BBA 75561

REVERSIBLE INHIBITION OF INTESTINAL ACTIVE SUGAR TRANSPORT BY DECONJUGATED BILE SALT *IN VITRO*

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(Received August 3rd, 1970)

SUMMARY

1. The studies reported here were undertaken to establish whether the inhibition of intestinal sugar transport caused by deconjugated bile salts *in vitro* is simply due to cellular destruction, and therefore irreversible.

2. Because Arbutin (*p*-hydroxyphenyl-*β*-glucoside) is actively transported by the small intestine of the rat in the same manner as glucose and is not metabolised, it was used for these *in vitro* studies.

3. The addition of 10 mM sodium taurocholate to the incubation medium does not significantly affect the normal uptake pattern of Arbutin.

4. Low concentrations of sodium deoxycholate (0.5–1 mM) markedly inhibit the uptake of Arbutin by the gut.

5. Under the experimental *in vitro* conditions described, the inhibitory effect of deoxycholate is reversible and not simply the result of tissue damage.

INTRODUCTION

Previous *in vitro* studies showed inhibition of intestinal glucose uptake in the presence of conjugated bile salts[1,2]. However, POPE *et al.*[3] later showed that this effect could be accounted for by contamination with small amounts of unconjugated bile salts and fatty acids and clearly demonstrated the inhibitory effect to be confined to chromatographically pure, unconjugated bile salts. All other metabolic functions of the enterocyte investigated *in vitro* with unconjugated bile salts have also been inhibited and DIETSCHY[4] suggests that "substances such as deoxycholate simply kill *in vitro* preparations". Certainly, tissue disruption has been consistently demonstrated in preparations such as everted intestinal sacs incubated with unconjugated bile salts[5,6]. FORTH *et al.*[7] have shown impairment of glucose absorption by unconjugated bile acids in rats *in vivo*, but glucose is rapidly metabolised by rat intestine[8] and is not ideal for uptake studies in these animals. The effect of pure, unconjugated bile salts on intestinal sugar transport remains to be elucidated.

The present studies were undertaken to re-evaluate the effect of bile salts on intestinal sugar absorption in experiments where tissue damage would not explain all the effects observed by investigating the uptake of a nonmetabolised analogue of glucose in the plexiglass chambers devised by SEMENZA[9].

METHODS AND MATERIALS

Incubation technique

Female adult Wistar rats weighing 200–300 g were used in all experiments. They were fasted for 24 h then killed by a blow on the neck. The small intestine was immediately removed and gently washed through with physiological saline; the gut was everted and divided transversely into segments 1.5 cm long, discarding areas containing nodular Peyer's patches. The segments were randomized[10] in a beaker containing KREBS–HENSELEIT[11] bicarbonate buffer (pH 7.4) at 37°. Tissue specimens were then removed, gently blotted and mounted in plexiglass chambers[9]. This part of the experiment was completed within 5 min.

The mounted specimens were preincubated for 2 min in KREBS–HENSELEIT buffer then transferred to beakers containing 30 ml of incubation medium; four specimens were incubated in each beaker.

The incubation medium consisted of KREBS–HENSELEIT bicarbonate buffer at pH 7.4, gassed with O_2–CO_2 (95:5, by vol.) 3 mM Arbutin except when indicated differently, 1.5 mM 2-deoxy-D-glucose and the bile salts as indicated. Incubations were performed in a Dubnoff-type shaker (Gallenkamp) at 37 ± 1° in an atmosphere of O_2–CO_2 (95:5, by vol.). After incubation the chambers were rinsed in buffer at room temperature, the exposed tissue removed by means of a metal punch, very gently blotted to remove excess fluid and immediately weighed. The specimens were then homogenised and deproteinised according to the method of SOMOGYI[12].

Materials

Arbutin (*p*-hydroxyphenyl-*β*-glucoside) and 2-deoxy-D-glucose of analytical grade were obtained from Sigma Chemical Company, London. Sodium salts of deoxycholate and taurocholate were obtained from Maybridge Chemical Company, Cornwall. Purity was checked by thin-layer chromatography.

