EFFECT OF LITHIUM AND OTHER MONOVALENT CATIONS ON THE ADP INDUCED PLATELET AGGREGATION IN HUMAN PLATELET RICH PLASMA*

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1. Introduction

It is widely accepted that divalent cations are essential for platelet aggregation [1–4]. Within the group of monovalent cations, only the effect of potassium on platelet aggregation has been described: K^+ accelerates the ADP induced platelet aggregation, but is not essential [1]. In an earlier report we compared the effects of: Li^+, Na^+ and K^+ on various platelet functions, including release of serotonin and ATPase activity of thrombosthenin [5, 6]. This communication deals with the effects of the five alkali cations Li^+, Na^+, K^+, Rb^+ and Cs^+ on ADP induced platelet aggregation.

We were specially interested in the effect of lithium. With respect to its chemical properties and to the radius of the hydrated ion, lithium resembles the divalent cations and magnesium [7, 8]**. Furthermore, lithium plays an important role in the treatment of manic depressive disorder [7].

Our results show that lithium in concentrations above 5 mM inhibits ADP induced platelet aggregation. This effect occurs without preincubation of platelet rich plasma with lithium.

2. Methods

Platelet rich plasma (PRP) was obtained from venous blood of healthy donors by anticoagulation with 1/10 volume of 3.8% sodium citrate or with heparin (2.5 units per ml) and subsequent centrifugation at 360 *g* for 15 min at room temp.

Platelet aggregation was followed by means of the turbimetric method [9] measuring the change in light absorbance with an automatic six-channel-aggregometer at 37°. The turning speed of stirring magnet was 350 rpm. Citrate PRP and heparin PRP were stored for a maximum of 2 hr at 22° and at 37°, respectively.

3. Results

Fig. 1 shows the influence of Li^+, Na^+ and K^+ on the ADP induced platelet aggregation in citrated platelet rich plasma (PRP). Isotonic solutions of LiCl, NaCl or KCl are added to PRP, in final conc. of 50 mM, immediately before addition of ADP. The monovalent cations enhance the ADP induced platelet aggregation in the order $K^+ > Na^+ > Li^+$, as can be clearly seen from fig. 1. As compared with sodium, lithium inhibits and potassium stimulates platelet aggregation. The first phase as well as the second phase of aggregation are affected by the ions in the same order.

In citrate PRP the effects of Li^+, Na^+ and K^+ are obtained at alkali ion concentrations above 5 mM, in heparin PRP above 10 mM.

The inhibitory effect of Li^+ can be compensated by increasing the ADP concentration, the stimulating influence of K^+ by reducing the ADP concentration. The aggregation curves in the presence of 50 mM LiCl, NaCl or KCl, respectively, become identical, when the corresponding ADP concentrations are in the ratio of

* Part XVI of a series on biochemistry of platelets; for part XV see [6].

** Effective hydrated radii (Å): Mg^{2+} 5.9; Ca^{2+} 4.5; Li^+ 4,5; Na^+ 3.4; K^+ 2.2; Rb^+ 1.9; Cs^+ 1.9 [8].

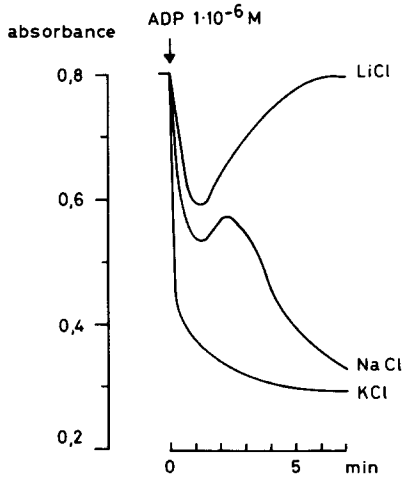

Fig. 1. Effects of Li^+, Na^+ and K^+ on the ADP induced platelet aggregation in citrate PRP; final concentrations of added alkali salts: 50 mM.

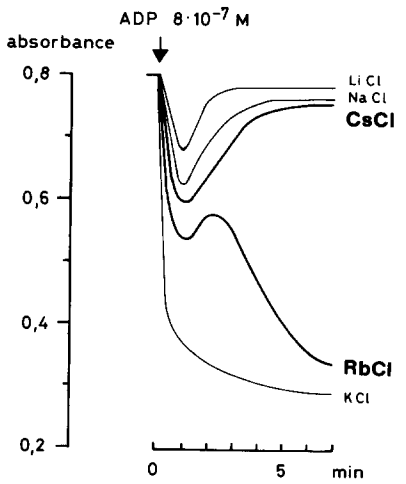

Fig. 2. Effects of Rb^+ and Cs^+ on the ADP induced platelet aggregation in heparin PRP, compared to the effects of Li^+, Na^+ and K^+; final concentrations of added alkali salts: 50 mM.

