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INTERACTION OF THALLOUS IONS WITH THE CATION TRANSPORT MECHANISM IN ERYTHROCYTES

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SUMMARY

1. Tl^+ can replace K^+ in the activation of the ATPase of fragmented red cell membranes. In the presence of Na^+, equal activation is obtained with Tl^+ concentrations only 1/10 of those of K^+. In all other respects the $(Na^+ + K^+)$- and $(Na^+ + Tl^+)$-ATPases were found to be identical.

2. The transport of Tl^+ into erythrocytes was found to be independent of ouabain and glucose. The stationary cell/medium distribution of $^{204}Tl^+$ was only 1.8–2.0, *i.e.* close to reciprocal values of the passive distribution of small anions. The inward rate constants were 9.36 h^{-1} for $^{204}Tl^+$ and 0.153 h^{-1} for K^+. The corresponding outward rate constants were 4.68 h^{-1} and 0.0066 h^{-1}, respectively.

3. Tl^+ could substitute for K^+ in supporting the ouabain-sensitive outflux of $^{22}Na^+$ from red cells. A similar outflux of $^{22}Na^+$ was observed at 1 mM Tl^+ and 10 mM K^+. At higher concentrations Tl^+, unlike K^+, inhibited the ouabain-sensitive $^{22}Na^+$ outflux. The inhibition was greater in fresh erythrocytes than in cells stored in the cold for about 1 week or more. The difference in the degree of the Tl^+ inhibition may be attributed to the different Na^+ concentrations in fresh and old erythrocytes.

4. On the basis of the results obtained it is suggested that the mechanism of active ion transport involves a destruction of the ion hydration shells.

INTRODUCTION

The chemical and biochemical behavior of Tl^+ resembles that of K^+ and Rb^+ (ref. 1). The membranes of frog sartorius are about as permeable to Tl^+ as to K^+ (ref. 2). Tl^+ and K^+ have a similar influence on frog heart functions[3]. In *Chlorella* Tl^+ can be accumulated by a K^+ pump[4]. This similarity can be accounted for by the identity of charges and the closeness of the ion sizes. The crystal radius of Tl^+ is 1.47 Å, and those of K^+, 1.33 Å, and Rb^+, 1.47 Å (ref. 5).

Unlike the alkali ions, Tl^+ possesses two 6-s electrons and is capable of a more appreciable association with anions and of complex formation[1,6]. The difference between Tl^+ and K^+ is also manifested in studies on the activation of K^+-dependent

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enzymes by Tl^+. The affinity of Tl^+ to these enzymes, including the $(Na^+ + K^+)$-ATPases, is about 10-fold or more than that of K^+ (refs 6–10).

In red blood cells the common K^+ substitutes, such as Rb^+, Cs^+ and NH_4^+, participate in the ouabain-sensitive K^+ transport system with a similar affinity as they show for the red cell membrane $(Na^+ + K^+)$-ATPase[2]. Experiments on frog skin revealed that Tl^+, when substituted for K^+ at the serosal side, rapidly inhibited the ouabain-sensitive transport of Na^+ across the frog skin, while, on the contrary, the frog skin $(Na^+ + K^+)$-ATPase was activated by Tl^+ (ref. 12).

In the present investigation the ability of univalent Tl^+ to replace K^+ in activating the fragmented membrane $(Na^+ + K^+)$-ATPase and in the ion transport processes across the membrane was studied in human red blood cells, where dominant pathways for potassium accumulation and sodium extrusion are known to be coupled *via* an ouabain-sensitive mechanism.

MATERIAL AND METHODS

Normal fresh heparinized human or rabbit blood was used in most experiments. The blood was centrifuged at $3000 \times g$ for 15 min, whereafter plasma, buffy coat and the topmost cell layer were discarded. The cells were then washed 2–3 times with a 2-fold volume of the buffered saline described below, and the cells were subsequently resuspended in the same solution. In general, methods described earlier in detail[13,14] were applied.

The cells were usually incubated in a water bath at 38 °C and mixed gently. Experiments at low temperature were carried out by placing the incubation vessels in a water bath containing ice-cubes.

