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Ouabain-Na^+ relation in the inhibition of the active transport of sugars by the intestine in vivo*

by

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It is well known that ouabain and other cardiac glycosides inhibit the active transport of Na^+ by several biological systems (3, 4, 11, 18). It has been also verifield that active transport of sugars by the intestine is depending on the Na^+ concentration at the mucosal side (1, 2, 6, 9, 10, 12, 16). On the other hand, there are some observations using *in vitro* techniques, showing an inhibition of the active transport of sugars by these glycosides. In strips of hamster intestine (6), K-strophanthidin strongly inhibit it but at rather high concentrations (10^{-3} M), and the same result has been obtained in rat intestine (13). Amphibian intestine seems to be however much more sensible, since 10^{-6} M ouabain or 10^{-7} M thevetin already inhibit the active transport of 3-methylglucose (7, 8).

In the present paper the *in vivo* action of ouabain added to the sugar solutions on the absorption of glucose or fructose is studied, working with different levels of Na^+ at the intestinal lumen.

Material and Methods

White rats, 150-200 g weight, have been used, by the SOLS and PONZ method of succesive absorptions (20). Each animal was utilized for 4 succesive periods of absorption, with a 10 ml volume of solution to be absorbed and a repletion pressure of 8 cm H_2O.

Sugar solutions were 300, 55 or 2.77 mM glucose and 2.77 mM fructose. These solutions, incluted NaCl at different concentrations and were made isotonic, adding when necessary, sorbitol (Roche) or mannitol (Merck). Ouabain (G-Strophanthidin**) was also dissolved in the sugar solutions to be absorbed in a part of the absorption periods.

Determinations of sugar were made by

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SOMOGYI method (19) and those of Na^+ by flame photometry.

The absorption in the 1st. period which is taken as control, is given in μM/cm. (21) and the absorption in the next ones are indicated as + or — deviations per cent respect to the control. In the tables are only given the mean values and its standard errors (15).

Results **

1. *Absorption of 300 and 55 mM glucose.*

In a first group of animals the washing of the intestinal segment before and after each absorption period was made with 9 °/₀₀, NaCl and the absorptions of 0.3 M glucose were carried out in the presence of 13 meq. Na^+/l. Ouabain was included at the 2nd and 4th periods of absorption. All the periods were of 30 minutes. As Table I shows, it was not found inhibition by ouabain but at concentrations 7.5×10^{-6} M and higher. This inhibition increases on in the next periods of absorption, there being or not ouabain in the sugar solutions. With ouabain 1.5×10^{-5} M the inhibition reaches a 21 % and 40 % in the 2nd and 4th period respectively.

To discuss this result, it was interesting to know if the inhibition could be imputed to a local effect of ouabain on the sugar absorbing intestinal segment or rather to a more general pharmacological action of the absorbed glycoside. For it, experiments were carried out preparing two different contiguous segments of intestine in each animal, the proximal being used for sugar absorption from solutions without ouabain and the distal one for ouabain absorption from saline solutions. Under these condition was observed t hat ouabain at 7.5×10^{-6} M and higher concentrations, put in the distal segment in the meantime of the 2nd and 4th periods of glucose absorption, produces very similar effects to those observed when it was present in the same solutions to be absorbed, indicating that these inhibitions may be imputable to general pharmacological actions (Table I).

Another experiment was performed with 0.3 M glucose, in which the 1st period the sugar solution contained Na^+ (13 meq./l) and the successive ones did not. The washing was made with water. Ouabain was present in the 2nd and 4th period. Now, ouabain 1.5×10^{-6} M, a

TABLE I

Absorption of 0.3 M glucose by rat intestine in vivo. *Effect of ouabain added in 2nd and 4th absorption. Initial Na+ in lumen 13 meq./l. Time of absorption 30 min. Inhibitions referred to the first absorption (control).*

Animals	Abs. control μM/cm	Successive Absorptions			
		Ouabain M	Variations %		
			2nd Abs.+Ouab.	3rd Abs.	4th Abs.+Ouab.
3	43±1.0	1.5×10^{-6}	—7±5.0	—1±1.0	—1±1.0
6	45±1.3	4.5×10^{-6}	—1±0.5	—3±1.0	—1±0.4
9	37±2.0	7.5×10^{-6}	—12±1.8	—26±2.2	—32±1.4
9	34±2.0	1.5×10^{-5}	—21±2.4	—26±2.1	—40±2.8
4*	42±1.5	7.5×10^{-6}	—10±5.1	—22±4.0	—25±4.0
7*	35±1.4	1.5×10^{-6}	—2±1 0	—2±1.0	—4±1.0

* Ouabain put in a intestinal segment different from that with glucose solution.