1.2:1:0.65 for heparin PRP and 2.25:1:0.65 for citrate PRP.

Reducing the concentration of ionized calcium in PRP by EGTA†, the sequence $K^+ > Na^+ > Li^+$ is maintained. However, the inhibitory effect of Li^+ and the stimulating effect of K^+ are increased.

Fig. 2 shows the effects of 50 mM RbCl and CsCl in heparin PRP. Rb^+ is more efficient in promoting ADP induced platelet aggregation than Cs^+. The five alkali ions enhance the first phase of aggregation in the order $K^+ > Rb^+ > Cs^+ \geqslant Na^+ > Li^+$. At the ADP concentration used, in presence of LiCl, NaCl or CsCl, respectively, the first phase of aggregation is too small to induce the second phase of aggregation; therefore deaggregation occurs in these cases.

In citrate PRP the same sequence of the five alkali ions is found.

4. Discussion

The five alkali ions can be arranged in 5 ! = 120 possible combinations. However, the theory of selectivity developed by Eisenman [10] predicts that for physicochemical reasons only 11 sequences of the 120 permutations can occur. The orders found in various (living and non-living) systems [11] agree satisfactorily with Eisenman's theory. The order of effectiveness in ADP induced platelet aggregation $K^+ > Rb^+ > Cs^+ > Na^+ > Li^+$ belongs to the 11 possible sequences.

With washed platelets, alkali ions promote platelet aggregation in the order $K^+ > Na^+ > Li^+$, when platelet aggregation is induced with Ca^{2+} or Mg^{2+} alone at 20° or with thrombin/Ca^{2+} or thrombin/Mg^{2+} at 37°. As compared with sodium, lithium inhibits and potassium stimulates platelet aggregation [5, 6]. It could be shown that ADP induced platelet aggregation also is enhanced by the ions in the sequence $K^+ > Na^+ > Li^+$ (fig. 1). Thus, it may be assumed that the identical effects of Li^+, Na^+ and K^+ on different types of Ca^{2+} or Mg^{2+} dependent platelet aggregation result from interactions between monovalent and divalent cations.

The effects of the alkali ions on PRP as well as on washed platelets are brought about without any preincubation. The prompt effects suggest that the interactions of alkali and earth alkali ions take place at surface structures of platelets. There, monovalent cations and Ca^{2+} may compete at the same binding sites. Such a competition has been demonstrated for K^+, Rb^+

† Ethylene glycol bis-(β-aminoethyl ether)-N,N'-tetraacetic acid.

and Ca^{2+} in submitochondrial particles of rat liver cells [12]. In skeletal muscle microsomes, among the monovalent cations, Li^+ most effectively inhibits uptake and binding of Ca^{2+} [13].

Common binding sites for alkali and earth alkali ions might also be localized at calcium sensitive enzyme systems involved in the aggregation process, e.g. thrombosthenin ATPase and/or adenylate cyclase.

ATPase activity of bovine thrombosthenin, which is stimulated by Ca^{2+} [14], in the presence of 1×10^{-3} M $CaCl_2$ is enhanced by the alkali ions in the order $K^+ > Na^+ > Li^+$ [5, 6].

Adenylate cyclase, which is inhibited by Ca^{2+} [15], is promoted by the alkali ions in the reserve order $Li^+ > Na^+ > K^+$, as has been demonstrated for epinephrine stimulated adenylate cyclase of rat glial tumour cells [16]. Adenylate cyclase of platelets may be stimulated by the alkali ions in the same way ($Li^+ > Na^+ > K^+$). Thus, the different effects of alkali ions on platelet aggregation would be caused by different levels of cyclic AMP.

Apparently, monovalent cations compete with calcium in the order $Li^+ > Na^+ > K^+$, corresponding to the size of the hydrated ions. A possible competition between Li^+ and Ca^{2+} at specific membrane receptor sites of platelets or/and at Ca^{2+} sensitive enzyme systems is the subject of further investigations in our laboratory.

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