	−97.2
	±0.4	±0.6
	n = 5	*n* = 10
Krebs solution	−94.9	−98.0
	±0.5	±0.4
	n = 10	*n* = 9

These results were obtained by incubation of platelets in plasma or Krebs solution containing [^{14}C]-taurine (1 nmol/ml) for 2 h in the presence of 0.1 μmol/ml each of DNP and iodoacetic acid (IAc) as described in the results section.
Each value is the mean (with s.e.) obtained from the experiments indicated. The results are expressed as % inhibition (or stimulation +) of the uptake of [^{14}C]-taurine that occurred with absence of DNP and iodoacetic acid.

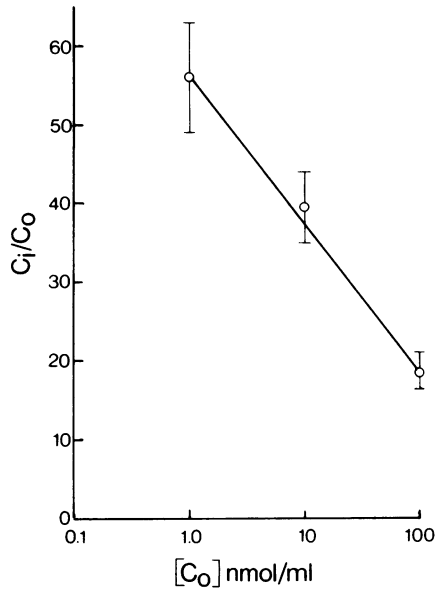

Fig. 3 The relationship between $C_i : C_o$ and (C_o). Results were obtained by incubation of platelets suspended in plasma containing [^{14}C]-taurine 1.0, 10.0 or 100 μmol/ml for 120 min at 37° C as described in methods section. Results are expressed as $C_i : C_o$ (ordinates) plotted against (C_o) (μmol/ml, abscissae) and are mean of 4 to 6 observations. Vertical bars indicate s.e. mean.

energy-dependent process. Thus, data obtained following incubation of platelets for 2 h, with a range of medium concentrations (1-100 nmol/ml), indicated that the accumulation process is saturable (Figure 3). In addition experiments in which platelets were suspended in a modified Krebs solution with the Na^+ concentration reduced from 150 to 3.0 μmol/ml showed that the uptake process has a requirement for Na^+. In this low Na^+ medium, taurine was accumulated by the platelets very slowly and C_i/C_o did not significantly exceed unity after 2 h incubation at 37° C.

Kinetics of taurine transport

The above results showing that taurine is transported by a Na^+- and energy-dependent, saturable, mechanism raise the question as to whether the process fulfils the criteria of Michaelis & Menten normally applicable to saturable enzyme/substrate interactions. Analysis of the data obtained following incubation of platelets with [^{14}C]-taurine (7 to 100 nmol/ml) in plasma for 5 min at 37° C shows that the Michaelis/Menten equation can be applied. Table 2 gives the values for the 'apparent K_m' hereafter referred to as K_m, and V_{max} obtained for taurine transport in plasma and Krebs solution. The effect of resuspending platelets in Krebs solution was greatly to increase the affinity of taurine for the transport process (decrease in the value for K_m) without significantly affecting the velocity constant V_{max}.

Decline in taurine content of the incubation medium

In all experiments, the taurine content of the incubation medium declined. Although the decreases were not significant during the first 5 min of the experiments, there were significant changes later. As might be supposed from the results described earlier, the decline was greatest in the experiments with Krebs solution. For example, the following results were obtained in the experiment which showed maximal effects: after 2 h incubation with [^{14}C]-taurine (1 nmol/ml), C_i/C_o was 247 : 1, and the taurine content of the Krebs solution (C_o) declined to 34.6% of the original value. Similar but quantitatively smaller reductions were also seen with higher initial concentrations in the medium; thus, (C_o) declined to 68.5% of an original value of 10 nmol/ml and to 92.8% with 100 nmol/ml. The cumulative data for experiments with plasma and Krebs solution are shown in Table 3. The ability of platelets to accumulate sufficient exogenous radio-labelled taurine *in vitro* to result in a highly significant decrease in medium concentrations, suggests that