The composition of the incubation medium was that of Ringer-type buffered saline in which Cl^- was replaced by SO_4^{2-} in order to prevent precipitation of TlCl. The medium is referred to as Ringer–SO_4. The composition of the base medium (as mmoles/l) was as follows; Na_2SO_4, 80: $MgSO_4$, 1.0: $CaSO_4$, 0.5: K_2SO_4, 5.0: $NaHCO_3$, 20: glucose, 14. The actual osmolarity of the medium measured from freezing-point depression was about 280 mosM. Final adjustments of the medium composition were made by adding suitable concentrates to the final incubate. The pH of the medium during experiments was 7.4.

When tracer inflow was studied, samples of the cell suspension were pipetted at intervals into isotope counting tubes and after separating the cells by centrifugation, a sample of the supernatant was removed. The cells were then rapidly washed once with a 15-fold volume of ice-cold saline. Care was taken not to remove cells during the above procedure. The cell volume in the sample was determined by measuring separately the packed red cell volume by microcapillary centrifugation. Backward movement of the tracer during the wash with ice-cold saline was negligible. Even with the rapidly transported $^{204}Tl^+$ equal radioactivities were observed in the cells if the washed cells were counted directly or if calculated from the loss of radioactivity in the medium. However, if the relative cell volume is low, direct counting of the cellular radioactivity is preferred.

For the tracer outflow experiments cells were loaded by incubation from 3 h to 2 days after adding the tracer ions to whole blood. The outflow experiment was then initiated by rapidly washing the cells twice with ice-cold saline. The medium

into which the outflow was studied was prepared beforehand and the cells were suspended into it with a fractional volume of about 0.10–0.05. Aliquots of the suspension were removed at intervals, centrifuged, and the supernatant was discarded. The relative amount of radioactivity of the trapped medium after centrifugation in the cell samples was small, and usually cells were not washed before counting the radioactivity.

The radioactivity was determined with an automatic dual channel well-type scintillation spectrometer (Wallac Decem.). If several isotope species were simultaneously present, they were separated by their different half-times and radiation energies. The $^{22}Na^+$, $^{86}Rb^+$ and $^{204}Tl^+$ isotopes were supplied by the Radiochemical Centre (Amersham) and $^{42}K^+$ by the Reactor Laboratory, Helsinki University of Technology.

Non-radioactive K^+ and Na^+ bulk ions were determined with an internal standard flame photometer (Beckman, 105).

The tracer rate constants were calculated according to Ekman *et al.*[14].

Fragmented membranes for the assay of the ATPase activity were prepared according to Wiley[15]. After washing cells for three times in saline they were lysed at room temperature in a 10-fold volume of 6 mM Tris–HCl, adjusted to pH 7.5. The lysate was allowed to stand for about 10 min, whereafter the membranes were separated by centrifugation at $20000 \times g$ for 15 min. All visible traces of haemoglobin were removed by four further washes in this medium. The white stroma was then resuspended in the previous medium. The reaction was initiated by adding 10 mM of Mg–ATP and the additional co-factors. The ATPase activity was calculated from the increase of inorganic phosphate (P_i) concentration during 60 min incubation at 38 °C: P_i was determined according to Järnefelt[16]. Increase of P_i liberation over the baseline value was taken to indicate the amount of specific activation of ATPase by the substance in question.

RESULTS

Activation of the red cell membrane ($Na^+ + K^+$)-ATPase by Tl^+

When K^+ was substituted by Tl^+ in the incubation medium equal activation of the ($Na^+ + K^+$)-ATPase could be reached at Tl^+ concentrations of about 1/10 of those of K^+ (Fig. 1). This ($Na^+ + Tl^+$)-ATPase was found in all respects to behave like the ($Na^+ + K^+$)-ATPase. If Na^+ was omitted, there was no activation of the ATPase by Tl^+. In the presence of 100 mM Na^+ and 10 mM K^+ there was no additional activation of the enzyme by Tl^+. Ouabain inhibited ($Na^+ + Tl^+$)-ATPase in a similar way as it inhibited the ($Na^+ + K^+$)-ATPase.

