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EFFECTS OF MONOSACCHARIDES ON THE SODIUM ACTIVATION CURVE OF THE INTESTINAL (Na^+–K^+)-ATPASE

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SUMMARY

1. (Na^+–K^+)-ATPase was purified from the mucosa of rat small intestine; its final specific activity of about 1.2 I.U. The principal enzymatic properties of the enzyme were similar to those of (Na^+–K^+)-ATPases from other sources.

2. In the presence of actively transported monosaccharides such as glucose, galactose, 3-*O*-methylglucose and mannose the K^+-dependent transition of the hyperbolic Na^+ activation curve into a sigmoidal one takes place at lower K^+ concentrations than in its absence.

3. Parallel to the change in kinetics an inhibitory effect of these monosaccharides on the (Na^+–K^+)-ATPase is observed, which is significantly dependent on the Na^+ and K^+ concentrations as well as on the Na^+:K^+ ratio. At low concentrations of these ions a total inhibition caused by these monosaccharides takes place.

INTRODUCTION

Inhibitory effects of monosaccharides and phloridzin on (Na^+–K^+)-ATPase were described by several authors[1–5]. Robinson[4] discussed phloridzin as an allosteric effector, which increases the affinity of the enzyme towards K^+ and decreases it towards Na^+. Such interactions are of special interest regarding the close coupling of Na^+ and monosaccharide transport in the intestinal mucosa.

In an earlier publication[5] we discussed the possibility of a direct involvement of (Na^+–K^+)-ATPase in intestinal active sugar transport. Also Kimmich[6] in a recent paper drew similar conclusions. In order to have a more precise basis for the investigation of the coupling mechanism between monosaccharide and ion transport, we purified the ATPase from rat small intestine and studied the influence of actively and passively transported sugars on this enzyme. Actively transported sugars alter the Na^+ activation curve and inhibit the (Na^+–K^+)-ATPase. This inhibition is dependent on Na^+ and K^+ concentrations.

METHODS

Preparation procedure

Homogenate. Male BD III rats were fed *ad libitum* with a standard diet. The rats were killed by a blow on the neck. After bleeding the small intestine was removed quickly, rinsed with 5 mM Tris–EDTA solution and opened with scissors. The

mucosa was scraped off, weighed and homogenized in an Ultra-Turrax in 9 vol. of 0.25 M sucrose, buffered with 30 mM Tris–HCl buffer (pH 7.4).

100 000 × g sediment. The homogenate was centrifuged at 100 000 × *g* for 30 min in order to include all subcellular particles with (Na^+–K^+)-ATPase activity. The supernatant was discarded, the pellet resuspended in buffered sucrose and centrifuged again at 100 000 × *g*. This procedure was repeated once more.

Deoxycholate treatment. The 100 000 × *g* sediment was resuspended in Tris-buffered sucrose, which contained 0.15 % deoxycholate. The suspension was allowed to stand for 1 h at 3°. It was then spun down at 10 000 × *g* for 10 min. The sediment was discarded and the supernatant centrifuged at 100 000 × *g* for 30 min.

Gel filtration. The pellet was resuspended in a small volume of sucrose and applied on a Sephadex G-200 column. The elution buffer contained 30 mM Tris–HCl buffer (pH 7.4), 100 mM Na^+ and 5 mM EDTA. The fractions of the first peak were pooled and centrifuged at 100 000 × *g* for 30 min. The sediment was resuspended in a small volume of Tris-buffered sucrose and frozen at −20°.

We obtained a (Na^+–K^+)-ATPase preparation with an average specific activity of about 1.2 I.U. The ratio Mg^{2+}-ATPase:(Na^+–K^+)-ATPase activity was about 1:1. At −20° the preparation was found to be stable for more than 1 month. The basic enzymatic properties were similar to those of (Na^+–K^+)-ATPases from other sources.