Table 2 Kinetics of taurine transport by platelets

Incubation medium	*Apparent* K_m *(μM)*	V_{max} *(nmol ml packed cells^{-1} min^{-1})*
Plasma	199.3	59.1 ± 7.3
Krebs solution	25.4	39.6 ± 6.7

The values shown are based on results obtained by incubating normal platelets in autologous plasma or Krebs solution containing [^{14}C]-taurine (1, 10 or 100 nmol/ml) for 5 min at 37° C. Each value is based on 4 experiments and where appropriate is expressed as mean with s.e.

Table 3 Decline in taurine content of the incubation medium following incubation of platelets with various concentrations of [^{14}C]-taurine for 2 hours

Incubation medium	*Initial taurine concentration (nmol/ml)*		
	1	*10*	*100*
Plasma	82.1 ± 1.4 *n* = 4	72.7 ± 3.6 *n* = 4	83.5 ± 3.0 *n* = 4
Krebs solution	65.8 ± 2.7 *n* = 3	73.6 ± 4.9 *n* = 3	91.8 ± 3.8 *n* = 3

The results are from experiments in which platelets were incubated in autologous plasma or Krebs solution for 2 hours. The radioactivity in the incubation medium was counted before and after incubation. Values shown are % of the original value (= 100%) present in the incubation medium after 2 h and are the mean with s.e. of 3 or 4 experiments.

in vivo the transport process may be partly responsible for maintenance of the high platelet : plasma concentration gradient (see discussion section).

Metabolism of accumulated taurine by platelets

The results of 3 experiments where taurine was separated from other amino acids by the procedure of Agrawal *et al.* (1971) (see methods section) showed little metabolism. After 2 h incubation with [^{14}C]-taurine (10 nmol/ml), 89-94% of the radioactivity in the platelets was unmetabolized taurine.

Endogenous platelet taurine

Endogenous taurine was measured in platelets from 6 ml PRP in 4 normal subjects. The cells contained 44.8 ± 7.4 μmol/g after correction for 46% recovery of authentic taurine added to duplicate samples. These values are midway between those of Frendo, Koj & Zgliczynski (1959) who reported 1.5 mg/g platelets (equivalent to approximately 78 μmol/g platelets) and those of Maupin (1969) (21 μmol/g platelets). Plasma content was also measured, but was below the limit of sensitivity of the analytical method (<0.01 μmol/ml).

Discussion

The results described clearly show that human platelets transport radioactively-labelled taurine by a sodium-dependent, energy-requiring process that is readily saturable and conforms to Michaelis-Menten kinetics, at least during the first 5 min of experiments. The data also support the view that the high platelet content of endogenous taurine described previously by Frendo *et al.* (1959) and Maupin (1969) and confirmed again here, may involve the operation *in vivo* of the taurine transport mechanism that we have studied *in vitro*. The reason for this is that after prolonged incubation, the platelets accumulate sufficient [^{14}C]-taurine to bring about a significant decline in the concentration of the amino acid in the incubation medium.

On the basis of the metabolic data, it is reasonable to assume that [^{14}C]-taurine was not metabolized significantly by platelets under our experimental conditions. Accordingly, the platelet : medium concentration ratios for total, as opposed to radioactive, taurine may be calculated using the endogenous value 44.8 μmol/g. Although there was considerable accumulation of radioactive taurine by the platelets, the concentrations were very low in proportion to the endogenous concentration. Even when the medium concentration was 100 nmol/ml the total platelet taurine content was increased from 44.8 to 46.6 μmol/g. These data do not alter our contention that platelets may accumulate taurine *in vivo*.

One of the interesting aspects of our experiments concerns the effects of changes in the incubation medium on taurine transport. Changing the incubation from autologous plasma to Krebs solution had two main effects: firstly, it resulted in alterations to the kinetic constants, K_m and V_{max}, describing the transport process, and secondly, it influenced the effectiveness of the metabolic inhibitors, DNP and iodoacetic acid.