Accumulation of $^{204}Tl^+$, $^{42}K^+$ and $^{86}Rb^+$ tracer ions by red cells

Fig. 2 shows the accumulation of $^{204}Tl^+$ and $^{42}K^+$ tracer ions by human red cells from Ringer–SO_4 solution when both radioisotopes were added simultaneously to the medium. Tracer Tl^+ reached the stationary state distribution across the membrane much more rapidly than $^{42}K^+$. The calculated inward rate constants were 9.36 h^{-1} for $^{204}Tl^+$ and 0.153 h^{-1} for $^{42}K^+$. The outward rate constants were calculated as

$$k_{out} = \frac{k_{in}}{r^{\infty}} \tag{1}$$

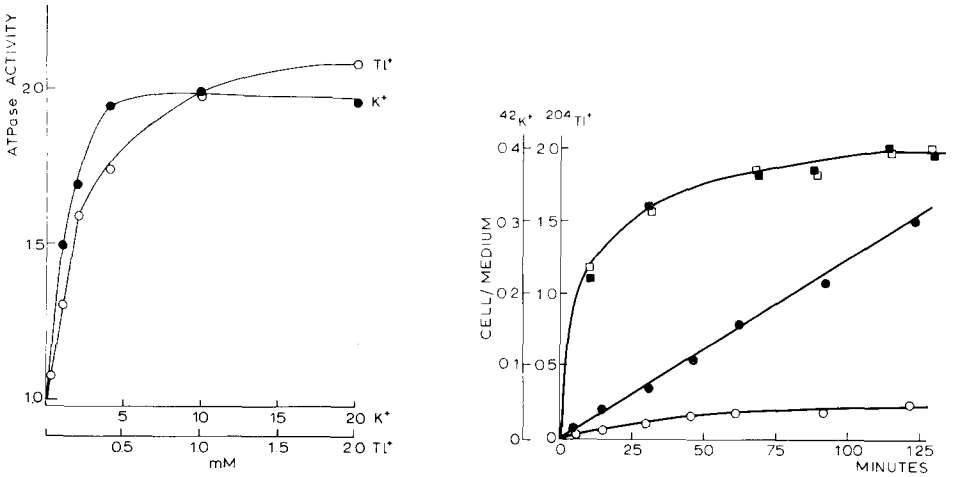

Fig. 1. Activation of the ATPase in fragmented human red cell membranes by K^+ or Tl^+ in the presence of 100 mM Na^+. ATPase is expressed as the ratio of P_i liberation in presence of Tl^+ or K^+ to that in absence of Tl^+ or K^+, respectively.

Fig. 2. Cell/medium concentration ratios of $^{42}K^+$ and $^{204}Tl^+$ in human red cells when initially added simultaneously into the medium. The Tl^+ concentration is about 10^{-2} mM, the K^+ concentration 5 mM; temperature 38 °C. ■, $^{204}Tl^+$, control; □, $^{204}Tl^+ + 1 \cdot 10^{-5}$ M ouabain; ●, $^{42}K^+$, control; ○, $^{42}K^+ + 1 \cdot 10^{-5}$ M ouabain.

TABLE I

UPTAKE OF $^{86}Rb^+$ AND $^{204}Tl^+$ TRACER IONS, ADDED INITIALLY TO THE MEDIUM, BY RABBIT ERYTHROCYTES AT LOW EXTERNAL K^+ CONCENTRATIONS

Hematocrit 0.11, 38 °C.

Time of incubation (min)	K^+_{out}* *(mM)*	*Cell/medium concentration ratios*			
		Ringer–SO_4		*Ringer–SO_4 + $1 \cdot 10^{-5}$ M ouabain*	
		$^{86}Rb^+$	$^{204}Tl^+$	$^{86}Rb^+$	$^{204}Tl^+$
10	0.3	0.105	2.10	0.020	2.05
20	0.4	0.150	2.16	0.023	2.24
30	0.4	0.210	2.29	0.025	2.36
50	0.4	0.380	2.62	0.036	2.57
70	0.6	0.580	2.46	0.042	2.38
90	0.7	0.865	2.90	0.064	2.83
120	1.0	1.110	3.02	0.080	2.93
160	1.2	1.420	2.96	0.111	2.98

* There was some outward leakage of K^+ during the experimental run, as manifested by the increase of external K^+ concentration. This outflow of K^+ was not connected with Tl^+.

where r^∞ is the stationary cell/medium concentration ratio for $^{204}Tl^+$ and K^+. The outward rate constant was 4.68 h^{-1} for $^{204}Tl^+$ and 0.0066 h^{-1} for K^+. Stationary cell/medium concentration ratios were about 1.8–2.0 for $^{204}Tl^+$ and about 20 for

$^{42}K^+$. Ouabain decreased markedly the accumulation of $^{42}K^+$ but had no effect on that of $^{204}Tl^+$. The same results were obtained with rabbit red cells at very low external bulk K^+ concentrations (Table I).

