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SPECIES DIFFERENCES IN INFLUENCE OF DIVALENT CATIONS AND OF OUABAIN ON ATPase ACTIVITY OF INTESTINAL BRUSH BORDERS OF RAT AND GUINEA PIG*

G. LEOPOLD, E. FURUKAWA, W. FORTH and W. RUMMEL

Institut für Pharmakologie und Toxikologie der Universität des Saarlandes, 665 Homburg/Saar Germany

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Abstract—Five mM Ca^{2+} reduce the activity of the Na,K-ATPase of brush borders in rat by 1/3 and in guinea pig by 2/3. Five mM Ca^{2+} increase the ouabain inhibition in rat. That is not the case in guinea pig. In guinea pig, however, the inhibition of the Na,K-ATPase by Ca^{2+} can be reduced by ouabain. In guinea pig Mg^{2+} can be substituted by Zn^{2+} without influence on the activity of residual ATPase. In rat, however, 2 mM Zn^{2+} increase the activity of residual ATPase three times more than 2 mM Mg^{2+}. In both species Zn^{2+} does not alter the activity of the Na,K-ATPase. In both species in the presence of Zn^{2+} alone ouabain is without influence on the Na,K-ATPase, whereas Mg^{2+} in addition to Zn^{2+} inhibits the activity of the Na,K-ATPase over more than 90 per cent. This inhibition by Mg^{2+} can be reduced by ouabain.

IN INTESTINAL absorption cardiac glycosides contact at first the brush border membrane of the mucosal epithelium. Therefore, the binding of cardiac glycosides to isolated jejunal brush borders from rat and guinea pig has been investigated.[1,2] With regard to the role of the Na,K-ATPase of the small intestine in active absorption mechanisms,[3–5] also the inhibitory activity of different cardiac glycosides on the membrane ATPase system has been studied.[2]

In this connection the influence of divalent cations seemed to be interesting especially in view of species differences. Therefore, the ATPase activity of isolated jejunal brush borders of rat and guinea pig has been studied in the presence of Mg^{2+}, Ca^{2+} and Zn^{2+} respectively as well as the modification of the ouabain inhibition under these conditions.

MATERIALS AND METHODS

Female rats (150–200 g, Wistar, from R. Reupohl, Lage/Lippe) and female guinea pigs (250–300 g, from E. Stock, Gelnhausen) were used unfasted. (Nutrition: "ssniff", G. Plange, Soest).

In all experiments analytical grade chemicals were used. Tris–ATP was prepared as described by Clausen and Formby.[6]

Preparation of brush borders

Isolated brush borders from the proximal 30 cm of the small intestine (beginning at the pylorus) were prepared according to the method of Forstner *et al.*[7] This method

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combines repeated homogenization of mucosal scrapings in 5 mM EDTA solution and centrifugation[8] and the purification of brush borders by treatment with saline buffer and passage through a pad of glass wool.[9] The saline buffer contained 90 mM LiCl and 0·8 mM EDTA pH 7·4. Each step of the preparation and purification of the brush borders was monitored in a phase contrast microscope. The degree of purification has also been proved by means of electron microscopy.

Protein was determined according to Lowry *et al.*[10]

Determination of adenosinetriphosphatase activity

(EC 3.6.1.3, EC 3.6.1.4). The ATPase activity was assayed by measuring the rate of release of inorganic phosphate (P_i) by a modification of the method of Matsui and Schwarz[11] and Fiske and Subbarow.[12] The ATPase activity and the influence of ouabain on it was measured under two conditions: (1) in presence of one (or two) divalent cations alone ("residual ATPase") and (2) in presence of one (or two) divalent cations and Na^+ and K^+ ("total ATPase"). The Na^+, K^+-activated part of ATPase is the difference between the total and the residual ATPase. The influence of Na^+ or K^+ alone was not investigated. The incubation volume of 1·0 ml contained 50 mM tris–HCl pH 7·4, 5 mM tris–ATP, 5 mM $MgCl_2$, 100 mM NaCl, 10 mM KCl, 0·25 mM EDTA and brush border preparation (25–35 μg protein). After 3 min preincubation at 37° the reaction was started by the addition of ATP. The P_i was determined after an incubation period of 15 min at 37°. ATP hydrolysis in control tubes without enzyme or substrate or incubation were measured simultaneously. The reaction was stopped by adding 2·0 ml of ice-cold 10% TCA. Specific ATPase activity is expressed as μmoles P_i liberated per mg protein and per min.

The amount of substrate hydrolysed was linearly related to the time of incubation. Under these conditions ATP was hydrolysed three times faster than ADP.

