Sodium–bicarbonate cotransport occurs in rat kidney cortical membranes but not in rat small intestinal basolateral membranes

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Basolateral membrane vesicles were isolated from rat kidney cortex and small intestinal enterocytes. Both membrane preparations show ATP-dependent calcium uptake and cytochalasin B-sensitive D-glucose transport. In renal membranes, sodium influx is stimulated by bicarbonate; bicarbonate-dependent sodium flux is membrane-potential-dependent and inhibited by 4,4'-di-isothiocyanato-2,2'-stilbenedisulphonic acid ('DIDS'). Small intestinal basolateral membranes do not show bicarbonate-dependent sodium fluxes.

INTRODUCTION

Studies on intact proximal tubular preparations of the tiger salamander (Boron & Boulepaep, 1983), rats (Yoshitomi *et al.*, 1985) and rabbits (Sasaki *et al.*, 1985) provided evidence for a coupling between sodium and bicarbonate fluxes in the basolateral membrane of the epithelial cell. Recently, studies on rabbit renal cortical basolateral membranes identified a bicarbonate–sodium cotransport mechanism with a stoichiometry of 3 (Grassl & Aronson, 1986; Akiba *et al.*, 1986). Until now such studies have not been performed in intact small intestinal epithelia or on membrane vesicles isolated thereof.

Transport studies on isolated renal proximal tubular and small intestinal basolateral membrane vesicles revealed many similarities in transport functions (Murer & Kinne, 1980; Murer *et al.*, 1984). Thus, we have performed in the present study parallel experiments with rat kidney cortical and small intestinal basolateral membranes in order to test for the existence of the sodium–bicarbonate cotransport mechanism. We report that rat proximal tubule, as in the rabbit, possesses the cotransport mechanism. Small intestinal membranes are lacking this transport system.

EXPERIMENTAL

Methods

Basolateral membranes were isolated from kidney cortex slices and small intestinal enterocytes from male Wistar rats (180–200 g body weight) by a Percoll-centrifugation method (Hagenbuch *et al.*, 1985). Transport was analysed by a rapid filtration method (Hopfer *et al.*, 1973). ($\text{Na}^+ + \text{K}^+$)-ATPase (EC 3.6.1.37) was determined according to Berner & Kinne (1976), and leucine aminopeptidase (EC 3.4.11.2) according to Haase *et al.* (1978). Transport experiments were performed on unfrozen membrane preparations immediately after isolation; enzyme analysis was performed after storage in liquid N_2 . Protein was determined by the Bradford (1976) method. Further experimental details are given in the legends to the Tables.

Materials

Radiolabelled substances were purchased from NEN (Dreieich, Germany). All other chemicals were at least analytical grade and were obtained either from Fluka (Buchs, Switzerland), Merck (Darmstadt, Germany) or from Sigma (Munich, Germany).

RESULTS

The basolateral membrane fraction was characterized by using the two marker enzymes for basolateral and brush border membranes, ($\text{Na}^+ + \text{K}^+$)-ATPase and leucine aminopeptidase respectively (Murer & Kinne, 1980). Similar to the values reported previously for isolation of rat small intestinal (Scalera *et al.*, 1980) and rat renal proximal tubular basolateral membranes (Scalera *et al.*, 1981), ($\text{Na}^+ + \text{K}^+$)-ATPase was enriched 11- and 15-fold, respectively, and brush border contamination was minimal (results not shown).

ATP-dependent calcium uptake and sodium-independent D-glucose transport are located at the basolateral surface of the proximal tubular and small intestinal epithelial cell (Murer & Kinne, 1980; Murer *et al.*, 1984). As can be seen from the data presented in Table 1, ATP stimulates calcium uptake in basolateral membranes isolated either from small intestine or renal cortex. ATP-dependent uptake of calcium at 1 and 2 min of incubation is against its concentration gradient and therefore higher than the values in the presence of the ionophore A23187. We have also analysed sodium-independent D-glucose transport and its inhibition by cytochalasin B (Murer & Kinne, 1980; Murer *et al.*, 1984). The results for the two membranes were similar: after 20 s of incubation at 25 °C, 30 % equilibration was reached and this initial uptake value of D-glucose (0.05 mM) was reduced by 75 % by the presence of 0.1 mM-cytochalasin B (results not shown). Thus, both membrane preparations are apparently functionally intact.

Finally, we have tested for the presence of a sodium–bicarbonate cotransport system (Table 2). In small intestinal membranes, sodium influx rate is apparently not affected by bicarbonate nor by the imposition of an

Abbreviation used: DIDS, 4,4'-di-isothiocyanato-2,2'-stilbenedisulphonic acid.