The comparative effects of plasma and artificial media upon platelet amino acid transport have not been studied. The use of physiological saline media excludes both plasma proteins which may bind the amino acid, and also the 18-20 amino acids normally found in plasma. Our group has now investigated the transport of 6 amino acids by human platelets in physiological saline solutions; there are wide variations in transport kinetics

(Table 4). The depression of taurine transport in plasma compared with saline (Fig. 2) and a decreased affinity for the transport system (Table 2) may well be due to competition between amino acids for platelet transport systems. Boullin, Votavova & Green (1972) showed that arginine and leucine transport is dramatically reduced by the addition of 19 other amino acids to the Krebs solution in concentrations of 20 nmol/ml. Although in our case we were unable to detect taurine in the plasma, Maupin (1969) had earlier reported values of 48 nmol/ml. As our detection limit was 10 nmol/ml, the presence of lower concentrations would not have been discovered; had values been in the range 1 to 10 nmol/ml they would have substantially altered our determinations of K_m and V_{max} made in plasma. It seems, therefore, that the use of artificial saline incubation media for investigation of platelet transport mechanisms is likely to give quite different results from those obtained with autologous plasma. If practicable, the use of plasma would appear preferable because of the changes in platelet shape and ultrastructure which take place in saline solutions. However, when investigating amino acid transport, the most reliable information on transport kinetics may be obtained at the present time with the use of physiological saline solutions, which obviates the effects of amino acids invariably present in plasma.

The apparent stimulation of taurine transport produced in plasma by the metabolic inhibitors DNP and iodoacetic acid, was a surprising finding. An analogous effect has been noted occasionally during the accumulation of some guanidine-based drugs such as amiloride and debrisoquin (D.J. Boullin & R.A. O'Brien, unpublished observations), whilst cocaine enhances the binding of chlorpromazine to platelet membranes (O'Brien & Boullin, 1974). One possible explanation of this phenomenon is that the metabolic inhibitors affect the binding of amino acids and drugs to plasma proteins, thereby influencing the quantities of free drug available for transport (O'Brien & Boullin, 1974). Another possibility that applies particularly to amino acid transport concerns the multiplicity of transport systems involved. It seems unlikely that platelets possess separate transport systems for each amino acid. As several amino acids probably share a common dose-dependent, carrier mediated, transport process, then the metabolic inhibitors may have differential effects; this is known to be the case for arginine and leucine (Boullin *et al.*, 1972).

Another possibility is that DNP stimulates Na^+-dependent ATPases (Tanaka & Abood, 1962, 1964; Abood, 1969) which are involved in various transport systems (Crane, 1965; Bogdanski & Brodie, 1969). The platelet protein, thrombasthenin, is known to act as an ATPase, and ultrastructural studies have shown that ATPase activity is confined to platelet surfaces (for references, see Marcus & Zucker, 1968). Thus, resuspension of platelets in saline media could result in alterations to ATPase activity.

Although these suggestions are speculative, we can conclude that taurine is actively transported into platelets under experimental conditions that closely resemble those likely to occur *in vivo* and a taurine transport system probably accounts for the very high taurine concentrations reported previously.

It is a pleasure to thank the Sigrid Juselius Foundation for financial support of this work, and for a Fellowship for one of us (D.J.B.).

Table 4 Some apparent affinity and velocity constants of transport processes by human platelets

Substance transported	*Incubation medium*	*Apparent* K_m *(nmol/ml)*	V_{max} *(nmol ml packed platelets^{-1} min^{-1})*	*Authors*
Taurine	Plasma	199.0	59.0	This paper
	Saline	25.0	40.0	This paper
L-arginine	Saline	3.9	59.0	Boullin *et al.* (1972)
L-leucine	Saline	0.7	13.1	Boullin *et al.* (1972)
Dopamine	Plasma	0.7	1.9	Boullin & O'Brien (1970)
	Saline	67.0	33.0	Solomon, Spirt & Abrahams (1970)
L-tryptophan	Saline	0.2	7.1	Boullin & Green (1972)
L-phenylalanine	Saline	7.7	132.0	Boullin & Green (1972)
L-DOPA	Saline	25.0	303.0	Boullin & Green (1972)

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