Scanning Microscopy

Volume 7 | Number 1 Article 19

2-11-1993

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P. Fraunberger
Universität München

A. Dörge
Universität München

P. Buchinger
Universität München

F. X. Beck
Universität München

K. Thurau
Universität München

Scanning Microscopy, Vol. 7, No. 1, 1993 (Pages 177-189)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0891-7035/93$5.00+.00

IDENTIFICATION OF ION TRANSPORT COMPARTMENTS IN TURTLE URINARY BLADDER

P. Fraunberger, A. Dörge*, P. Buchinger, F.X. Beck, K. Thurau

Physiologisches Institut der Universität München
Pettenkoferstr. 12, D-8000 München

(Received for publication May 11, 1992, and in revised form February 11, 1993)

Abstract

To identify the turtle urinary bladder cells involved in Na and Cl absorption and H and HCO_3 secretion cellular electrolyte concentrations and uptake of Br and Solutrast were determined using electron microprobe analysis. Whereas inhibition of transepithelial Na transport by ouabain (reversion of short circuit current) led to a pronounced K-Na exchange in granular, and most of the basal cells, surface CA-cells and some basal cells were ouabain insensitive. Surface CA-cells could be divided into a large Cl-rich and a small Cl-poor population. Since the ouabain-induced K-Na exchange could be completely prevented by blocking passive luminal Na entry by amiloride, granular and most of the basal cells seem to form a syncytial Na transport compartment. Luminal uptake of Br only occurred in Cl-poor surface CA-cells, indicating the sole responsibility of these cells for electrogenic and electroneutral Cl absorption and HCO_3 secretion.

Serosal Br was taken up into all cell types. Whereas H secretion and serosal Br uptake into all cell types could be inhibited by 4-isothiocyano-4'-acetamido-2,2'-disulfonic stilbene (SITS), blockade of H secretion by lowering luminal pH to 4.5 diminished Br uptake only in Cl-rich surface CA-cells. Theses results indicate: a) Only Cl-rich surface CA-cells have a serosal anion exchanger involved in H secretion and b) granular and basal cells also possess a serosal anion exchanger, possibly responsible for cellular pH regulation.

Luminal endocytosis of the I-containing Solutrast was observed in apical regions of Cl-rich surface CA-cells after inhibition of H secretion, but not under steady-state conditions, indicating a transport related but not a constitutive endo-exocytosis.

KEY WORDS: Electron microprobe analysis; cellular electrolyte concentrations; 4-isothiocyano-4'-acetamido-2,2'-disulfonic stilbene (SITS); acetazolamide; Solutrast; Na-, Cl-, H- and HCO_3-transport; cellular Br uptake; endo-exocytosis; fluid phase markers.

*Address for correspondence:
A. Dörge
University of Munich, Institute of Physiology
Pettenkoferstr. 12
8000 Munich 2, Germany Phone No.: 089-5996-504

Introduction

Like the renal collecting tubule, the epithelium of turtle urinary bladder consists of different cell types with different transport functions. According to morphological and histochemical studies, the two-layered turtle bladder epithelium contains granular and carbonic anhydrase rich (CA) cells in the luminal layer and basal cells in the second layer (Rosen 1970a, 1970b; Husted et al. 1981; Schwartz et al. 1982). In analogy to other tight, Na-transporting epithelia, it has been supposed that granular cells are responsible for the amiloride sensitive Na absorption (Schwartz et al. 1982; Durham and Nagel 1986). Since H and HCO_3 secretion and Cl absorption are independent of Na absorption (Husted and Steinmetz 1979; Satake et al. 1983; Solinger et al. 1968; Steinmetz et al. 1967) and can be blocked by carbonic anhydrase inhibitors (Steinmetz 1969; Schwartz et al. 1972; Leslie et al. 1973; Satake et al. 1983), it is likely that CA-cells mediate these transepithelial transports. Ultrastructural characterization of CA-cells has provided evidence that an α-type might be responsible for H, and a β-type for HCO_3, secretion (Stetson and Steinmetz 1985). In both transport routes, a Cl/HCO_3 exchanger seems to be involved. Whereas in the α-CA-cells an anion exchanger is located in the serosal membrane to eliminate HCO_3 generated by the H secretion process, in the β-cells a luminal anion exchanger seems to be the decisive step for HCO_3 secretion and Cl absorption (Steinmetz 1986; Husted und Fischer 1987; Stetson et al. 1985; Kohn et al. 1990).

Studies have shown stimulation of H secretion to be accompanied by exocytosis of vesicles located in the apical region of α-CA-cells (Gluck et al. 1982; Stetson and Steinmetz 1983, 1986) and, conversely, inhibition of this transport by endocytosis of luminal membrane vesicles (Husted et al. 1981; Dixon et al. 1986, 1988). This implies that variation in the number of H pump units in the α-CA-cell luminal membrane is an important mechanism for regulating H secretion.

In order to identify the different transport compartments in turtle urinary bladder by electron microprobe analysis, cellular elemental concentrations were determined under various experimental conditions. To elucidate the Na transport pathway by the induction of characteristic changes of the electrolyte composition in the Na transport compartment,

Na absorption was blocked by the diuretic amiloride and by the glycoside ouabain. Determination of cellular Br uptake (as an index for anion exchange) was used to identify the cells responsible for H and HCO_3 secretion. To obtain an insight into the endo-exocytotic processes involved in the regulation of H secretion, the uptake of the I-containing contrast medium "Solutrast" was studied. Whereas most of the results regarding the Na, H and HCO_3 transport compartments have already been published (Buchinger et al. 1989; Fraunberger et al. 1992), the present study also reports new findings related to cellular Cl absorption and regulation of H secretion.