Analytical methods

Arbutin was determined as free phenol[13,14]; 2-deoxy-D-glucose was determined by the formation of malonic dialdehyde with periodate and on its condensation with thiobarbituric acid[15].

Reversibility of inhibition

To determine whether the inhibition of Arbutin uptake we demonstrated with sodium deoxycholate, was reversible we studied Arbutin uptake from a solution free of bile salts after preincubation in a medium containing deoxycholate. Segments of intestine were incubated in KREBS–HENSELEIT buffer containing 3 mM Arbutin with 0.5 mM sodium deoxycholate for 25 min. The tissues were then removed from this medium, rinsed with buffer and immediately transferred to a medium of 3 mM Arbutin, without added bile salts, in the usual experimental conditions. Uptake of Arbutin was then studied at 5–25 min from commencing the second incubation.

Kinetics of the inhibitory effect of deoxycholate

The K_i for the inhibitory effect of 0.5 mM sodium deoxycholate on Arbutin

uptake at different substrate concentrations at 45 min was determined and recorded on a LINEWEAVER-BURK[16] plot.

Histological methods

After incubation in media consisting of buffer or buffer containing bile salts, tissues were fixed in formol–saline, mounted in paraffin-wax and stained with Haematoxylin–eosin prior to histological examination.

Calculation of data

Results are expressed as μmoles of substrate accumulated per ml tissue in a given period of time, assuming a water content of approx. 80% of the wet weight of the tissue. These values were used after correction for 2-deoxy-D-glucose space. The results are also expressed as percent filling which equals 100 $\times$ μmoles per ml tissue per μmoles per ml medium. The percentage inhibition was calculated as

$$\frac{\text{Transport (Control)} - \text{Transport (Deoxycholate)}}{\text{Transport (Control)}} \times 100$$

Standard arithmetical methods were used to calculate arithmetical means and standard deviations (S.D.). Significance values were achieved by using student's t test. P values of < 0.05 are taken as being significant.

RESULTS

Histological pattern of tissues after incubation

Tissues from each category of incubation experiment showed a similar histological pattern. Mucosal integrity was essentially maintained in each group. There was a moderate degree of sub-mucosal swelling only. The appearances were identical in control experiments and in those after incubation with taurocholate or deoxycholate.

TABLE I

UPTAKE OF 3 mM ARBUTIN BY NORMAL RAT SMALL INTESTINE IN THE PRESENCE AND ABSENCE OF ADDED BILE SALT

Time (min)	*Tissue arbutin concentration*					
	Without bile salt added ($n = 3$)		*With 10 mM sodium taurocholate* ($n = 3$)		*With 0.5 mM sodium deoxycholate* ($n = 5$)	
	Mean $\pm$ *S.D.*	*% Filling*	*Mean* $\pm$ *S.D.*	*% Filling*	*Mean* $\pm$ *S.D.*	*% Filling*
5	2.0 $\pm$ 0.2	66	2.0 $\pm$ 0.5	66	0.4 $\pm$ 0.2	13
10	2.6 $\pm$ 0.5	86	2.4 $\pm$ 0.3	80	0.8 $\pm$ 0.3	25
15	3.0 $\pm$ 0.4	100	2.7 $\pm$ 0.2	90	1.0 $\pm$ 0.3	32
20	3.6 $\pm$ 0.4	120	3.4 $\pm$ 0.3	113	1.1 $\pm$ 0.3	37
25	4.2 $\pm$ 0.6	140	3.6 $\pm$ 0.3	120	1.4 $\pm$ 0.4	47
30	4.7 $\pm$ 0.8	158	4.2 $\pm$ 0.2	140	1.6 $\pm$ 0.4	53
35	5.0 $\pm$ 1.2	166	4.4 $\pm$ 0.1	147	2.0 $\pm$ 0.4	66
40	—	—	4.8 $\pm$ 0.2	160	—	—

Normal uptake pattern of Arbutin

The normal uptake pattern was reproducible within close limits and the results are shown in Table I. There was rapid tissue accumulation of Arbutin in the first 15 min to 100 % tissue filling. Following this there was progressive accumulation at a less rapid rate; more than 150% tissue filling was achieved after 30 min.