ATPase assay

The tubes containing the reaction mixture were incubated at 37° for 5 min. The reaction was then started by addition of the enzyme. The incubation medium contained, if not otherwise stated, 30 mM Tris–HCl buffer (pH 8.0), 3 mM Mg^{2+}, 3 mM Tris–ATP, 100 mM Na^+ and 20 mM K^+. The final volume was 1.0 ml. After 5 min the reaction was stopped by addition of 0.5 ml phosphate reagent. The latter was prepared freshly each day by adding 2 parts of 10 % ammonium molybdate to 2 parts of 5 M H_2SO_4 and 1 part of 0.1 M silicotungstic acid. The solution was shaken vigorously with 3 ml isobutanol–benzene (1:1 v/v) for 20 sec. 2.0 ml of 95 % ethanol, 0.3 M in H_2SO_4 and 0.1 ml 0.25 % $SnCl_2$ in 0.5 M H_2SO_4 were added to 0.5 ml of the extract[7,8]. Tris–ATP was prepared by passing ATP–Na^+ through a column of Dowex 50-X8, and the free acid was neutralized with Tris. Sucrose and monosaccharides were found to be free of Na^+ and K^+ as demonstrated by flame photometry.

Protein determinations were carried out by the method of Lowry *et al.*[9] using bovine serum albumin as standard.

The determinations were always performed in duplicate. Each point represents the average value of three different enzyme preparations. Under conditions of low enzyme activity the incubation time was extended up to 20 min to improve the precision of the determinations. Within this range inorganic orthophosphate was liberated linearily with time.

RESULTS

Glucose, galactose, 3-*O*-methyl glucose and mannose have been found to significantly change the kinetics of the Na^+ activation curve of the (Na^+–K^+)-ATPase.

At low K^+ concentrations this activation curve follows normal Michaelis–Menten kinetics. In the presence of one of these monosaccharides (10 mM), however, the Na^+ activation curve becomes sigmoidal.

In Fig. 1 these effects are demonstrated using the Eadie plot. It is evident, that arabinose as a non-actively transported monosaccharide does not change the curve. In further experiments glucose was selected from this group for studying this effect in relation to K^+ concentration. In the presence of 10 mM glucose the Na^+ activation curve shifts to sigmoidicity at lower K^+ concentrations than in its absence.

In Fig. 2 the results of a series of experiments are expressed in terms of Hill coefficients and $K_{0.5}$ values. Without glucose the Hill coefficients increase linearly in the range of 10–100 mM K^+. However, in the presence of 10 mM glucose the curve is steeper, reaching a plateau with maximal values about $n = 3$ already at

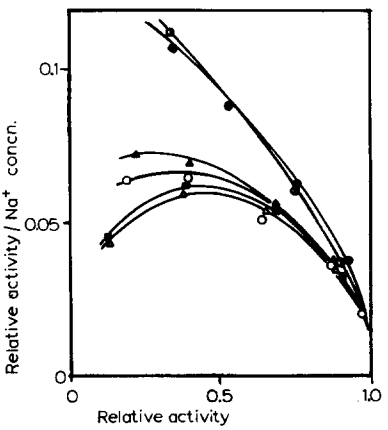

Fig. 1. Effects of monosaccharides on the Na^+ activation curve at 20 mM K^+. Na^+ concentrations were 3.2, 6.3, 12.5, 25, 50 mM. ●—●, without monosaccharide; ◑—◑, with 10 mM L-arabinose; △—△, with 10 mM 3-O-methylglucose; ○—○, with 10 mM D-galactose; ■—■, with 10 mM D-glucose; △—△ with 10 mM D-mannose.

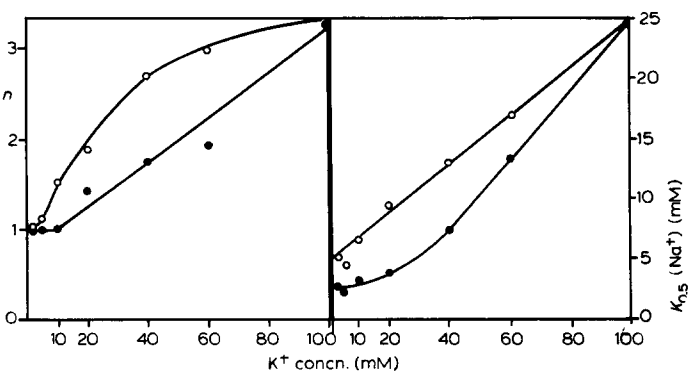

Fig. 2. Effects of glucose on the Na^+ activation curve at different K^+ concentrations. The figure shows the Hill coefficients and the $K_{0.5}$ (Na^+) values as a function of K^+ concentrations. ●—●, without glucose; ○—○, with 10 mM glucose.