Materials and Methods

Experiments were performed on isolated urinary bladders obtained from adult fresh water turtles *(Pseudemys scripta elegans)*. Prior to use, the animals were fed twice a week with rabbit liver or a commercial amphibian/reptile food (Tetra Repto Min, Tetra Werke, Melle, FRG) and kept in tap water at room temperature. Food was withheld for the 7-10 days prior to the experiment, so that the turtles can be regarded as "postabsorptive". After decapitation, the plastron and the peritoneum were removed and the bladder dissected. The isolated bladder was cut into several pieces which were then mounted on lucite rings and inserted in modified Ussing-type chambers (exposed area 0.75 cm^2). In the chamber, the tissue piece was positioned between O-rings covered with silicon grease to minimize edge damage. During chamber incubation both sides of the bladder were perfused continuously with solutions and the short-circuit current (SCC) was measured using an automatic voltage clamping device (Frankenberger, Germering, FRG). After stabilization of the SCC, pieces from the same urinary bladder were subjected to various treatments. In each experimental group, one bladder piece remained untreated and served as control.

To localize the Na transport compartment, the transepithelial Na transport was inhibited by serosal ouabain or successive applications of amiloride and ouabain. To discriminate between the H secreting and HCO_3 and Cl absorbing cells the uptake of Br from either the luminal or serosal side was determined. Luminal Br-Ringer was applied under control conditions and after successive application of serosal ouabain and Cl-free Ringer to both sides. In all cases in which serosal Br-Ringer was used, the transepithelial Na transport was blocked by serosal ouabain. Measurements of cellular elemental concentrations were performed after ouabain alone (normal H secretion) and when H secretion was blocked by inhibition of the serosal anion exchanger with the stilbene derivative SITS or by decreasing the luminal pH to 4.5 with citrate-buffered Ringer. To assess endocytotic processes involved in the regulation of H secretion, the luminal uptake of I, in the form of the contrast medium Solutrast, was measured. In all cases ouabain was applied to block transepithelial Na transport. Luminal Solutrast solution was applied under steady-state conditions of H secretion with either normal or CO_2-free Ringer on the serosal side and during inhibition of H secretion by serosal SITS or acetazolamide (ACZL) or by exchanging the serosal normal Ringer with CO_2-free Ringer.

The composition of the normal Ringer was (in mM): 80 NaCl, 20 $NaHCO_3$, 4 KCl, 2 $CaCl_2$, 0.8 $MgCl_2$, 0.53 Na_2HPO_4, 0.21 NaH_2PO_4 and 11 glucose. In the Br-Ringer, all Cl was replaced by Br on an equimolar basis. The citrate-Ringer contained (in mM): 80 NaCl, 4 KCl, 2 $CaCl_2$, 1 Na_2HPO_4, 13 citric acid, 7 Na citrate and 5 glucose. All these solutions were gassed with 95% O_2 and 5% CO_2 and had a pH of 7.38 (normal and Br-Ringer) and 4.5 (citrate-Ringer). CO_2-free Ringer had the same composition as the normal Ringer, except that $NaHCO_3$ was replaced by NaCl. This solution was bubbled with synthetic air (80% N_2, 20% O_2) which had been passed through KOH to remove CO_2. The Solutrast solution contained (in mM): 216 iopamidol, 10 NaCl and 4 imidazol; the sucrose solution 216 sucrose, 10 NaCl and 4 imidazol. The I concentration of the Solutrast solution was about 650 mM. The perfusion solutions used to study endocytosis were maintained at 25°C; all other solutions were used at room temperature. Amiloride, ouabain and SITS were applied at a concentration of 10^{-4}M and ACZL at a concentration of $5x10^{-4}$M. These drugs were obtained from Sigma (Heidelberg, FRG). Solutrast was a gift from Byk Gulden (Konstanz, FRG).

At the end of incubation, the bladder pieces were quickly removed from the chambers, and after covering the luminal side with a thin layer of an albumin standard solution, snap frozen in a propane/isopentane mixture cooled to the temperature of liquid N_2 (Jehl et al. 1981). Less than 10 s elapsed between removal of the tissue from the chamber and immersion in the freezing mixture. The standard solution consisted of 1 g albumin dissolved in 4 ml of the same solution used to bathe the luminal side. From the frozen tissue, 1 μm-thick sections were cut in a modified Reichert cryomicrotome (OmU 3, Vienna, Austria) at -90°C. After sandwiching the sections between two collodion films, they were freeze-dried at -80°C and 10^{-6} mbar.

The analysis of the freeze-dried cryosections was performed in a scanning electron microscope (S150, Cambridge Instruments, Cambridge, UK) with an energy dispersive X-ray detector (LINK Systems, High Wycombe, UK). The acceleration voltage was 20 kV and probe current 0.3 nA. Small areas were scanned within the albumin standard layer and the cells for 100 s and the emitted X-rays were analysed between 0.5 and 5 keV. This energy range encompasses the K_α-lines of Na, Mg, P, Cl, K and Ca, and the L_α-lines of Br and I. Discrimination between the element characteristic X-ray peaks and the background radiation was performed by a computer program (Bauer and Rick 1978). Cellular element concentrations in mmol/kg wet weight were obtained by comparing the element characteristic radiations obtained in the cells with those of the albumin standard. The cellular dry weight contents in g/100g were estimated by comparing the background radiation of cell and standard spectra. More detailed descriptions of tissue preparation, electron microprobe analysis, and quantification procedure may be found elsewhere (Dörge et al. 1978; Rick et al. 1982). Intracellular concentrations and SCC are given as means ± SD. In figures means ± 2 SEM are shown. Student's t-test was used to assess

the significance of differences in means. A p value of less than 0.05 was regarded as statistically significant.

