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SODIUM EFFECT ON BILIRUBIN UPTAKE BY THE RAT INTESTINAL MUCOSA

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SUMMARY

1. Decreasing the incubation medium Na^+ concentration below physiological levels resulted in a decrease in bilirubin uptake by the rat intestinal mucosa.

2. Preincubation in Na^+-free medium which lowered the intracellular Na^+ content had no effect on bilirubin uptake during incubation in normal Krebs–Ringer–Tris.

3. Ethacrynic acid inhibited the bilirubin uptake from a 142 mM Na^+ medium without altering the tissue Na^+ level.

4. The uptake of bilirubin depended more on the extracellular than on the intracellular Na^+ concentration. The results suggest the existence of a Na^+-dependent, ethacrynic acid-sensitive mucosal uptake of bilirubin. This process may be mediated by a Na^+–Na^+ exchange mechanism across the luminal membrane of intestinal cells.

INTRODUCTION

Previous investigations[1,2] demonstrated the *in vitro* capacity of rat intestinal mucosa to take up bilirubin and to translocate the pigment transmurally. The previous findings suggested the existence of a facilitated diffusion mechanism for bilirubin Coupling effects with Na^+ are known to occur for a great variety of solutes that are wholly or partially transported by facilitated diffusion[3]. The present report demonstrates the role of Na^+ in bilirubin uptake by intestinal mucosa. The uptake of bilirubin depended most on the extracellular Na^+ concentration.

METHODS

The uptake of bilirubin by intestinal slices was determined by a tissue accumulation method described in detail previously[2]. Briefly, the intestine was excised from a rat sacrificed by cervical dislocation, rinsed with physiological saline and cut into 100–200 mg wet weight slices open lengthwise to prevent trapping of media inside

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the tissue. A Krebs–Ringer solution[4] buffered with Tris–HCl instead of phosphate and bicarbonate served as the Na^+-containing control medium. The experimental media contained 0.142 M choline chloride or 0.284 M mannitol in place of 0.142 M NaCl. It was necessary to use sodium taurocholate to solubilize bilirubin, so that the experimental media contained in some experiments 5 mM Na^+. All media were considered osmotically equivalent.

The slices were equilibrated in the buffer in which they were incubated. Some samples were preincubated during 30 min prior to incubation with 0.3 mM bilirubin in one of the media. The 10-min equilibration period as well as the pre- and incubation periods were done under oxygen atmosphere. Only the mucosal fraction was assayed because more than 75% of the total intestinal wall incorporation of bilirubin corresponds to this fraction[2].

Na^+, K^+ and tissue water were determined in slices shaken for 45 min (100 oscillations/min, 5 cm throw) in a water bath at 37 °C in the media stated in Table III. The slices were removed from the bath, rinsed thrice with an isoosmotic iced sucrose solution and gently blotted on filter paper. The mucosal fraction was sliced[5] and weighed before and after being dried at 105 °C overnight. Then, the slices were immersed in 2 ml of 2 M HNO_3 and shaken at room temperature for 24 h. Na^+ and K^+ were measured by flame photometry in aliquots of the extraction fluid and expressed in mequiv/kg dry tissue wt. Total tissue water content was expressed as g/g dry tissue wt. The other techniques were described previously[2].

Ethacrynic acid and ouabain were analytical grade and provided by Merck Sharp and Dohme and Sigma, respectively.

RESULTS

Bilirubin uptake as a function of time

The mucosal bilirubin uptake rate was constant during 0–15 min where the tissue uptake was linear as shown in Fig. 1. The uptake at 15 min gives a reasonably good measure of medium–tissue unidirectional bilirubin flux.

Effect of Na^+ concentration in incubation medium and in tissue on bilirubin uptake

The data shown in Table I demonstrate that the mucosal bilirubin uptake rate was significantly decreased by incubation in the experimental media containing 5 mM Na^+, regardless of the Na^+ concentration in the preincubation media. When Na^+ was replaced by choline chloride, the results (0.065 ± 0.009 (4)) did not differ from those obtained when Na^+ was replaced by mannitol. There was no significant difference in the bilirubin uptake of tissues incubated in the control media after preincubation in media containing no Na^+ or 142 mM Na^+.

Effect of varying medium bilirubin concentration

Uptake was measured at bilirubin concentrations varying from 0.3 mM to 20 mM in the incubation medium. This was done at two levels of Na^+ concentration (142 mM and 5 mM). The Lineweaver–Burk plot[6] in Fig. 2 shows that a linear relationship for bilirubin uptake occurred at the two levels of Na^+ concentration. The K_m corresponding to 142 mM and 5 mM were 0.90 and 2.86 mM, respectively. The V value was the same (2 μmoles/g wet wt per h) in the media containing 142 mM or 5 mM Na^+.