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TABLE II

Absorption of 0.3 M glucose by rat intestine in vivo. *Ist absorption with 13 meq. Na+/l. 2nd, 3rd and 4th without initial Na+. Ouabain added in absorption 2nd and 4th. Time of absorption 30 min. Inhibitions referred to the first absorption (control).*

Animals	Abs. control μM/cm	Successive Absorptions		
		Variations %		
		2nd Abs.+Ouab.	3rd Abs.	4th Abs.+Ouab.
10	38±2.5	—18±2.0	—33±3.1	—53±3.2

TABLE III

Absorption of 55 mM glucose by rat intestine in vivo. *Sugar solution isotonized with sorbitol. Ist absorption with 13 meq. Na+/l. 2nd, 3rd and 4th without initial Na+; 2nd and 4th with ouabain 1.5 × 10^{-6} M. Time of absorption 20 min. Inhibitions referred to the first absorption (control).*

Animals	Abs. control μM/cm	Successive Absorptions		
		Variations %		
		2nd Abs.+Ouab.	3rd Abs.	4th Abs.+Ouab.
6	10±1.0	—31±2.0	—26±3.0	—35±1.0

concentration showing no effect in the preceding experiments with Na^+, exerts a clear inhibitory action on the glucose absorption (Table II). Of course, this inhibition can be ascribed both to the ouabain and to the initial absence of Na^+ in lumen, however, the last factor, as has been previously tested (14) cannot explain an inhibition so great as of 53 % (4th period). From these results, it seems may be deduced that the initial absence of Na^+ in the intestinal lumen sensibilizes the absorption of 0.3 M glucose to the ouabain.

Also ouabain 1.5 × 10^{-6} M inhibited the absorption of glucose from solutions 55 mM without initial Na^+ isotonized with sorbitol (table III).

2. *Absorption of 2.77 mM glucose.*

From a 2.77 mM concentration in lumen, glucose must be transported to the blood against a gradient, thus actively. All the solutions to be absorbed were made isotonic by addition of variable quantitaties of NaCl and mannitol. Four successive periods of absorption of 2.77 mM glucose, in the presence of 154 meq. Na^+/l, gave constant values of transported sugar (16). If in 2nd, 3rd and 4th periods, ouabain was present in the sugar solutions (table IV), the glucose absorption resulted not affected by 1.5 × 10^{-6} M ouabain, and was only found inhibition at 1.5 × 10^{-5} M and higher concentrations. But these last levels of concentration of ouabain had also effect when they are absorbed from an intestinal segment different of that absorbing glucose.

If the first absorption period was carried out with 13 meq. Na^+/l, the 2nd without initial Na^+ and the 3rd. and 4th with again 13 meq. Na^+/l but also with 1.5 × 10^{-6} M ouabain, the glucose absorption in the 2nd period was about a 13 % less than during the first, due to the initial absence of Na^+, and the absorption in the 3rd. and 4th became pro-

TABLE IV

Absorption 2.77 mM glucose (with 154 meq. Na+/l) by rat intestine in vivo. *Ouabain added in absorptions 2nd, 3rd and 4th. Time of absorption 20 min. Inhibitions referred to the first absorption (control).*

Animals	Abs. control μM/cm	Successive Absorptions			
		Ouabain	Variations %		
			2nd Abs.+Ouab	3rd Abs.+Ouab.	4th Abs.+Ouab.
6	0.32±0.02	1.5×10^{-6}	—4±2.8	—7±3.0	—3±3.2
6	0.29±0.03	1.5×10^{-5}	—10±3.4	—9±3.7	—13±2.6
6	0.36±0.05	3×10^{-5}	—9±1.8	—24±1.1	—23±1.5

TABLE V

Absorption of 2.77 mM glucose isolonized with sorbitol by rat intestine in vivo. *1st, 3rd and 4th absorption 13 meq. Na+/l, no Na+ in 2nd absorption. Ouabain 1.5 × 10^{-6} M in 2nd and 4th absorptions. Time of absorption 20 min. Inhibitions referred to the first absorption.*

Animals	Abs. control μM/cm	Successive Absorptions		
		Variations %		
		2nd Abs.	3rd Abs.	4th Abs.
7	0.43±0.01	—13±1.0	—21±2.0	—29±3.0

gressively inhibited (20-30 %) by effect of the ouabain (Table V). So, it was again observed that glucose absorption, now at 2.77 mM concentration, is more sensible to the ouabain when the luminal Na^+ levels are low.