Results and Discussion

Identification of different cell types

Morphological and histochemical studies have shown that the two-layered epithelium of turtle urinary bladder comprises 3 main cell types, granular and CA-cells in the surface layer and basal cells in the serosal layer (Rosen 1970a, 1970b). Although freeze-dried cryosections of 1 μm-thickness do not yield detailed information on cellular ultrastructure, these main cell types can be identified. Figure 1 shows a scanning transmission electron micrograph of such a freeze-dried cryosection of turtle urinary bladder.

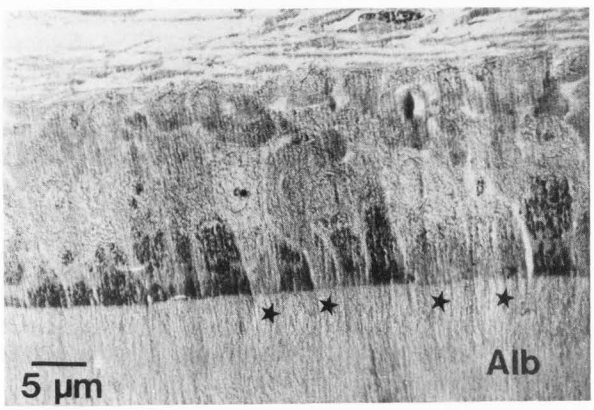


Figure 1. Scanning transmission electron micrograph of a freeze-dried cryosection of turtle urinary bladder. Alb: Albumin layer on surface; Asterisks: surface CA-cells.

Most of the surface cells contain large dense particles in their apical region. Electron microprobe analysis (see below) revealed that the electron dense particles represent mucin granules. Some surface cells (marked by asterisks) do not contain these dense particles. Under control conditions and after exchanging normal luminal Ringer with Br-Ringer morphological criteria alone were used to discriminate between the different epithelial cell types. Surface cells with large electron dense granules were regarded as granular cells; those without as CA-cells. Cells in the serosal layer were assumed to be basal cells. In all other cases in which serosal ouabain was applied to block transepithelial Na transport, the ouabain insensitivity of CA-cells (see below) was also used to distinguish between the different cell types.

Localization of Na transport compartment

Since amiloride is known to block the passive entry of Na at the luminal side and ouabain active Na exit at the serosal side of the turtle urinary bladder (Solinger et al. 1968; Wilczewski and Brodsky 1975; Husted and Steinmetz 1979; Nagel et al. 1981), these substances were used to provoke Na transport dependent electrolyte concentration changes in the Na transport compartment.

Figure 2 illustrates the experimental protocol by means of typical time courses for the SCC. Both ouabain and amiloride decrease the SCC to negative values. In the postabsorptive state, the decrease in SCC is a measure of Na absorption; the negative SCC corresponds to H secretion (Schwartz 1976). When ouabain was applied in addition to amiloride, it had no further significant effect on the reversed SCC. The SCC in 14 experiments decreased from 26.5 ± 17.7 to -3.9 ± 2.9 after ouabain and from 25.4 ± 17.2 to -8.0 ± 5.1 $\mu A/cm^2$ after successive application of amiloride and ouabain. Figure 3 shows the major results obtained by electron microprobe analysis of electrolyte concentrations in the different epithelial cell types under these experimental conditions. The data given for basal cells after ouabain only comprise ouabain sensitive cells (see below). Under control conditions the Na, K and Cl concentrations do not vary substantially between the three cells types. The data shown for granular cells were obtained from the basal part of the cell. Measurements performed below the apical membrane where the granules are localized, revealed somewhat lower K (70 versus 110 mmol/kg w.w.) and much higher Ca concentrations (14 versus 0.7 mmol/kg w.w.) under control conditions. Similar electrolyte patterns have been obtained for other mucin granules containing epithelial cells such as the goblet cells, in toad urinary bladder (Rick et al. 1978a).

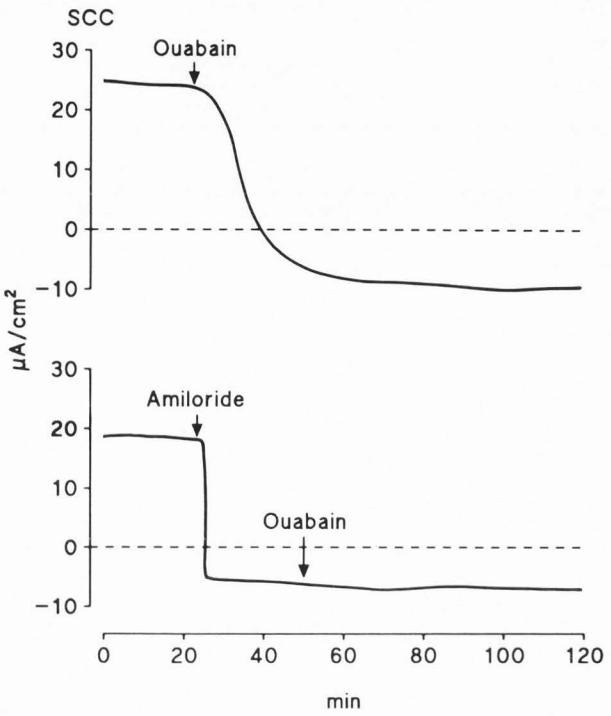


Figure 2. Time courses of short-circuit current (SCC) after the application of serosal ouabain (10^{-4}M) and the successive application of luminal amilorde (10^{-4}M) and serosal ouabain.