Effect of bile salts on Arbutin uptake

Sodium taurocholate, in a concentration of 10 mM, had no significant effect on the uptake of Arbutin throughout the period of the experiments.

Sodium deoxycholate, in a concentration of 0.5 mM, had a significant effect on the uptake of Arbutin ($P < 0.05$) throughout the experiments (Table I). The effect of various concentrations of sodium deoxycholate on Arbutin uptake was studied after

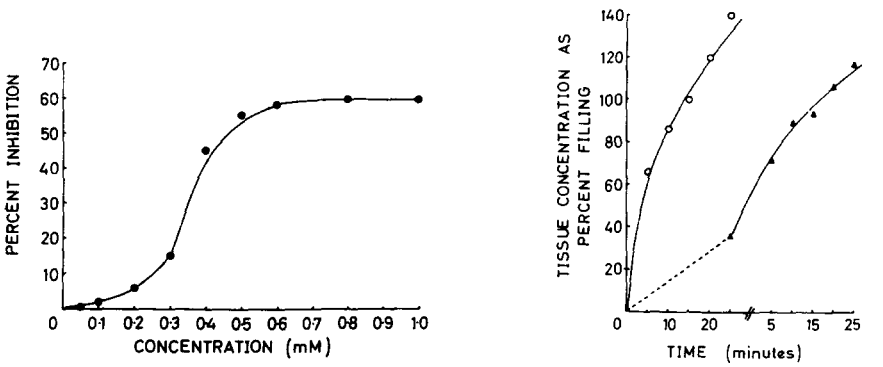

Fig. 1. Effect of various concentrations of sodium deoxycholate on the uptake of 3 mM Arbutin by the rat small intestine (incubation period, 30 min). Results indicate the mean of three experiments.

Fig. 2. Reversibility of deoxycholate inhibition of 3 mM Arbutin uptake, following the transfer of tissue to a bile salt-free medium in the second part of the experiment. ○, normal uptake pattern of 3 mM Arbutin; ▲, after incubation in 0.5 mM deoxycholate and 3 mM Arbutin for 25 min, transfer to bile salt-free medium containing 3 mM Arbutin shows rapid recovery of the normal uptake pattern. Results indicate the mean of three experiments.

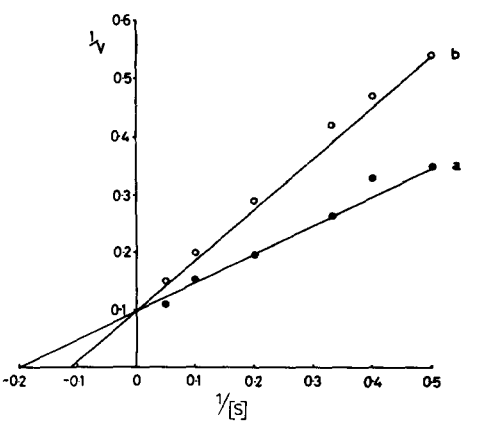

Fig. 3. Kinetics of entry of 3 mM Arbutin into the rat small intestine in the absence (a) and presence (b) of 0.5 mM sodium deoxycholate. Results indicate the means of two and four experiments respectively.

30 min incubation. Concentrations of less than 0.5 mM caused less than 50% inhibition and this effect was considerably less marked at lower concentrations. However, 50% or more inhibition was consistently achieved at concentrations in excess of 0.5 mM (Fig. 1).

Reversibiltiy of deoxycholate inhibition

The inhibitory effect of 0.5 mM sodium deoxycholate was reversible. After incubation for 25 min in 0.5 mM deoxycholate, subsequent incubation in 3 mM Arbutin without bile salts allowed a rapid and progressive accumulation of Arbutin, approximating the normal Arbutin uptake pattern (see Fig. 2).

Kinetics of inhibition of Arbutin uptake

The LINEWEAVER–BURK plot showed the inhibitory effect of 0.5 mM sodium deoxycholate to be competitive with a K_i of $6 \cdot 10^{-4}$ M (Fig. 3).