In order to see better this phenomenon experiments were performed in which at the first absorption period the sugar solution contained 154 meq. Na^+/l, and at the next three only 8.5 meq./l. In some animals ouabain was added for the 3rd. and 4th periods; in other, ouabain was not. Also determinations of the nett Na^+ movement in the different periods of absorption were made in many cases, to test if ouabain had any effect on it.

The absorption diminished in about a 54 % with 8.5 meq. Na^+/l in respect to the 1st absorption with 154 meq./l, as it had been shown previously (16). But if ouabain 1.5×10^{-6} M was also present, the inhibition increased up to 81-82 % and at concentration 1.5×10^{-5} M the inhibition by ouabain was total (Table VI, fig. 1).

If the absorptions of glucose with or without ouabain at Na^+ levels of 8.5 meq./l are directly compared, inhibitions of 59 % and 100 % are foun with 1.5×10^{-6} and 1.5×10^{-5} M respectively (fig. 2).

The determinations of initial and final Na^+ present in the luminal solution, corresponding to each period of absorption in these experiments (Table VI) revealed that in the first period (154 meq./l initial Na^+ there was not a nett passage of Na^+) (20 min.); but in the there consecutive periods (8.5 meq./l, initial Na^+), a net exit of Na^+ to the lumen was meas-

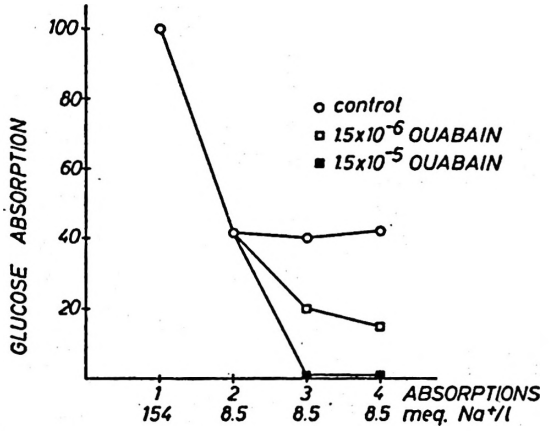

FIG. 1. Effect of Na^+ and ouabain on the glucose absorption (2.77 mM). Rat intestine, *in vivo*.

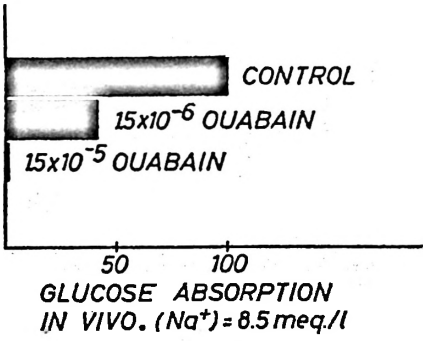

FIG. 2. Inhibition of glucose absorption (2.77 mM) by ouabain, at initial Na^+ levels of 8.5 meq./l. Rat intestine, *in vivo*.

ured. This exit of Na^+ reached 5.4 mg. when ouabain was not present, and 3.5 mg. when the sugar solutions contained 1.5×10^{-6} or 1.5×10^{-5} M ouabain. The differences between both mean values of nett exit of Na^+ according to the presence or absence of ouabain are statistically significative ($p<0.01$). These results demonstrate that ouabain does exert some action on the movement of Na^+ through the intestine conducing to a 35 % diminishion of its nett exit from blood to the intestinal lumen, at the same time that strongly inhibits the active transport of glucose to the blood.

3. *Absorption of 2.77 mM fructose.*

Similar experiments to the preceeding ones, were carried out with 2.77 mM fructose. Ouabain 1.5×10^{-6} M did not show effect on the absorption of fructose (Table VI).

TABLE VI

Absorption of 2.77 mM glucose or fructose through rat intestine in vivo, and Na+ luminal contents at the beginning and the end of each absorption period (20 minutes), 154 meq. Na+/l in absorption 1; 8.5 meq. Na+/l in the 2nd, 3rd and 4th. Ouabain added in 3rd and 4th absorptions. Solutions isotonized with mannitol. Inhibitions referred to the first absorption (control). The Na+ values are given in parenthesis (initial-final) in mg.