40–60 mM K^+. Moreover, glucose clearly lowers the affinity of the enzyme towards Na^+. Apparently the differences in the $K_{0.5}$ values for Na^+ become smaller with increasing K^+ concentrations and disappear completely at 100 mM K^+. These effects make it seem most likely, that the extent of inhibition of this enzyme by glucose is also dependent on Na^+ and K^+ concentrations.

Fig. 3 demonstrates the results of the inhibition experiments. Total inhibition is obtained at low Na^+ and K^+ concentrations. L-arabinose shows no inhibitory effect and has no influence on the kinetic behaviour of the enzyme.

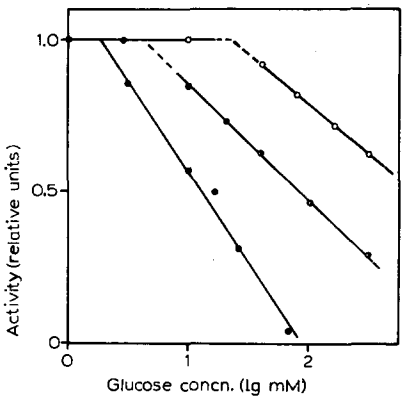

Fig. 3. Inhibition of the (Na^+–K^+)-ATPase by glucose at different Na^+ and K^+ concentrations. ○—○, 100 mM Na^+, 20 mM K^+; ◑—◑, 50 mM Na^+, 2 mM K^+; ●—●, 6.25 mM Na^+, 2 mM K^+.

DISCUSSION

LARIS *et al.*[1], Ewers *et al.*[2]. described the inhibition of (Na^+–K^+)-ATPase from erythrocytes by glucose, phloridzin and phloretin. Ewers *et al.*[2] observed that this inhibition is reduced after sonic disruption of the membranes. These authors discussed the effect in terms of a tight region of (Na^+–K^+)-ATPase- and glucose-binding sites in the membrane. Owing to the high concentrations of glucose used by these authors (60–700 mM) a nonspecific inhibition cannot be excluded. A more detailed description of the monosaccharide inhibition of (Na^+–K^+)-ATPase from rat kidney was given by Britton and Blank[3]. Some sugars, at concentrations between 0.1 and 0.5 M, were found to inhibit the enzyme with decreasing inhibitory power in the order D-mannose > D-arabinose > D-xylose > D-glucose > D-fructose > L-arabinose > D-galactose. The authors discussed a possible change in the structure of the enzyme caused by sugar concentrations used in their experiments (alteration of hydrogen bonding by polyhydroxy compounds). The effect of phloridzin was dependent on the Na^+:K^+, ratio. This inhibitor apparently shifted the substrate concentration velocity curve towards lower affinities. According to Robinson[4] phloridzin acts as a heterotropic allosteric modifier increasing the apparent affinity to K^+ and lowering it towards Na^+. In comparison with the investigations of Britton and Blank[3], Laris *et al.*[1], and Ewers *et al.*[2] we found inhibitory effects of glucose in the range of 10–100 mM, which can be considered as being closer to physiological concentrations. It was shown that total inhibition of the enzyme can take place under suitable conditions. The strong dependence of this inhibition on the Na^+

and K^+ concentrations together with the characteristic change of the Na^+ activation curve makes it likely that these interactions between actively transported sugars with (Na^+–K^+)-ATPase are specific.

The fact that also mannose behaves like an actively transported sugar is in accordance with the results of Bihler[10]. The kinetic effects are of special interest with regard to possible allosteric properties of (Na^+–K^+)-ATPase. Squires[11], Robinson[14], and Jardetzky[12] discuss (Na^+–K^+)-ATPase as an allosteric system. The altered kinetic behaviour of the Na^+ activation curve in the presence of glucose, galactose, 3-*O*-methylglucose and mannose justify that these effects can be classified as heterotropic allosteric effects. Studies are in progress regarding the possible relations between kinetic and allosteric behaviour of intestinal (Na^+–K^+)-ATPase and the phenomenon of Na^+-dependent monosaccharide transport observed in intact preparations of intestine.

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