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THALLIUM INHIBITION OF OUABAIN-SENSITIVE SODIUM TRANSPORT AND OF THE $(Na^+ + K^+)$-ATPase IN HUMAN ERYTHROCYTES

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SUMMARY

The influence of Tl^+ on Na^+ transport and on the ATPase activity in human erythrocytes was studied. 0.1–1.0 mM Tl^+ added to a K^+-free medium inhibited the ouabain-sensitive self-exchange of Na^+ and activated both the ouabain-sensitive ^{22}Na outward transport and the transport related ATPase. 5–10 mM external Tl^+ caused inhibition of the ouabain-sensitive ^{22}Na efflux as well as the $(Na^+ + Tl^+)$-ATPase. Competition between the internal Na^+ and rapidly penetrating thallous ions at the inner Na^+-specific binding sites of the erythrocyte membrane could account for the inhibitory effect of Tl^+. An increase of the internal Na^+ concentration in erythrocytes or in ghosts protected the system against the inhibitory effect of high concentration of Tl^+. A protective effect of Na^+ was also demonstrated on the $(Na^+ + Tl^+)$-ATPase of fragmented erythrocyte membranes studied at various Na^+ and Tl^+ concentrations.

INTRODUCTION

Thallous ions can replace potassium ions in the activation of the $(Na^+ + K^+)$-ATPase and in the stimulation of the ouabain-sensitive outward sodium transport in human erythrocytes [1–3]. The $(Na^+ + K^+)$-ATPase and the $(Na^+ + Tl^+)$-ATPase were found to be identical in all respects [2]. The same level of enzyme activity and ouabain-sensitive Na^+ efflux could be reached at Tl^+ concentrations only one-tenth of those of K^+ (1 and 10 mM, respectively). Increasing the Tl^+ concentration up to 5–10 mM resulted in a progressive inhibition of the ouabain-sensitive Na^+ efflux, though the $(Na^+ + Tl^+)$-ATPase activity of the fragmented erythrocyte membranes was not reduced. The inhibitory effect of thallous ions was found to be far greater in fresh erythrocytes than in cold stored cells. This difference in the degree of Tl^+-inhibition was suggested as arising from the different sodium concentrations in fresh and cold-stored erythrocytes [2].

In the present investigation the influence of Tl^+ on the ouabain-sensitive Na^+ transport and on the $(Na^+ + K^+)$-ATPase was studied at various intra- and extra-

cellular concentrations of Na^+. The main purpose of the work was to compare sodium transport with the enzyme activity in similar conditions in order to understand the role played by Na^+ in the inhibition caused by thallium.

MATERIALS AND METHODS

In addition to the experimental procedures described earlier [2], the ouabain-sensitive ^{22}Na transport from low and high sodium ghosts was measured. Ghosts were prepared in accordance with Godemann and Passow [4]. Erythrocytes were hemolyzed at 0 °C for 5 min in a 20 mosM solution containing (in mM/l): Tris · Cl 5, Tris · ATP 2, $MgCl_2$ 2.5 at pH 7.6. Sufficient amounts of 3 M KCl, ^{22}Na tracer and bulk Na^+ were added to restore the initial osmolarity and to obtain ghosts loaded with ^{22}Na and containing various Na^+ and K^+ concentrations. After an incubation at 38 °C for 30–40 min the ghosts were centrifuged at 20 000 × *g*, washed three times with K^+-free sulfate/Ringer (see below) and put in incubation media of various composition for the measurement of ^{22}Na-outflow.

The inhibition caused by Tl^+ was also studied in fresh and cold-stored human erythrocytes, the ^{22}Na efflux and P_i production being measured in the same erythrocyte suspension. The erythrocytes were loaded with Na^+ during cold storage at 4 °C for various time intervals in initially K^+-free sulfate/Ringer containing ^{22}Na and were then preincubated at 37 °C for 3 h with 1 mM adenosine + 1 mM inosine in order to restore the original level of intracellular ATP and to decrease the background of inorganic phosphate in the erythrocyte suspension [5]. The rate of the ouabain-sensitive sodium transport was compared with the ATPase activity in media containing 10 mM Tl^+ or 10 mM K^+ at the various cell sodium concentrations developed during cold storage. The results were expressed as the ratio of the ouabain-sensitive efflux of ^{22}Na induced by Tl^+ to the efflux induced by K^+. The activity of the $(Na^+ + Tl^+)$-ATPase was compared to that of the $(Na^+ + K^+)$-ATPase. The ATPase activity in intact erythrocytes was determined by measuring P_i production in accordance with Whittam and Ager [6].