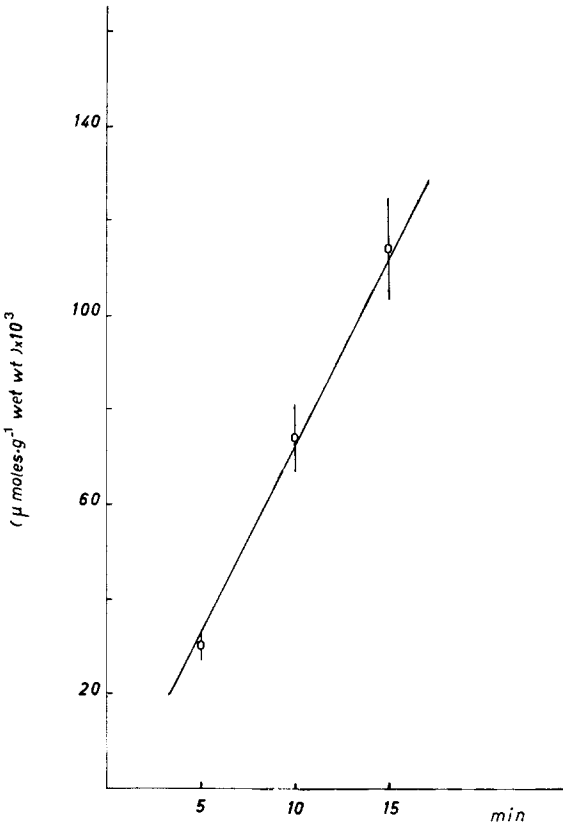

Fig. 1. Bilirubin uptake as a function of incubation time. Each point is the mean ±S.E. of four observations.

TABLE I

The effect of varying the Na^+ concentration in the preincubation and incubation media on bilirubin uptake (μmoles/g wet wt per 15 min). Preincubation time, 30 min; incubation time, 15 min; initial bilirubin concentration 0.3 mM; number of observations in parentheses.

Incubation	*Preincubation*		
	—	*142 mM Na^+*	*0 mM Na^+*
142 mM Na^+	0.143 ± 0.011 (24)	0.157 ± 0.011 (12)	0.147 ± 0.015 (10)
5 mM Na^+ + 284 mM mannitol	0.051 ± 0.004 (21)		0.064 ± 0.012 (12)

Doubling taurocholate concentration in the incubation medium did not affect bilirubin uptake as a function of medium bilirubin concentration nor the kinetic parameters.

Effect of ethacrynic acid and ouabain on bilirubin uptake

These two drugs which alter Na^+ transport in epithelial tissues[7,8] have different effects on tissue bilirubin uptake. Table II shows that a significant difference

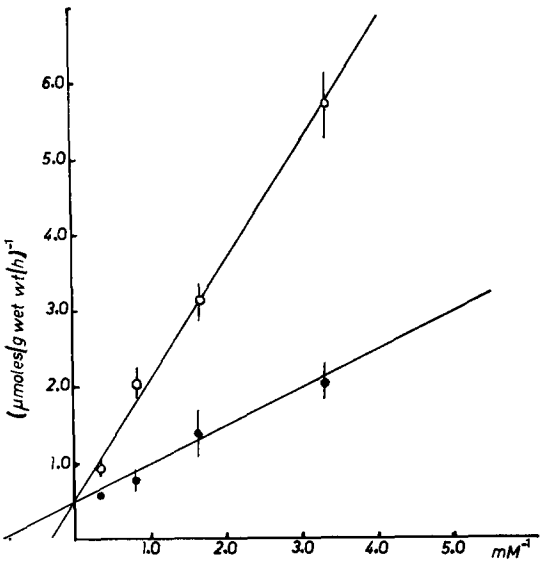

Fig. 2. The inverse of the bilirubin uptake rate estimated from 15-min uptake as a function of the inverse of the bilirubin concentration in the incubation medium. ●, 142 mM Na^+; ○, 5 mM Na^+ *plus* 284 mM mannitol.

TABLE II

The effect of ethacrynic acid (0.031–1.0 mM) or ouabain (1.0 mM) on bilirubin uptake (μmoles/g wet wt per 15 min). The tissue was preincubated in 142 mM Na^+ media containing the stated concentration of ethacrynic acid or ouabain. The incubation period (15 min) began with the addition of 0.3 mM bilirubin to the media. Number of observations in parentheses.

mM	*Bilirubin uptake*	
	Ethacrynic acid	*Ouabain*
0.000	0.145 ± 0.015 (16)	
0.031	0.140 ± 0.015 (7)	
0.125	0.103 ± 0.007 (6)	
0.500	0.092 ± 0.008 (9)	
1.000	0.073 ± 0.003 (7)	0.146 ± 0.012 (10)

in bilirubin uptake was observed from media containing 1 mM ethacrynic acid compared to 1 mM ouabain ($P<0.001$). The uptake of bilirubin was dependent on the dose of ethacrynic acid in the medium.