In contrast to granular and basal cells, large variations in the Cl concentrations of surface CA-cells were found under

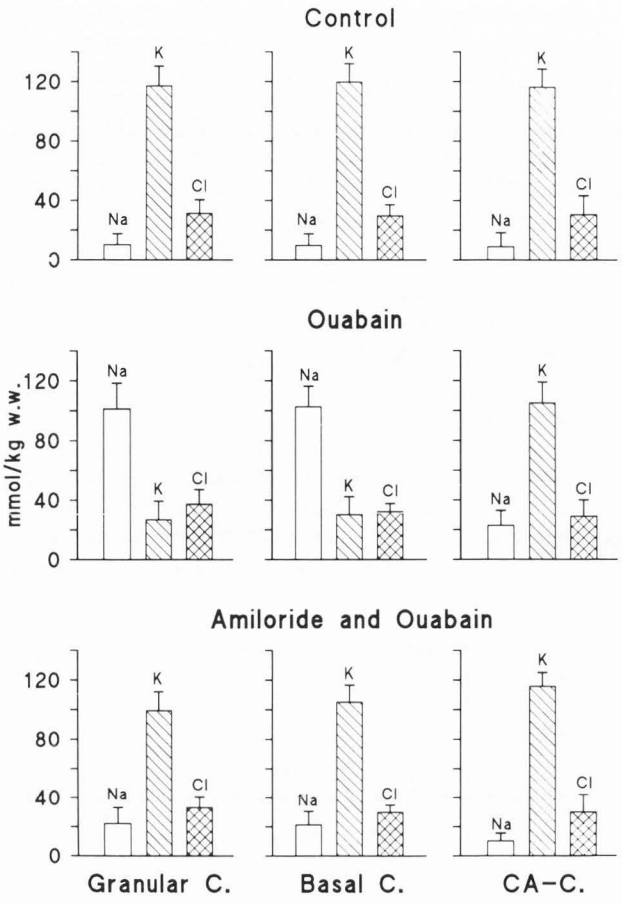


Figure 3. Electrolyte concentrations of granular, basal, and surface CA-cells under control conditions, after serosal ouabain (10^{-4}M) and after the successive application of luminal amiloride (10^{-4}M) and serosal ouabain (adapted from Buchinger et al. 1989 and Fraunberger et al. 1992).

all experimental conditions. Whereas about 30% of surface CA-cells had a low Cl concentration of about 13 mmol/kg w.w., the rest exhibited a high Cl concentration of 30 mmol/kg w.w. The division of surface CA-cells into a Cl-poor and a Cl-rich population seems to be justified since only very few cells had a Cl concentration between 15 and 20 mmol/kg w.w.

After serosal application of ouabain, the Na concentration in granular and most of the basal cells increased from about 10 to 100 mmol/kg w.w. whereas the K concentration decreased by about the same amount from about 115 to 25 mmol/kg w.w. The Cl concentration in granular cells increased by about 10 and in most of the basal cells by about 5 mmol/kg w.w. This increase in Cl concentration was accompanied by a decrease in the cellular dry weight content of about 2 g/100g w.w. It might therefore be concluded that ouabain not only causes exchange of K for Na, but also cellular influx of NaCl which then leads to cell swelling. Compared with granular and most of the basal cells, the Na and K concentrations of surface CA-cells were not much affected by ouabain. Only small increases in Na and decreases in K concentrations were found after 90 min of ouabain application. However, ouabain insensitivity was not only found in morphologically identified surface CA-cells, but also in some basal cells (about 20% of all ouabain insensitive cells) without visible contact to the luminal surface. The idea that these cells are precursors of surface CA-cells is also supported by recently published morphological and histochemical findings (Fritsche et al. 1991a, 1991b). The reason for the ouabain insensitivity might be due to a low membrane Na and/or K permeability of CA-cells. If this were so, only a relatively low Na-K pump activity would be required to maintain the Na and K concentration gradients between extra- and intracellular spaces. The nearly unaltered electrolyte composition of CA-cells after ouabain infers that the cellular electrical potentials are also not much altered. This would account for the lack of effect of ouabain on the voltage dependent H secretion process (Al-Awqati et al. 1977).

To establish whether the ouabain-induced Na accumulation is mainly due to a cellular influx of Na from the luminal or the serosal side, the Na influx from the luminal side was inhibited by amiloride. From transepithelial potential measurements it was deduced that amiloride retarded cellular Na increase caused by ouabain (Wilczewski and Brodsky 1975). As obvious from Fig. 3 amiloride almost completely prevented the ouabain-induced changes in the electrolyte composition in all cell types. Accordingly, all epithelial cells of the turtle urinary bladder exhibited the typical properties of a Na transport compartment. However, CA-cells seem not to contribute substantially to Na absorption. The cellular Na influx calculated from the small Na increase after ouabain was only about 0.5% of the total transepithelial Na transport as measured by the SCC. The observation that both granular