The basic incubation medium was Ringer-type buffer saline in which Cl^- was replaced be SO_4^{2-} in order to prevent precipitation of TlCl. This is referred to a Ringer/SO_4^{2-} or sulfate/Ringer in which the composition (in mmol/l) was as follows: Na_2SO_4 80, $MgSO_4$ 1.0, $CaSO_4$ 0.5, $NaHCO_3$ 20, glucose 14. Various concentrations of K_2SO_4 and Tl_2SO_4 were used as stated below, and the osmolarity of the media measured by freezing-point depression ranged from 280 to 300 mosM. The pH of the medium during experiments was 7.4. A sulfate/Ringer with 5 mM Na^+ was obtained by partial replacement of Na_2SO_4 by sucrose (about 220 mM) and 20 mM choline chloride. The latter was added to prevent a non-specific loss of Na^+ found to occur in the sucrose medium at very low Cl^- concentrations [7]. There was no precipitation of TlCl at 20 mM Cl^- in the incubation medium.

RESULTS

1. Inhibition of Na^+-Na^+ self-exchange by Tl^+

In order to clarify in more detail the potential of Tl^+ as a K^+ substitute, the influence of Tl^+ on the ouabain-sensitive ^{22}Na transport was studied in K^+-free

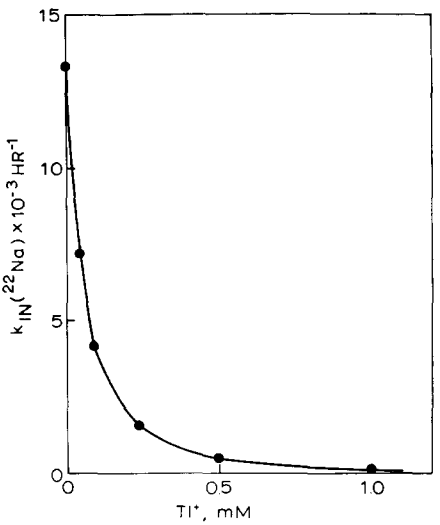

Fig. 1. Effect of Tl^+ on the ouabain-sensitive influx of ^{22}Na into human erythrocytes from a K^+-free medium. The flux corresponding to the value 10 in the graph is 1.8 mmol · l^{-1} · h^{-1}.

media. It is known that at low concentrations of external K^+ an ouabain-sensitive self-exchange of Na^+ appears instead of the ouabain-sensitive sodium-potassium coupled transport [8]. The results presented here (Fig. 1) show that the self-exchange of Na^+ measured as an ouabain-sensitive influx of ^{22}Na was suppressed by very low concentrations of Tl^+. It follows that an addition of Tl^+ to K^+-free media known to stimulate the ouabain-sensitive coupled Na^+ transport [2] would at the same time inhibit the ^{22}Na efflux occurring via this ouabain-sensitive self-exchange mechanism.

2. Tl^+-dependent outward transport of Na^+

To simplify the interpretation of the ^{22}Na outflow data, we decreased the external concentration of sodium to 5 mM at which the ouabain-sensitive exchange of Na^+ has been reported to be minimized [8]. Fig. 2 shows that at these conditions the ouabain-sensitive fraction of the ^{22}Na efflux into K^+-free medium was very low indeed, unless Tl^+ was added to the external solutions. It was thus possible to study the influence of Tl^+ on the ouabain-sensitive ^{22}Na outward transport without the complication caused by the ouabain-sensitive self-exchange of ^{22}Na. Since Tl^+ was found to affect the ouabain-insensitive ^{22}Na efflux (Fig. 2), the ouabain-sensitive Na^+-transport shown in Fig. 3 was calculated taking this effect into account. In the K^+-free sucrose/choline medium containing 5 mM Na^+ the ouabain-sensitive ^{22}Na outward transport increased to a maximal level at 0.1 mM external Tl^+, equal to the level obtained with 10 mM K^+ (Figs 2 and 3).