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inside positive potassium diffusion potential (potassium gradient plus valinomycin) nor by DIDS. In contrast, in renal cortical basolateral membranes, an inwardly directed bicarbonate gradient accelerates sodium influx. In the presence of bicarbonate, an inside positive diffusion

potential stimulates sodium influx and 0.5 mM-DIDS inhibits bicarbonate-dependent sodium influx. The observations in renal membranes are best explained by a sodium-bicarbonate cotransport mechanism which is sensitive to DIDS. The coupling between bicarbonate and sodium flux exceeds unity. Therefore, cotransport is electrogenic and carries negative charges across the membrane, i.e. its transport rate is affected by the membrane potential. This cotransport mechanism is lacking from intestinal basolateral membranes.

Table 1. ATP-dependent transport of calcium in purified basolateral membrane vesicles

Membranes were suspended in 300 mM-mannitol/100 mM-KCl/20 mM-Mops, adjusted with Tris to pH 7.0, containing 5 mM-MgCl₂ and 2 mM-ouabain. Uptake of calcium was studied at 37 °C at a concentration of 50 µM (⁴⁵CaCl₂) in a buffer containing in addition 5 µg of oligomycin/ml and either no ATP or 5 mM-ATP, or no A23187 or 5 µg of A23187/ml. Uptake was initiated by mixing 20 µl of membrane suspension with 200 µl of incubation medium and stopped by pipetting 20 µl of the incubation mixture on a pre-wetted 0.65 µm cellulose nitrate membrane filter. To remove non-vesicular radioactivity, filters were washed with 5 ml of a solution containing 300 mM-mannitol, 100 mM-KCl, 20 mM-Mops/Tris, pH 7.0, 2 mM-EGTA and 1 mM-MgCl₂. The values are means ± S.D. of a typical experiment performed in quadruplicate. Similar data were obtained in two other experiments.

Incubation time...	Calcium uptake (pmol/mg of protein)	
	1 min	2 min
Small intestine		
– ATP	383 ± 102	504 ± 93
+ ATP	2018 ± 226	2796 ± 244
+ ATP + A23187	1124 ± 146	1096 ± 113
Renal cortex		
– ATP	632 ± 94	724 ± 16
+ ATP	1963 ± 151	3154 ± 201
+ ATP + A23187	1083 ± 102	1106 ± 16

DISCUSSION

Studies on intact epithelia provided evidence for the existence of an electrogenic cotransport mechanism for sodium and bicarbonate in basolateral membranes of proximal tubular epithelial cells of rats and rabbits (Yoshitomi *et al.*, 1985; Sasaki *et al.*, 1985; Alpern & Chambers, 1986; Alpern, 1985); a direct documentation of this transport mechanism became recently available for isolated rabbit proximal tubular basolateral membranes (Grassl & Aronson, 1986; Akiba *et al.*, 1986). The present report extends these observations and documents the existence of a sodium-bicarbonate cotransport mechanism also for the isolated rat proximal tubular basolateral membrane. Although we did not perform a detailed characterization of this cotransport mechanism, it can be assumed that the properties of the rat transport mechanism are very similar to those in the rabbit (Grassl & Aronson, 1986; Akiba *et al.*, 1986). The cotransport mechanism carries excess negative charges via a stoichiometry exceeding unity. Furthermore, it is inhibited by DIDS. These properties are also entirely consistent with the observations on intact rat proximal tubule (Yoshitomi *et al.*, 1985).

In view of the many similarities between transport functions in small intestinal and renal proximal tubular basolateral membranes (Murer & Kinne, 1980; Murer *et*

Table 2. Bicarbonate-dependent transport of sodium in purified basolateral membrane vesicles

Membranes were suspended in 300 mM-mannitol/50 mM-Hepes/Tris, pH 7.5. Incubation media contained 200 mM-mannitol, 50 mM-Hepes/Tris, pH 7.5, 50 mM-potassium gluconate, 25 mM-KHCO₃, 5 mM-[²²Na]sodium gluconate (5 µCi/100 µl) and were continuously gassed with CO₂/air (1:19). A second set of incubation media contained 25 mM-potassium gluconate instead of KHCO₃ and was not gassed. All incubation media contained 1.0% ethanol and either no valinomycin, 10 µg of valinomycin/ml, or 10 µg of valinomycin/ml plus 0.5 mM-DIDS. Uptake was initiated by mixing 10 µl of membrane suspension with 90 µg of incubation media and terminated by pipetting the incubation mixture on a pre-wetted 0.65 µm cellulose nitrate membrane filter. Non-vesicular radioactivity was removed by washing the filters with 5 ml of 200 mM-mannitol/100 mM-potassium gluconate/5 mM-HCl, pH 7.5. The values are means ± S.D. of a typical experiment performed in quadruplicate. Similar data were obtained in two other experiments.