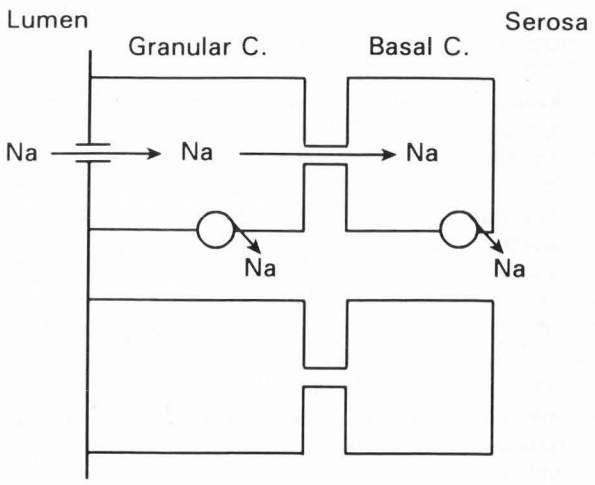


Figure 4. Proposed pathway of transepithelial Na transport.

and basal cells exchange Na mainly with the luminal bathing solution is consistent with the idea of a syncytial Na transport compartment as shown in Figure 4. Na enters the epithelium passively across the apical membranes of granular cells, diffuses through intercellular bridges into the basal cells and

is then actively extruded by Na-K pumps into the interstitial spaces. With respect to the Na transport compartment, similar results have been obtained for other multilayered Na transporting epithelia such as frog skin and toad and rabbit urinary bladder (Rick et al. 1978a, 1978b; Dörge et al. 1988).

Identification of H and HCO_3 secreting and Cl absorbing cells

The 3:1 ratio of Cl-rich to Cl-poor ouabain insensitive surface CA-cells was similar to that obtained for the morphologically characterized α- and β-CA-cells. Based on this agreement, we suggested that Cl-rich surface CA-cells are involved in H secretion and that Cl-poor surface CA-cells are responsible for HCO_3 secretion and Cl absorption. To test this hypothesis, the uptake of Br into the different epithelial cell types from either the luminal or serosal side was determined. The rationale for this is the observation that Br is accepted instead of Cl by both the serosal and luminal anion exchangers (Husted and Fischer 1987; Kohn et al. 1990) thought to be involved in H and HCO_3 secretion, respectively. Since under control conditions (normal Ringer on both sides) HCO_3 secretion in bladders of postabsorptive turtles is equal to Cl absorption (Leslie et al. 1973), the luminal anion exchanger seems also to be involved in Cl absorption. However, in contrast to this electroneutral Cl absorption an electrogenic Cl transport could be provoked, when the serosal bathing solution was kept Cl-free (Durham and Matons 1984). In order to localize the Cl transport compartment of the electrogenic Cl absorption, Br-Ringer was applied to the luminal side also under conditions similar to those used by Durham and Matons (1984).

The exchange of normal for Br-Ringer on the luminal side had no significant effect upon the SCC. Moreover, luminal Br-Ringer neither influenced the electrolyte concentrations of granular and basal cells nor was Br detectable in these cells, even after 90 min of exposure. Assuming that Br is similarly transported as Cl, this infers that the luminal membrane of granular cells is impermeable to Cl. Similar anion impermeability has been demonstrated for other Na transporting epithelia such as frog and toad skin (Dörge et al. 1989).

Figure 5 demonstrates the luminal uptake of Br into individual surface CA-cells. In this Figure, the Br concentrations found in these cells are shown as a function of the sums of Cl and Br concentrations. Whereas substantial amounts of Br were taken up into surface CA-cells with low anion concentration (sum of Cl and Br concentrations) almost no Br was detectable in Cl-rich surface CA-cells. Since it appears that only Cl-poor surface CA-cells possess a significant luminal anion permeability, they are probably the sole transport pathway for Cl absorption and HCO_3 secretion.

The experimental protocol employed to evoke an electrogenic Cl or Br absorption is demonstrated by the time course of SCC shown in Figure 6. After blocking Na absorption by ouabain (SCC negative) and H secretion by incubating both sides of the epithelium with Cl-free Ringer (SCC practically zero), Cl- or Br-Ringer was applied to the luminal side. The negative SCC generated under these conditions is, according to Durham and Matons (1984), a measure of

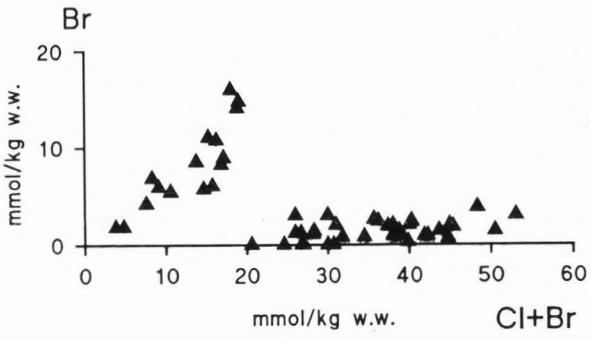


Figure 5. Relationship of Br concentrations to the sums of Cl and Br concentrations of surface CA-cells after 60 min of luminal incubation with Br-Ringer (adapted from Fraunberger et al. 1992).

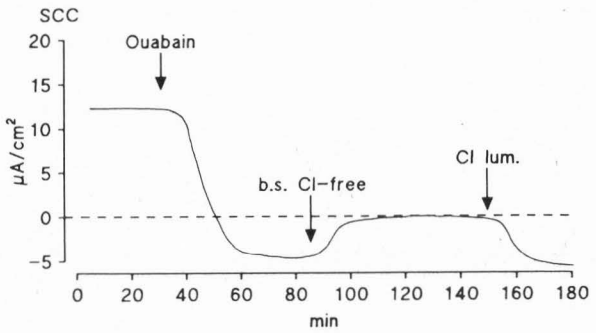


Figure 6. Time course of short circuit current (SCC) after application of serosal ouabain (10^{-4}M) and the successive application of Cl-free Ringer to both sides and Cl-containing Ringer to the luminal side (adapted from Fraunberger et al. 1992).