3. Inhibition of outward transport of Na^+ by Tl^+

When the external concentration of Tl^+ was increased beyond 0.1–1.0 mM up to 5–10 mM, a strong inhibition of the ouabain-sensitive ^{22}Na efflux from fresh erythrocytes was observed. In cold stored cells this effect of Tl^+ was only slight

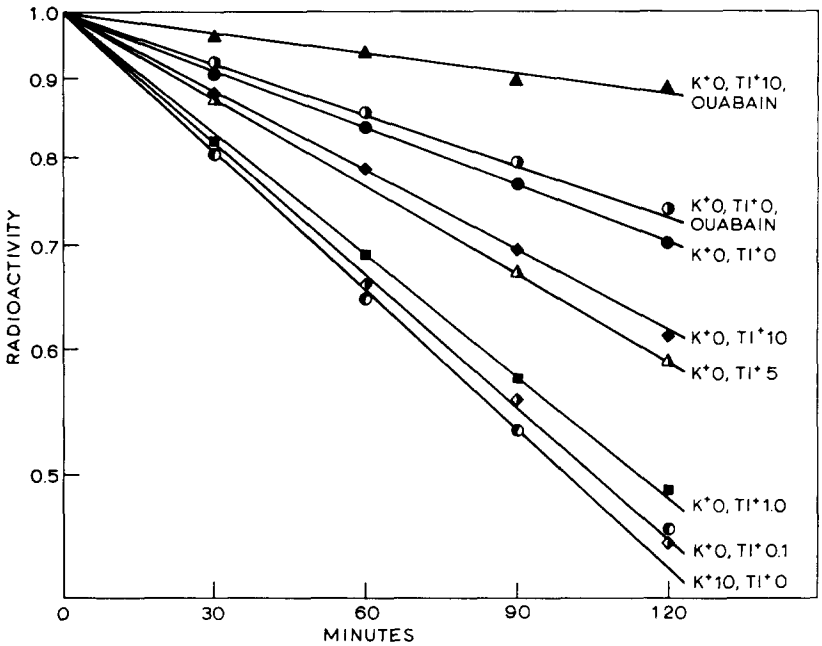

Fig. 2. Fraction of ^{22}Na tracer ions remaining in cells after incubation of erythrocyte suspension in sulphate/Ringer with various concentrations of thallium, potassium and ouabain.

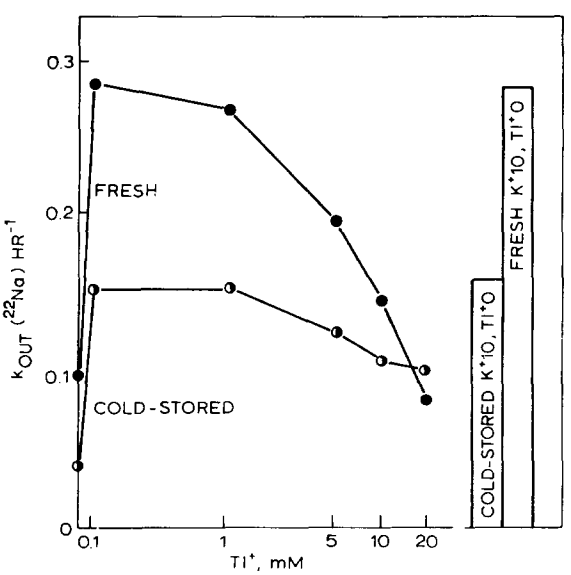

Fig. 3. Rate constants of ouabain-sensitive efflux of ^{22}Na from fresh and cold-stored red cells into K^+-free sucrose/choline media containing 5 mM Na^+. Maximal rates of efflux with 10 mM K^+ are depicted by the bars.

(Figs 2 and 3). Table I shows the effect of Tl^+ on ^{22}Na efflux from ghosts with low and high internal Na^+ concentration. The ouabain-sensitive ^{22}Na efflux from the ghosts containing 10–20 mM Na^+ (typical for fresh erythrocytes) was greatly inhibited by 10 mM Tl^+, while the same concentration of Tl^+ had no inhibitory effect in the ghosts with 60–90 mM Na^+ (typical for cold-stored cells).