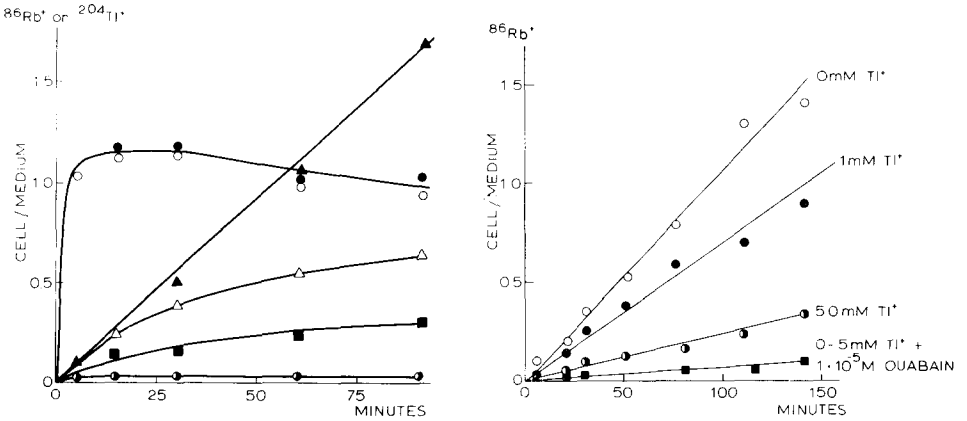

Fig. 3. Effect of glucose and temperature on the uptake of $^{204}Tl^+$ and $^{86}Rb^+$ in human red cells. When present, the glucose concentration was 15 mM. The uptake of $^{204}Tl^+$: ●, 38 °C, +glucose; ○, 38 °C, no glucose; ■, 4 °C, +glucose; and without glucose. The uptake of $^{86}Rb^+$: ▲, 38 °C, +glucose; △, 38 °C, no glucose. ◑, 4 °C, +glucose; and without glucose.

Fig. 4. Effect of external Tl^+ on the uptake of $^{86}Rb^+$ tracer ions in human red cells at 38 °C.

TABLE II

INFLUENCE OF EXTERNAL BULK K^+ ON THE UPTAKE OF $^{204}Tl^+$ TRACER IONS BY HUMAN ERYTHROCYTES

Hematocrit 0.16, 38 °C.

Time of incubation (min)	K^+_{out} *(mM)*	$^{204}Tl^+$ *cell/medium*	K^+_{out} *(mM)*	$^{204}Tl^+$ *cell/medium*	K^+_{out} *(mM)*	$^{204}Tl^+$ *cell/medium*
Ringer–SO_4						
5	0.8	1.56	4.1	1.60	13.2	1.56
15	0.9	1.79	4.1	1.81	13.4	1.83
30	1.3	1.86	4.5	1.50	13.8	1.72
60	1.7	2.45	5.0	2.19	14.6	2.17
100	2.4	2.17	5.6	2.16	15.0	2.30
150	3.2	2.24	6.2	2.14	15.6	2.12
Ringer–SO_4 + $1 \cdot 10^{-5}$ M ouabain						
5	1.1	1.60	4.3	1.56	13.4	1.48
15	1.4	1.79	4.5	1.71	13.8	1.69
30	1.7	1.75	5.4	1.75	14.0	1.71
60	1.9	2.07	5.6	2.13	14.8	1.95
100	2.6	2.06	6.3	2.16	15.6	2.16
150	3.6	2.20	7.5	2.06	17.0	2.18