an electrogenic active anion absorption, and was of similar magnitude whether carried by Cl or Br. The mean values obtained from 5 experiments were 7.1 ± 3.5 after luminal Cl and 6.0 ± 1.9 $\mu A/cm^2$ after luminal Br application. This infers that Br is as well accepted as Cl by the anion transport pathway. The changes in Na and K concentrations in the different cell types after ouabain and incubation of both sides with Cl-free Ringer were similar to those after ouabain alone. The Cl concentration decreased in granular cells from 25.3 ± 4.8 to 6.3 ± 3.6, in basal cells from 26.0 ± 3.8 to 2.8 ± 0.5 and in surface CA-cells from 25.7 ± 9.3 to 4.5 ± 2.2 mmol/kg w.w. During the subsequent period in which either Cl- or Br-Ringer was applied to the luminal side, granular and basal cells exhibited a further small decrease in Cl concentrations of some 1 to 2 mmol/kg w.w. As under control conditions, the application of luminal Br-Ringer did not result in any Br uptake into granular and basal cells. Table 1 shows the Cl and Br concentrations of CA-cells after incubating both sides with Cl-free Ringer and after applying Cl- or Br-Ringer to the luminal side. Under the latter conditions, CA-cells can be subdivided into two populations, one without

Table 1. Cl and Br concentrations in surface CA-cells of turtle urinary bladder after incubating both sides with Cl-free Ringer and after the successive application of Cl- or Br-Ringer to the luminal side.

		Cl	Br	n
		mmol/kg w.w.		
Cl-free		4.5 ± 2.2	---	30
Cl luminal	w.o.	3.9 ± 1.3	---	24
	w.	11.0 ± 2.1*	---	8
Br luminal	w.o.	2.7 ± 1.7	0.5 ± 0.5	30
	w.	3.5 ± 2.6	5.6 ± 2.4*	12

Mean values ± SD; * $p < 0.002$, significantly different from values after Cl-free incubation.
w. and w.o. indicates data from cells with or without Cl concentration increase or Br uptake.

any increase in Cl concentration or uptake of Br (w.o.); the other with an increase in Cl concentration or substantial Br uptake (w.). Whereas the Cl concentration in the majority of the surface CA-cells decreased further and no Br was taken up after applying Cl- or Br-Ringer to the luminal side, in about 1/4 of the cells Cl concentration increased and substantial amounts of Br were taken up. These results are consistent with the view that electrogenic Cl and Br absorption is accomplished by only about 1/4 of the CA-cells. The present experiments do not permit a clear answer as to whether these cells are the same as those responsible for anion transport under control conditions. However, the good agreement between the percentage distributions makes it very likely that the electrogenic anion absorption is also accomplished by the Cl-poor surface CA-cells. The present experiments do not provide informations regarding the basic transport mechanisms involved in Cl absorption. Therefore, the data are inappropriate to decide which of the transport models developed so far (Durham and Brodsky 1984; Stetson and Steinmetz 1985) is valid. It should be mentioned, however, that the theoretical model proposed by Durham and Brodsky (1984), which includes luminal Cl and HCO_3 pumps, would explain both, electroneutral and electrogenic Cl absorption.

Figure 7 demonstrates the experimental protocols used to determine cellular Br uptake from the serosal side. Transepithelial Na transport was first blocked by ouabain to obtain a measure of H secretion from the negative SCC and to yield a further criterion for the identification of CA-cells by their ouabain insensitivity. Exchanging the normal Ringer with a Br-Ringer on the serosal side had no significant effect upon the reversed SCC. Blocking the serosal anion exchanger by SITS or decreasing the luminal pH to 4.5 by

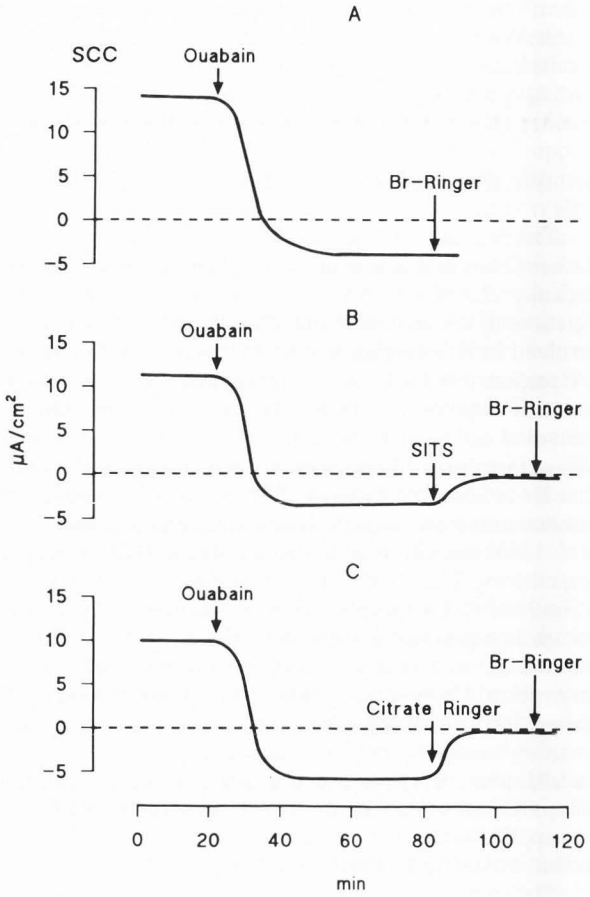


Figure 7. Time courses of the short-circuit current (SCC) after serosal application of ouabain (10^{-4}M) and Br-Ringer (A), after serosal application of ouabain, SITS (10^{-4}M) and Br-Ringer (B) and after the application of serosal ouabain, luminal citrate-Ringer (pH 4.5) and serosal Br-Ringer (C) (adapted from Fraunberger et al. 1992).

citrate-Ringer decreased the negative SCC (as a measure of H secretion) practically to zero. Br application lasted in all cases 7 min and was concluded by the shock-freezing of the tissues. In the mean of 5 experiments the SCC was -2.8 ± 1.3 after ouabain, 0.2 ± 1.6 after SITS, and -0.1 ± 0.3 $\mu A/cm^2$ after luminal citrate-Ringer.