TABLE I

RATE CONSTANTS (h^{-1}) OF ^{22}Na EFFLUX FROM LOW AND HIGH SODIUM GHOSTS

Na_i (mM) ($Na_i + K_i$ = 160 mM)	10 mM K^+	10 mM K^+, 10^{-4} M ouabain	Ouabain-sensitive efflux	10 mM K^+, 10 mM Tl^+	10 mM K^+, 10 mM $^+$ Tl^+, 10^{-4} M ouabain	Ouabain-sensitive efflux
Low sodium ghosts						
10	0.371	0.186	0.185	0.274	0.145	0.129
20	0.415	0.223	0.192	0.287	0.186	0.101
20	0.342	0.174	0.168	0.235	0.174	0.061
20	0.415	0.223	0.192	0.248	0.198	0.050
High sodium ghosts						
60	0.222	0.118	0.104	0.208	0.098	0.110
70	0.269	0.152	0.117	0.273	0.168	0.105
90	0.228	0.149	0.079	0.239	0.160	0.079

4. Competition between Na^+ and Tl^+ in the ATPase of erythrocyte membrane fragments

A similar sodium-thallium antagonism is apparent in the ($Na^+ + Tl^+$)-ATPase of fragmented erythrocyte membranes. The enzyme activity shown in Fig. 4 is expressed as a percentage of the activity measured in a Tl^+-free medium containing 100 mM Na^+ and 10 mM K^+. In agreement with previous reports [2, 3] low Tl^+ concentrations were found to substitute for K^+ in the ($Na^+ + K^+$)-ATPase at all Na^+ concentrations. The increase of Tl^+ concentration up to 10 mM caused only 10–20 % inhibition of the ($Na^+ + Tl^+$)-ATPase activity without any fixed relationship between sodium concentration and the degree of Tl^+ inhibition. A further increase of Tl^+ concentration to 20 mM led to strong inhibition of the enzyme activity in the low sodium media, while only a slight decrease was seen in the 40–80 mM Na^+ media. Therefore, both the ouabain-sensitive ^{22}Na outward transport and the transport related ATPase can be protected by high sodium concentrations against the inhibitory effect of high Tl^+ concentrations. At low sodium concentrations the ouabain-sensitive mechanism of $^{22}Na^+$-Tl^+ coupled transport appears to be somewhat less resistant towards the inhibitory effect of Tl^+ than the ($Na^+ + Tl^+$)-ATPase of the fragmented membranes.

5. Effect of Tl^+ on ATPase and on outward transport of Na^+ in intact erythrocytes

In order to compare the inhibition caused by Tl^+ in the ATPase and in the transport mechanism under identical conditions, the ^{22}Na efflux and P_i production were simultaneously measured in the same suspension of intact erythrocytes.

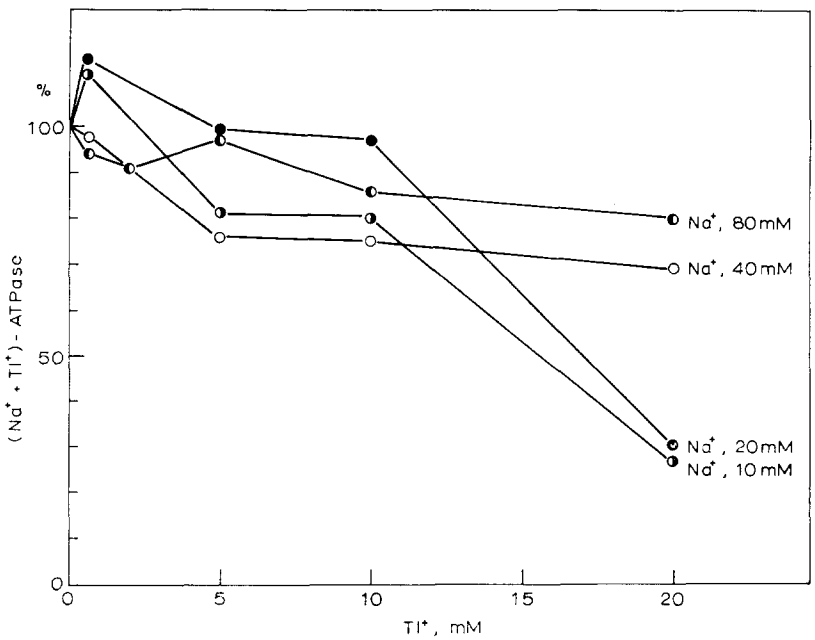

Fig. 4. Effect of Tl^+ on the ouabain-sensitive ATPase of fragmented red cell membranes incubated at different Na^+ concentrations in K^+-free media containing 2.5 mM Mg^{2+}. The results are expressed as percentages of the enzyme activity in the presence of 100 mM Na^+, 2.5 mM Mg^{2+} and 10 mM K^+ in a Tl^+-free medium.