In glucose-depleted red cells the uptake of $^{204}Tl^+$ did not differ from that obtained in experiments with glucose, while the accumulation of $^{86}Rb^+$ was markedly decreased (Fig. 3). In experiments at 4 °C both $^{86}Rb^+$ and $^{204}Tl^+$ accumulation were slower than at 38 °C and their accumulation was independent of glucose. At 4 °C the rate of $^{204}Tl^+$ accumulation remained consistently higher than that of $^{86}Rb^+$. Though the $^{204}Tl^+$ accumulation was insensitive to ouabain (Fig. 2, Table I), increasing the external concentration of non-radioactive bulk Tl^+ decreased the ouabain-sensitive part of the $^{86}Rb^+$ influx, while the ouabain-insensitive influx of $^{86}Rb^+$ was unaffected by Tl^+ (Fig. 4). On the other hand, increasing the concentration of bulk K^+ in the medium from 1 to 15 mM had no effect upon the accumulation of $^{204}Tl^+$ (Table II).

Influence of external bulk Tl^+ on the extrusion of $^{22}Na^+$ from human red cells

The outflow of $^{22}Na^+$ tracer ions from red cells into K^+-free medium containing 1.0 mM of bulk Tl^+ was about as rapid as in a medium containing 10 mM K^+ (Fig. 5). Further increasing the Tl^+ external concentration up to 5 and 10 mM resulted in a progressive decrease in the rate of $^{22}Na^+$ outflow. Inhibition of the $^{22}Na^+$ outflow by higher Tl^+ concentrations was more pronounced in fresh red cells (Fig. 5), than in those after a prolonged cold-storage (Fig. 6). The fresh red cells contained about 60 mM Na^+.

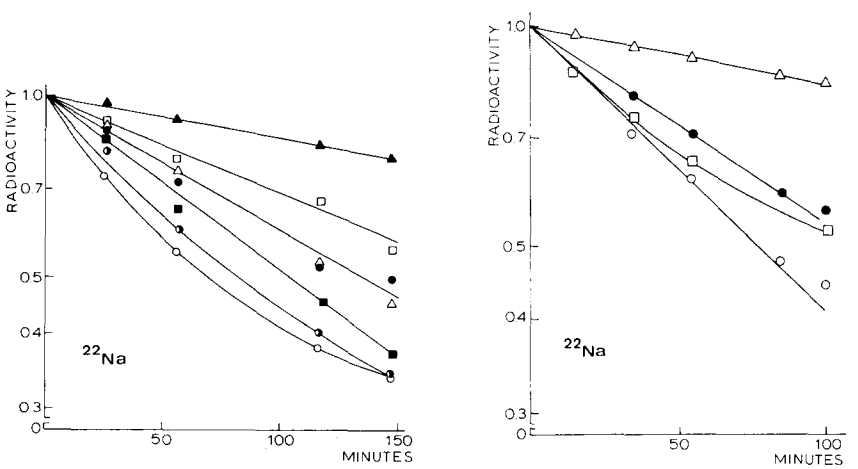

Fig. 5. Outflow of $^{22}Na^+$ from fresh red cells as a function of K^+ and Tl^+ concentrations. Cell Na^+ concentration was 9 mM; hematocrit 0.05, temperature 38 °C. ○, 10 mM K^+; ◑, 0.2–0.5 mM K^+, 1 mM Tl^+; ■, 0.2–0.5 mM K^+, 5 mM Tl^+; ●, 0.2–0.5 mM K^+, 10 mM Tl^+; △, 0.2–0.5 mM K^+, no Tl^+; □, 0.1 mM K^+ (achieved by adding the K^+-selective ion-exchanger Resonium-A to the medium); no Tl^+; ▲, 10 mM K^+, $1 \cdot 10^{-5}$ M ouabain. The fraction of the initial radioactivity remaining in the cells is plotted against time.

Fig. 6. Outflow of $^{22}Na^+$ from cold-stored red cells. Cell Na^+ concentration was about 60 mM. Hematocrit 0.05, temperature 38 °C. ○, 10 mM K^+; □, 0.2–0.5 mM K^+, 10 mM Tl^+; ●, 0.2–0.5 mM K^+, no Tl^+; △, 10 mM K^+, $1 \cdot 10^{-5}$ M ouabain. The fraction of the initial radioactivity remaining in the cells is plotted against time.