Under all three experimental conditions, the Na and K concentrations of granular and basal cells were similarly altered as after ouabain alone. The Cl and Br concentrations found in these cells are listed in Table 2. Under normal conditions of H secretion (after ouabain), granular and basal cells take up Br to a concentration of some 12 mmol/kg w.w. Assuming that this Br replaces equimolar amounts of Cl, more than 30% of cellular Cl was exchanged by Br. This exchange was reduced by SITS to less than 10%, whereas luminal citrate-Ringer had no effect. Figure 8 demonstrates serosal Br uptake into individual CA-cells. Br concentrations are again shown as function of the sums of the Cl and Br concentrations. The filled symbols represent data from surface

Table 2. Cl and Br concentrations of granular (G.C.) and basal cells (B.C.) of turtle urinary bladder after 7 min application of Br-Ringer to the serosal side. The bladders were pretreated with serosal ouabain (normal H secretion), serosal ouabain and SITS, and serosal ouabain and luminal Citrate-Ringer.

	n	Cl	Br	Σ(Cl+Br)
		mmol/kg w.w.		
Serosal Ouabain				
G.C.	102	25.2 ± 6.6	11.9 ± 5.9	37.1 ± 8.3
B.C.	25	22.6 ± 5.4	12.7 ± 4.7	35.3 ± 8.3
Serosal Ouabain, serosal SITS				
G.C.	117	35.0 ± 5.4*	2.8 ± 3.3*	37.8 ± 6.2
B.C.	25	31.9 ± 8.2*	2.7 ± 3.0*	34.6 ± 6.6
Serosal Ouabain, luminal Citrate-Ringer				
G.C.	44	22.3 ± 3.1	13.4 ± 3.3	35.7 ± 4.2
B.C.	16	24.8 ± 3.4	13.0 ± 2.3	37.8 ± 2.3

Mean values ± SD; * $p < 0.001$, significantly different from values after ouabain.

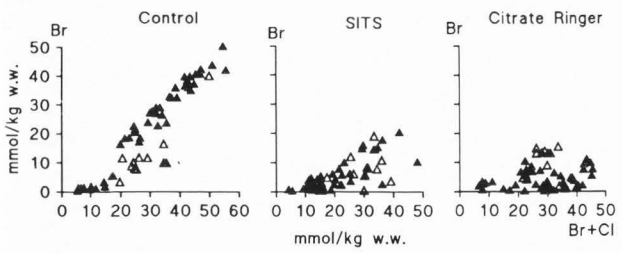


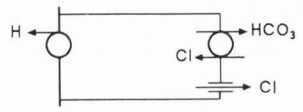
Figure 8. Relationship of Br concentrations to the sums of Cl and Br concentrations of CA-cells after 7 min incubation of the serosal side with Br-Ringer. Br-Ringer was applied after serosal ouabain (control), after serosal ouabain and SITS (10^{-4}M) and after serosal ouabain and luminal citrate-Ringer (pH 4.5). Solid and open triangles represent data from CA-cells with and without contact to the luminal surface, respectively (adapted from Fraunberger et al. 1992).

CA-cells, the open symbols those from ouabain insensitive cells located in the basal layer. Under normal conditions of H secretion, surface CA-cells seem to comprise two distinct subpopulations, a larger one with high anion concentration (sum of Cl and Br concentrations) and high Cl/Br exchange and a smaller one with low anion concentration and low Cl/Br exchange. About 20% of the ouabain insensitive cells had no visible contact to the luminal surface and exhibited an intermediate Cl/Br exchange. Serosal SITS and luminal citrate-Ringer drastically reduced the Br uptake into CA-cells with high anion concentration.

Under normal conditions of H secretion and after luminal citrate-Ringer, the mean anion concentration of all CA-cells was about 30 mmol/kg w.w. and thus not significantly different from the Cl concentration under control conditions. SITS, on the other hand, reduced this concentration to 22.4 mmol/kg w.w.

In order to assess which of the cells in contact with the luminal surface are involved in H secretion the measured Cl/Br exchanges were compared with those expected on the basis of compartmental analysis. The data so obtained are shown in Figure 9.

$$Br_c/Cl_c = 1\text{-}e^{-\frac{RSCC \cdot t}{Cl_c \cdot V_c}}$$



	V_c µl/cm²	Cl_c mmol/kg w.w.	Br_c/Cl_c meas.	pred.
Gran. + Basal C.	1.6	36	0.33	0.31
Cl-rich Surface-CA-C.	0.3	36	0.83	0.86
Cl-poor Surface-CA-C.	0.1	10	0.14	1.00

Figure 9. Proposed cellular model of H secretion and the exchanges of Cl with Br in the different cell types (Br_c/Cl_c) as measured (meas.) and predicted (pred.) from electron microprobe and compartment analysis, respectively. V_c and Cl_c, cell volumes and Cl concentration under control conditions.