DISCUSSION

Since Arbutin (*p*-hydroxyphenyl-*β*-glucoside) is transported *via* the active intestinal sugar transport pathway in the rat and is not metabolised[17], it is appropriate for studying the effects of bile salts on this process. The addition of 10 mM sodium taurocholate did not significantly affect the uptake of Arbutin, a finding in agreement with that of previous workers using pure bile salts[3]. Marked inhibition of uptake of Arbutin by the small intestine was produced by incubation with low concentrations of deoxycholate (Fig. 1). More than 50% inhibition occurred with a concentration as low as 0.5 mM. Similarly potent inhibitory effects on jejunal glucose transport have been found with low concentrations of deoxycholate by others[3,7].

The present studies clearly show that, in this experimental system, inhibition of sugar transport by unconjugated bile salts cannot be explained simply as a non-specific effect of tissue damage as has been previously suggested because of the effects observed when using other *in vitro* preparations[4]. There was no difference in the histological appearances of control tissues and those incubated with deoxycholate. That the inhibition is reversible indicates that integrity of intracellular metabolism was retained and excludes extensive tissue disruption as the explanation of the effects observed under the experimental conditions described. This finding, which has not been previously demonstrated, may be related to differences in the *in vitro* technique used. Apart from the advantages of the technique mentioned by SEMENZA[9] the exclusion of the cut ends of the tissue from the incubation medium removes a significant route of entry of bile salts into the preparation and this may account for the lack of tissue damage produced in these experiments.

The demonstration that the inhibitory effect of deoxycholate is not simply due to tissue damage suggests that this effect may be pertinent to *in vivo* situations, although this relationship remains to be firmly established. BARAONA *et al.*[18] have shown impaired glucose uptake *in vitro* in rats with intestinal blind loops. Unconjugated bile salts were present in the intestinal contents but were not quantitated. Their suggestion that impaired sugar absorption was due to the presence of high concentrations of these substances seemed to be supported by finding normal sugar absorption in blind-loop animals following biliary diversion. However, this latter

finding may be at least partly explained by the increased intestinal sugar absorption which follows biliary diversion, as shown in rats by ROY *et al.*[19] The occurrence of impaired urinary excretion of xylose in rats with intestinal blind loops[20] and monosaccharide malabsorption in infants with unconjugated intraluminal jejunal bile salts[21] lends support to the relevance of deconjugated bile salts to imapired intestinal sugar absorption *in vivo*.

The fact that unconjugated bile salts cause inhibition when added to normal intestinal tissue in our experimental system, but conjugated salts had no effect may have been related to the greater absorption of unconjugated compared with conjugated salts in areas of the small intestine where active transport of bile salts does not occur[22]. To test this possibility incubations of 10 mM sodium taurocholate with pieces of terminal ileum were performed in our experimental system and similarly did not show inhibition of Arbutin transport in spite of the greater absorption of the conjugated bile salt by this region, thus excluding this possibility.

The mechanism by which unconjugated bile salts cause inhibition of intestinal sugar uptake requires further investigation. The LINEWEAVER–BURK plot (Fig. 3) demonstrates that the mechanism is competitive, but the site of this action is uncertain. The inhibition of transport of water-soluble nutrients by unconjugated bile salts in the small intestine has been ascribed to suppression in the activity of membrane bound (Na^+–K^+)-ATPase[23], but pure preparations of conjugated bile salts similarly affect ATPase[3]. FORTH *et al.*[7] have demonstrated inhibition of Na^+ and water absorption in the rat intestine in the presence of unconjugated bile salts and since active sugar transport depends on the Na^+-pump[24] these observations suggest that interference with this mechanism by bile salts may explain the inhibition observed.

ACKNOWLEDGEMENTS

We thank Dr. Knowles Kerry for helping to establish the technical procedures, and Professor Charlotte M. Anderson for her advice and facilities. This work was supported by a Grant from the Endowment Fund of the United Birmingham Hospitals

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