RESULTS AND DISCUSSION

The species differences of jejunal brush borders from rat and guinea pig in the specific activities of the Mg-ATPase and the Na,K-ATPase and in sensitivity of the Na,K-ATPase to ouabain (g-strophanthin) are summarized in Table 2 of the preceding paper.[2]

In the following comparative studies on the influence of Mg^{2+}, Ca^{2+} and Zn^{2+} on the brush border ATPase of rat and guinea pig ouabain was used in equieffective doses (50 per cent inhibition in rat 10^{-4} M and in guinea pig 3×10^{-6} M).

Influence of Ca^{2+} on the ATPase activity

It is known for the red cell membrane, that the Na,K-sensitive component of the Mg-dependent ATPase is inhibited at all Ca^{2+} concentrations, whereas the Na,K-insensitive component is stimulated at low and inhibited at high Ca^{2+} concentrations.[13–15] In order to see whether this is also valid for the ATPase systems of the intestinal brush borders of rat and guinea pig two Ca^{2+} concentrations (0·2 mM and 5 mM) were tested. The results are summarized in Fig. 1.

Ca^{2+} can substitute for Mg^{2+}. In rat it is even more effective than Mg^{2+} (cf. bar 4 and 1). The addition of 0·2 mM Ca^{2+} does not alter significantly the activity of the Mg-ATPase in both rat and guinea pig (cf. bar 2 and 1). Five mM, however, increase the Mg-ATPase activity in rat and reduce it in guinea pig (cf. bar 3 and 1).

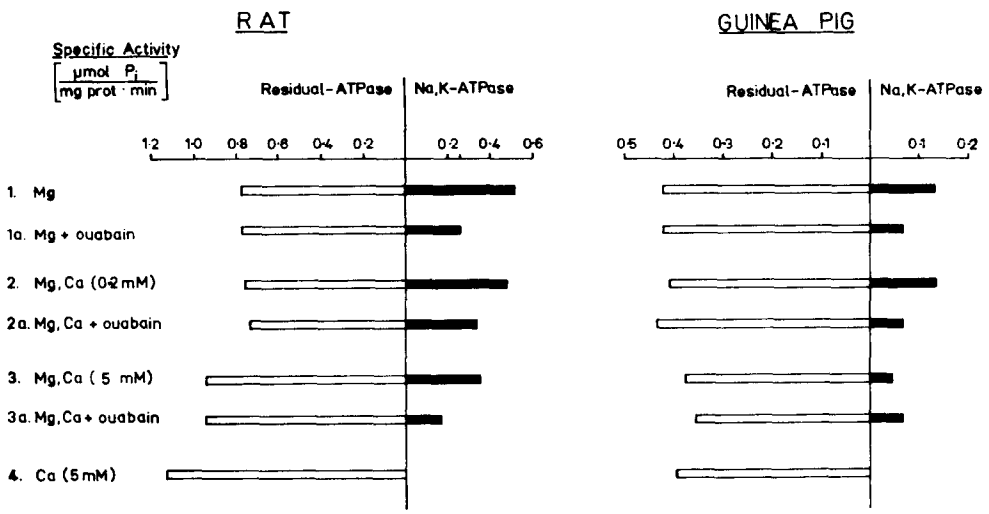

FIG. 1. Influence of Ca^{2+} on the ATPase activity of isolated intestinal brush borders in rat and guinea pig. Incubation volume of 1·0 ml contained 50 mM tris–HCl pH 7·4, 5 mM tris–ATP and as indicated 5 mM $MgCl_2$, 100 mM NaCl, 10 mM KCl, $CaCl_2$ and ouabain (rat: 10^{-4} M; guinea pig: 3×10^{-6} M), 0·1 ml brush border suspension (25–35 µg protein). Three min preincubation and 15 min incubation at 37°. Reaction started by adding ATP, stopped by adding 2 ml ice-cold 10% TCA. n = 8–30, shown difference: $P < 0{\cdot}02$ (paired observations).

The activity of the Na,K-ATPase is not (guinea pig) or only slightly (rat) diminished by 0·2 mM Ca^{2+}, while 5 mM Ca^{2+} reduce the Na,K-ATPase in rat by 1/3 and in guinea pig even by 2/3 (cf. bar 3 and 1).

As to the effect of Ca^{2+} on the *ouabain* inhibition of the Na,K-ATPase it can be stated that in rat 0·2 mM Ca^{2+} reduce slightly the inhibitory effect of ouabain, whereas 5 mM Ca^{2+} enhance the inhibition of the Na,K-activated part of the ATPase by ouabain (cf. bar 1a and 2a; 1a and 3a). In guinea pig, however, calcium did not influence the inhibitory activity of ouabain. Ouabain, on the other side, diminishes the inhibitory activity of calcium on the Na,K-ATPase (cf. bar 1, 3 and 3a).