DISCUSSION

Tl^+ can replace K^+ in activating the $(Na^+ + K^+)$-ATPase in fragmented membranes of human red cells. Activation of the ATPase by Tl^+ was not additive to that induced by K^+, but was in a similar way dependent on Na^+ and was inhibited by ouabain. Similar activation of the enzyme could be reached at Tl^+ concentrations in the medium only 1/10 of those of K^+, being consistent with the results obtained with the $(Na^+ + K^+)$-ATPases from various other sources such as kidney[7], brain microsomal fractions[8,9] and frog skin[12].

Indirect evidence was obtained concerning the participation of Tl^+ in ouabain-sensitive ion transport processes in red blood cells. For instance, the inhibition of the ouabain-sensitive portion of $^{86}Rb^+$ influx by Tl^+ (Fig. 4) suggests that there is an interaction between Tl^+ and the ouabain-sensitive K^+-binding sites in the red blood cell membranes. Tl^+ was also found to be capable of maintaining the ouabain-sensitive $^{22}Na^+$ outflow from the red cells into K^+-free medium with about the same efficiency as it activates the $(Na^+ + K^+)$-ATPase (Figs 1 and 5). At higher concentrations Tl^+, unlike K^+, decreased the outflow of $^{22}Na^+$ (Figs 5 and 6). The inhibitory effect of Tl^+ was found to be more pronounced in fresh low Na^+ red cells than in high Na^+ cold-stored cells, suggesting that Tl^+ and Na^+ would compete for the same sites at the inner side of the membrane.

Since Tl^+ and K^+ are indistinguishable in their wholly hydrated state, the ions being equal in charge and size[17], the much higher affinity of the ATPase and the Na^+ extrusion for Tl^+ than for K^+ suggests to us that the ion–membrane interactions involve a destruction of the ion hydration shells. The interaction might be of the type encountered in the formation of, for instance, the valinomycin–K^+ complex. But, as valinomycin is unable to replace the hydration shell of Tl^+, and Tl^+–valinomycin complexes thus cannot be formed in aqueous solutions[18], the nature of the K^+ and Tl^+ binding site of the membrane must involve other types of functional groups than those of valinomycin, *i.e.* carbonyl and ether oxygens. The nature of these functional groups of course remains unknown, but their interaction with the ions must provide enough free energy to remove the hydration shell of Tl^+. The free energy of the hydration of Tl^+ is 107 kcal/mole[19] while those of K^+ and Na^+ are 73.5 kcal/mole and 89.7 kcal/mole, respectively[20].

Gehring and Hammond[21,22] found that the rate of accumulation of $^{204}Tl^+$ by rabbit red blood cells was decreased by ouabain when external K^+ concentrations were not higher than 0.5–1.0 mM. In our experiments no ouabain-sensitive portion could be detected in the $^{204}Tl^+$ accumulation by human red blood cells (Figs 2 and 4) or by rabbit cells even at K^+ concentrations as low as 0.3 mM (Table I). Gehring and Hammond described the $^{204}Tl^+$ disappearance from the medium into the red blood cells by the equation:

$$-\frac{d(^{204}Tl^+)_{medium}}{dt} = k \times (^{204}Tl^+)_{medium} \qquad (2)$$

in which the backward flux of $^{204}Tl^+$ from the cells into the medium is neglected. The use of this equation can lead to erroneous results when the hematocrit is as high as 0.16–0.20 and the uptake is studied beyond the linear part of the accumulation curve, as was the case.

The stationary cell/medium concentration ratio of $^{204}Tl^+$ across the red cell membrane was found to be about 1.8–2.0, being close to the reciprocal concentration ratio of small anions, which in connection with the independence of Tl^+ uptake of ouabain and glucose suggests that Tl^+ is distributed passively. Rate constants, determined at the same concentrations of ions, lead to passive fluxes of Tl^+ exceeding the fluxes of K^+ or Rb^+ by about 50–100 times. An assumed ouabain-sensitive portion of the Tl^+ influx coupled with the ouabain-sensitive Na^+ outflux would thus be only 1–2% of the total passive influx of Tl^+. An accurate determination of the ouabain-induced decrement of the Tl^+ influx therefore seems hardly possible.

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