Incubation time...	Sodium uptake (pmol/mg of protein)					
	Bicarbonate gradient			No bicarbonate		
	5 s	15 s	30 min	5 s	15 s	30 min
Small intestine						
Control	311 ± 20	492 ± 38	2145 ± 126	303 ± 23	435 ± 22	1984 ± 108
+ Valinomycin	327 ± 23	509 ± 40	2373 ± 100	327 ± 46	445 ± 59	2043 ± 91
+ Valinomycin + DIDS	319 ± 18	452 ± 30	2162 ± 18	292 ± 38	386 ± 46	1807 ± 84
Renal cortex						
Control	586 ± 12	814 ± 27	2023 ± 108	403 ± 8	612 ± 23	1826 ± 12
+ Valinomycin	1110 ± 36	1632 ± 49	1986 ± 43	408 ± 13	643 ± 16	1946 ± 48
+ Valinomycin + DIDS	412 ± 10	793 ± 21	1913 ± 56	422 ± 9	598 ± 34	1902 ± 57

al., 1984), the lack of the sodium-bicarbonate cotransport system in intestinal membranes is surprising. In order to confirm this 'negative' finding we have always performed the transport studies on fresh preparations and have also performed some additional functional tests for the two membranes. As indicated by our experiments on ATP-dependent calcium transport and on sodium-independent D-glucose uptake, the two membrane preparations are apparently functionally intact, i.e. if damaged the inactivation phenomena should be similar for the two membranes.

The sodium-bicarbonate cotransport mechanism is apparently the major exit pathway for bicarbonate in the renal proximal tubular basolateral membrane (Yoshitomi *et al.*, 1985; Alpern & Chambers, 1986). For the small intestine, this efflux system is not available. Preliminary data reported by Faelli *et al.* (1982) have provided some evidence for a carrier-mediated, sodium- and membrane-potential-independent mechanism for bicarbonate flux in isolated rat small intestinal basolateral membranes. Lucas (1980) proposed on the basis of electrophysiological measurements the involvement of potassium/proton exchange and/or potassium-bicarbonate cotransport in basolateral bicarbonate exit in small intestine. Future experiments on bicarbonate and tracer potassium flux in small intestinal basolateral membranes should test for these mechanisms.

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REFERENCES

- Akiba, T., Alpern, R. J., Eveloff, J., Calamina, J. & Warnock, D. G. (1986) *J. Clin. Invest.* **78**, 1472-1478
- Alpern, R. J. (1985) *J. Gen. Physiol.* **86**, 613-636
- Alpern, R. J. & Chambers, M. (1986) *J. Clin. Invest.* **78**, 502-510
- Berner, W. & Kinne, R. (1976) *Pflügers Arch.* **361**, 269-277
- Boron, W. F. & Boullepaep, E. L. (1983) *J. Gen. Physiol.* **81**, 53-94
- Bradford, M. (1976) *Anal. Biochem.* **72**, 248-254
- Faelli, A., Tosco, M., Orsenigo, M. N., Esposito, G. & Capraro, V. (1982) *Abstr. Meet. Eur. Soc. Comp. Physiol. Biochem. (Bielefeld)*
- Grassl, S. M. & Aronson, P. S. (1986) *J. Biol. Chem.* **261**, 8778-8783
- Haase, W., Schaefer, A., Murer, H. & Kinne, R. (1978) *Biochem. J.* **172**, 57-62
- Hagenbuch, B., Stange, G. & Murer, H. (1985) *Pflügers Arch.* **405**, 202-208
- Hopfer, U., Nelson, K., Perrotto, J. & Isselbacher, K. J. (1973) *J. Biol. Chem.* **248**, 25-32
- Lucas, M. L. (1980) *J. Physiol. (London)* **257**, 645-662
- Murer, H. & Kinne, R. (1980) *J. Membr. Biol.* **55**, 81-95
- Murer, H., Biber, J., Gmaj, P. & Stieger, B. (1984) *Mol. Physiol.* **6**, 55-82
- Sasaki, S., Shiigai, T. & Takeuchi, J. (1985) *Am. J. Physiol.* **249**, F417-F423
- Scalera, S., Storelli, C., Storelli-Joss, C., Haase, W. & Murer, H. (1980) *Biochem. J.* **186**, 177-181
- Scalera, S., Huang, V. K., Hildmann, B. & Murer, H. (1981) *Membr. Biochem.* **4**, 49-61
- Yoshitomi, K., Burckhardt, B. C. & Froemter, E. (1985) *Pflügers Arch.* **405**, 360-366

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