Influence of Zn^{2+} on the ATPase activity

The motivation for studying the influence of Zn^{2+} on the Na,K-ATPase was the fact that brush borders contain significant amounts of Zn[16,17] and that Berg *et al.*[18] showed an inhibitory activity of Zn^{2+} on the Na,K-ATPase from rat intestinal epithelium. The experiments reported below, led to different results (Fig. 2). But a comparison of the results is not allowed because of several differences in conditions. (Berg *et al.*[18] for example used Na,K-ATPase of all components except clear soluble cytoplasma and a lower concentration of EDTA.)

In rat Zn^{2+} is three times more effective in activating the residual ATPase than Mg^{2+}, while in guinea pig Zn^{2+} and Mg^{2+} are in this regard equally effective (cf. bar 2 and 1). In both species Zn^{2+} does not alter the Na,K-sensitive ATPase activity (cf. bar 2 and 1). In the presence of Zn^{2+} alone ouabain is no more effective i.e. the Na,K-dependent part of the ATPase activity remains unchanged (cf. bar 2 and 2a). Mg^{2+} in the presence of Zn^{2+} produces a nearly total abolition of the Na,K-ATPase

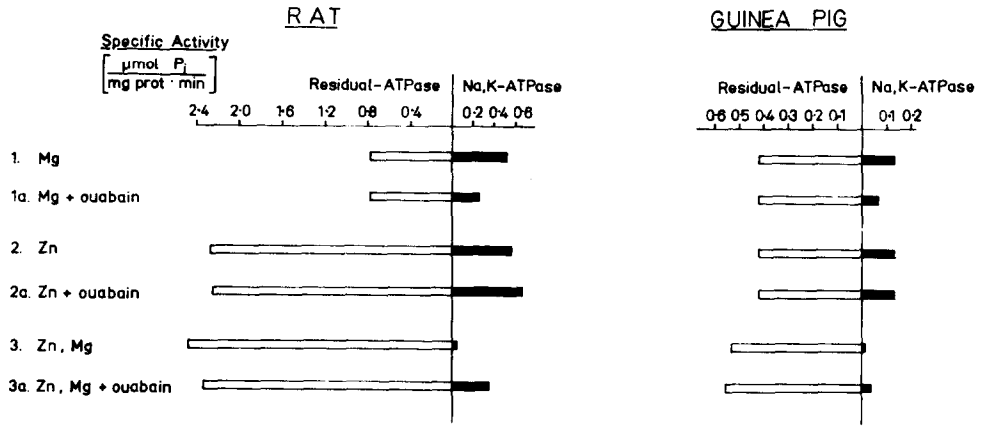

FIG. 2. Influence of Zn^{2+} on the ATPase activity of isolated intestinal brush borders in rat and guinea pig. Incubation volume of 1·0 ml contained 0·1 ml brush border suspension (25–35 µg protein), 50 mM tris–HCl pH 7·4, 5 mM tris–ATP and as indicated 2 mM $MgCl_2$, 100 mM NaCl, 10 mM KCl, 2 mM $ZnCl_2$ and ouabain (rat: 10^{-4} M; guinea pig: 3×10^{-6} M), 3 min preincubation and 15 min incubation at 37°. Reaction started by adding ATP, stopped by adding 2 ml ice-cold 10% TCA. $n = 8$–30, shown differences: $P < 0{\cdot}02$ (paired observations).

activity in both species (cf. bar 3 and 2). This Mg^{2+} inhibition is reduced partially by ouabain (cf. bar 2, 3 and 3a). The effect is more pronounced in rat.

From the results presented it can be clearly seen, that the residual ATPase of small intestinal brush borders is activated by divalent cations in rat in the order $Zn^{2+} > Ca^{2+} > Mg^{2+}$, whereas in guinea pig these three cations are equally effective. In both species 5 mM Ca^{2+} lead to a marked reduction in Na,K-ATPase activity. The inhibitory effect of ouabain, however, is increased in rat and decreased in guinea pig.

It seems necessary to avoid any interpretation of the inhibitory inactivity of ouabain observed in presence of Zn^{2+} and in the simultaneous absence of Mg^{2+} as well as of the inhibitory effect of Mg^{2+} on the Na,K-ATPase in the presence of Zn^{2+} and the reactivating effect of ouabain until further experiments are done.

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