All the results were subdivided into three groups according to the internal concentration of sodium developed during cold storage (Table II). There was good correlation between the inhibition of the ouabain-sensitive ^{22}Na efflux and that of the $(Na^+ + Tl^+)$-ATPase by 10 mM Tl^+. Thallium inhibition of both the transport and ATPase decreased concomitantly with an increase of the intracellular sodium concentration.

TABLE II

EFFECT OF 10 mM Tl^+ ON THE OUABAIN-SENSITIVE EFFLUX OF ^{22}Na AND ON THE OUABAIN-SENSITIVE P_i PRODUCTION IN ERYTHROCYTES WITH DIFFERENT INTERNAL Na^+ CONCENTRATIONS

The results are expressed as percentages of the ^{22}Na efflux and the enzyme activity obtained in Tl^+-free medium with 10 mM K^+. Mean values ± S.D.

No. of experiments	Na_i^+ (mM)	P_i production	^{22}Na efflux
5	10–20	20 ± 6	30 ± 8
7	25–40	54 ± 7	42 ± 10
5	55–80	100 ± 9	100 ± 12

DISCUSSION

The present work suggests that thallous ions interact with the active Na^+ transport mechanism both at the potassium site on the external side and at the sodium site on the internal side. In experiments where the external Na^+ concentration was only 5 mM, 0.1 mM external Tl^+ caused maximal stimulation of the ouabain-sensitive Na^+-efflux. At higher external Na^+ concentrations (160 mM), higher Tl^+ concentrations (1 mM) were required for maximal transport rates [2]. This interrelationship between the external Na^+ and Tl^+ seems to be of the same nature as that reported for Na^+ and K^+ [10, 11]. It is therefore assumed that at these relatively low concentrations, Tl^+ substitutes for K^+ at the K^+-site on the external surface of the membrane.

A further increase of Tl^+ concentrations in the incubation medium leads to an inhibition of the ouabain-sensitive Na^+ efflux. Since, presumably, the external K^+-sites have already been saturated with Tl^+ at much lower concentrations, the inhibition cannot be attributed to an interaction of Tl^+ with these sites. The inhibition suggests that Tl^+ competes with Na^+ at the Na^+ sites on the internal surface of the membrane. Since the membrane is highly permeable to thallous ions, internal concentrations of Tl^+ of about 10–20 mM would be reached with external concentrations of 5–10 mM [2]. This internal concentration would correspond to 100–200 mM K^+, if it is assumed that Tl^+ has 10 times the affinity of K^+ towards the ion-selective sites. High internal Na^+ concentrations, both in erythrocytes and in ghosts prevent the inhibition of Na^+-transport caused by Tl^+. Furthermore, it was shown that the ouabain-sensitive production of inorganic phosphate by intact erythrocytes was dependent on the Na^+/Tl^+ ratio to a similar extent as was the ouabain-sensitive Na^+ efflux. The tight coupling of Na^+ transport with the transport ATPase is strongly supported by this experiment, since Tl^+ interacts with transport and ATPase in parallel. The antagonism between Tl^+ and Na^+ in the ATPase was also demonstrated by the partial reversal of the Tl^+ inhibition by high Na^+ concentrations in the $(Na^+ + Tl^+)$-ATPase of fragmented erythrocyte membranes.

It should be noted that there was no correlation between the level of external Na^+ and the degree of inhibition by Tl^+. At 5 mM external Na^+ the inhibitory effect of Tl^+ was as high as that in a medium with 160 mM Na^+ (compare Figs 2 and 3 with Table I and ref. 2).

In the present work it has been possible to differentiate between two effects of Tl^+ on the coupled $(Na^+ + K^+)$-transport mechanism and on the $(Na^+ - K^+)$-ATPase of the erythrocyte membrane. The interaction of Tl^+ with the K^+-selective site on the external surface of the membrane becomes apparent at very low concentrations (0.1–1.0 mM) of Tl^+. At higher concentrations of Tl^+ (5–10 mM) the interaction with the Na^+ selective site on the internal side becomes predominant. The separation of the two effects of Tl^+ might provide opportunities for a more direct study of the interaction of the ions with their respective binding sites.

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