Tissue water, Na^+ and K^+ levels

Table III shows the water, Na^+ and K^+ levels found in the tissues under the experimental conditions. The values obtained under the 142 mM Na^+ and 284 mM mannitol conditions agree with those reported by Bosačková and Crane[9]. It is evident that the tissue Na^+ content was significantly decreased by incubation in the mannitol

TABLE III

The effect of mannitol, ouabain or ethacrynic acid on the water content and Na^+ and K^+ content in the mucosal slices. Incubation time, 45 min; mean ±S.E.; number of observations in parentheses.

Treatment	*Water content (g/g dry wt)*	*Na^+ content (mequiv/kg dry wt)*	*K^+ content (mequiv/kg dry wt)*
142 mM Na^+	4.62 ± 0.09 (12)	365 ± 19 (12)	367 ± 10 (12)
284 mM mannitol	3.04 ± 0.14 (12)	62 ± 14 (12)	276 ± 19 (12)
142 mM Na^+ *plus* 1 mM ouabain	4.78 ± 0.20 (15)	383 ± 23 (8)	378 ± 21 (15)
142 mM Na^+ *plus* 1 mM ethacrynic acid	4.35 ± 0.15 (15)	388 ± 9 (8)	334 ± 10 (12)

medium. The addition of 1 mM ouabain or 1 mM ethacrynic acid to the 142 mM Na^+ incubation medium failed to significantly alter the tissue Na^+ content.

The extracellular water content of the slices was estimated on the basis of the previously determined tissue extracellular space[2]. These data were subtracted from the corresponding total water content of the slices to obtain the intracellular water content. This last value and the Na^+ tissue content was used to estimate the intracellular Na^+ concentration (mM). The mean difference between the extracellular and intracellular Na^+ concentration after treatment with 1 mM ethacrynic acid was 28.03 ± 4.68 mM (14). This value did not differ significantly from that corresponding to incubation in the 142 mM Na^+ medium alone (29.08 ± 6.51 mM (12)).

DISCUSSION

A great number of solutes, most of which present an uphill transport capacity, are translocated across membranes by cotransport systems which require Na^+ (refs 3, 10–14).

The present results indicate that bilirubin uptake by the rat intestinal mucosa depends on: (a) a Na^+-independent process which probably represents equilibrium distribution in the extracellular space and amounts to 1/3 of the total mucosal uptake and (b) a Na^+-dependent process which represents the rest of the mucosal uptake.

It is to be noted that preincubation and incubation in 142 mM Na^+ media which substantially lowered the difference in the medium–tissue Na^+ concentration was not accompanied by a decrease in tissue bilirubin uptake. On the other hand, tissue Na^+ depletion produced by preincubation in no Na^+ medium which increased the medium–tissue Na^+ difference was not accompanied by an increase in bilirubin uptake. These facts, together with the differences in uptake between tissues incubated in 142 mM Na^+ or 5 mM Na^+, irrespective of the previous treatment of the tissue (Table I), supports the view that one important parameter which influences bilirubin uptake is the external Na^+ concentration.

The effect of a low external Na^+ concentration was not nonspecific or irreversible. Bilirubin uptake was completely reactivated within 15 min by placing a Na^+-deficient intestinal segment in a 142 mM Na^+ medium (Table I). This low Na^+ effect

did not depend on the decrease of medium ionic strength when mannitol was used as the same result was obtained when choline chloride replaced NaCl.

The linear relationship of the double-reciprocal plot suggests the existence of saturation kinetics of the Michaelis–Menten type for the interaction of bilirubin with its binding sites in the tissue. Lowering the Na^+ concentration in the incubation medium increased the K_m of the system without altering the V value. This indicates that Na^+ increases the affinity of the transport mechanism for bilirubin.

Ethacrynic acid lowering of bilirubin uptake was not mediated by a modification of the intracellular Na^+ content[7]. It could have acted by blocking a Na^+–Na^+ exchange mechanism[15] or by altering the formation of a carrier–substrate–Na^+ complex for bilirubin translocation.

On the other hand, ouabain did not affect tissue bilirubin uptake nor electrolyte levels. This last is in accordance with the demonstration[16] that in this structure Na^+ movements are not mediated in a significant manner by an ouabain-sensitive (Na^+–K^+)-ATPase.

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