Animals	Abs. control μ M/cm	Successive Absorptions (8.5 meq. Na+/l)			
		Ouabain M	Variations %		
			2nd Abs.	3rd Abs.+Ouab.	4th Abs.+Ouab.
Glucose					
12	0.35 ± 0.04 (35.36→35.07)	1.5×10^{-6}	—54 ± 3.4 (1.98→7.4)	—81 ± 1.4 (1.98→5.6)	—82 ± 2.2 (1.98→5.5)
10	0.32 ± 0.09 (35.36→36.24)	1.5×10^{-5}	—55 ± 2.9 (1.92→7.7)	—100 ± 0.0 (1.92→5.7)	—100 ± 0.0 (1.92→5.5)
Fructose					
8	0.22 ± 0.02	1.5×10^{-6}	—18 ± 0.8	—18 ± 1.0	—20 ± 2.0

Discussion

The intestinal absorption of glucose *in vivo* by the rat, is inhibited by the ouabain added to the sugar solution. However, a rather general pharmacological action only indirectly affecting the absorption cannot be excluded unless the concentrations of ouabain used are lower than 7.5×10^{-6} M, since as from these levels a similar inhibition is found when the glycoside is absorbed from a intestinal segment different from the utilized for sugar absorption, evidencing that ouabain has reached the blood.

At concentrations lower than 7.5×10^{-6} M, ouabain has no effet *in vivo* when the levels of Na^+ in lumen are high. But the susceptibility of the absorption of glucose to the ouabain, notablv increases if the level of Na^+ is mantained very low. This fact has been shown in the experiments either with 0.3 M or 2.77 mM glucose. With 0.3 M glucose, by passing from 13 meq. Na^+/l in the initial solutions to absence of Na^+, the absorption is reduced about 10-20%; but 1.5×10^{-6} M ouabain has no action when Na^+ is present, and inhibits the absorption about 50 % when the initial solutions are free of Na^+.

With glucose 2.77 mM and 154 meq. Na^+/l, ouabain has only a slight inhibitory effect at 1.5×10^{-5} M concentration. But this last concentration completely inhibits the absorption when the initial level of Na^+ in lumen is of 8.5 meq./l, and even at 1.5×10^{-6} M it inhibits about 80 % the absorption.

The activity of ouabain on the active transport of glucose as tested with low levels of Na^+ *in vivo* is by two or three orders of magnitude higher than that observed *in vitro* with some mammal intestine preparations (6, 13). Perhaps that is due to the higher level of Na^+ used in the *in vitro* experiments.

It is conceivable that if the active transport of sugars by the intestine requires (5, 6) a penetration of the substrate into the cell as a complex with Na^+ and a carrier and then a sugar accumulation component by pumping out actively the Na^+, the experimental conditions of very low levels of Na^+ in lumen may do particularly sensible the whole process to any one situation disturbing the movements of the Na^+ ion.

A total absence of Na^+ at the mucosal side completely inhibits the active transport *in vitro* (1, 9, 17). But *in vivo* this circumstance is not found, because the initial absence of Na^+ in lumen produces a nett exit of this cation from blood and tissues to the intestine (16). Ouabain might inhibit the sugar absorption *in vivo* by means of some alterations of the movements of the Na^+. In fact at very low levels of Na^+ in lumen, the ouabain inhibits the nett exit of Na^+. The effective concentrations of ouabain are of a similar order to those inhibiting Na^+ transport in many biological systems (3, 4, 11, 18).

For the present, it is difficult to determine with precision the inhibitory mechanism of ouabain, as still there is a great ignorance on the process of active transport of sugars and the part played in it by the active transport of Na^+ (14). Our results seem to relate the inhibition of the sugar penetration to the decrease of the Na^+ exit to the lumen. On the other hand, absorption of fructose, a sugar not actively transported, is not inhibited by ouabain 1.5×10^{-6} M, a difference showing that the ouabain effect is concerned to the absorption of actively transportable sugars.

The fact of ouabain reduces the nett exit of Na^+, produces a concentration of Na^+ in lumen during the absorption period lower than when ouabain is not present. That may account for a part of the inhibition on the glucose absorption.

Summary

It has been tested the effect of ouabain added to sugar solutions on the sugar absorption by rat intestine *in vivo*.

Ouabain inhibits the active transport of glucose, excluded more general pharmacological effects, at conc. lower than 7.5×10^{-6} M. This ouabain activity requires to work with very low levels of Na^+ in lumen. 1.5×10^{-6} M ouabain has no action on active transport of glucose (2.77 mM) with 154 mq. Na^+/l and inhibits it a 59 % with initial level of Na^+ of 8.5 meq./l. At this level of Na^+, a nett exit of Na^+ to the lumen is produced during an absorption period, which is inhibited in a 35 % by ouabain 1.5×10^{-6} M. This effect may explain a part of the inhibition of the glucose absorption. The same concentration of ouabain has no effect on the absorption of fructose.

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