In the equation used to estimate the Cl/Br exchange, the reversed SCC, RSCC, is a measure of H secretion (3 µA/cm²), t is the time of Br exposure (7 min), Cl_c the cellular Cl concentration under control conditions and V_c the cellular volume. The volumes of granular and basal cells and that of surface CA-cells were estimated by inspection of the electron micrographs. The calculation of Cl/Br exchange based on the assumption that for each H secreted, one HCO_3 is exchanged by the serosal anion exchanger for one Cl.

The relatively small apparent Cl/Br exchange (14%) measured in the Cl-poor surface CA-cells compared with the predicted value of 100% argues against a substantial contribution of these cells to H secretion. As already demonstrated by the luminal Br uptake measurements, Cl-poor surface CA-cells seem to be responsible for HCO_3 secretion and Cl absorption. Consideration of the serosal Cl/Br exchange rates alone implies that both granular and basal cells and the Cl-rich surface CA-cells could be responsible for H secretion. Indeed, for granular and basal cells the

measured Cl/Br exchange was even greater than predicted. However, the observation that inhibition of H secretion by lowering luminal pH to 4.5 had no effect upon the Br uptake into granular and basal cells indicates that these cells are not involved in H secretion. This interpretation is also in good agreement with conclusions derived from equivalent circuit analysis based on cellular potential measurements in granular cells (Durham and Nagel 1986). The finding that Br uptake by these cells was inhibited by SITS might be construed as evidence for granular and basal cells also being equipped with a serosal anion exchanger possibly involved in homocellular pH regulation. For the Cl-rich surface CA-cells, the predicted and the measured Cl/Br exchanges are the same and the exchange was reduced both by serosal SITS or lowering the luminal pH to 4.5. These results show that these cells alone are probably responsible for H secretion. The good agreement between the measured and calculated Cl/Br exchanges makes it also very likely that the serosal anion exchanger transports electroneutrally and that no other transport pathway is needed for the serosal exit of HCO_3 (Fischer et al. 1983; Durham and Matons 1984).

The 8 mmol/kg w.w. decreased anion concentration in CA-cells after SITS might be due to an increased HCO_3 concentration after blockade of the serosal anion exchanger. This would agree with the increase in cellular pH measured after the application of SITS (Cohen et al. 1978). However, the hypothesis that SITS inhibition of H secretion results from a cellular pH increase of about 0.8 pH unit (Cohen et al. 1978) is not supported by the present finding. Under the incubation conditions employed in the present study, such a cellular pH increase would necessitate an increase in HCO_3 concentration of more than 100 mM. This would lead to a drastic reduction in cellular Cl concentration and/or cell swelling. However, beside the relatively small decrease in anion concentration the dry weight content of the tissue (22.0 ± 5.8 before and 23.3 ± 5.7 g/100g w.w. after SITS) does not indicate cell swelling. According to recent results inhibition of H secretion by SITS and ACZL is accompanied by a retrieval of H pumps (see below). It is interesting to note that at least the inhibitory effect of ACZL is independent of cell pH (Graber et al. 1989).

Regulation of H secretion by endo- and exocytosis

To characterize the acid and base secreting cells further, endocytosis of a luminal fluid phase marker was studied under control conditions and after inhibition of H secretion. This experimental approach is based on the observation that the insertion or recovery of vesicles containing H pumps into or out of the luminal membrane of CA-cells seems to be an important mechanism for H secretion.

Luminal surface area of CA-cells is markedly reduced after inhibition of H secretion by SITS or AZCL (Husted et al. 1981) and drastically enhanced when H transport is stimulated by the addition of CO_2. Fluorescence microscopy (Gluck et al. 1982) and morphometric measurements (Stetson and Steinmetz 1983, 1986) revealed that CO_2 induced stimulation of H secretion is accompanied by an exocytotic insertion of H pump containing cytoplasmic vesicle membranes into the luminal membrane of CA-cells. Furthermore, estimation of luminal membrane area by impedance analysis, together with uptake measurements of a fluid phase marker, indicate that inhibition of H secretion by the carbonic anhydrase inhibitor ACZL is associated by endocytosis of membrane vesicles derived from luminal membrane of CA-cells (Dixon et al. 1986, 1988). These latter studies also provide evidence for two distinct endocytotic pathways, a constitutive one involved in membrane shuttling and another related to H transport.

In the present study, Solutrast - a non ionized, I-containing radiographic contrast medium with a molecular weight of 777 - was chosen as a fluid phase marker. An isotonic Solutrast solution with an I concentration of about 650 mM was applied to the luminal side and Solutrast uptake by the different epithelial cell types was determined by measuring cellular I concentrations. In surface CA-cells, measurements were performed in both apical and basal regions of the cell. In each case, the scanning area comprised about half of the cell.

Typical time courses of the SCC as shown in Figure 10 illustrate the experimental protocols used to study the endocytotic process. In the examples shown in the first three panels, the perfusion solutions contained CO_2 throughout. Inhibition of transepithelial Na transport by serosal ouabain reversed SCC within 40 min to negative values (H secretion). Solutrast solution on the luminal side decreased the reversed SCC transiently. The new steady state of reversed SCC, however, was in all cases some 20% less than before Solutrast. Subsequent inhibition of H secretion by serosal ACZL, SITS or CO_2-free incubation resulted in a decrease of the reversed SCC to practically zero. In an additional set of experiments in which the incubation media were CO_2-free throughout, Solutrast solution caused similar inhibition of of the reversed SCC. After inhibtion of H secretion luminal incubation with Solutrast solution lasted usually 90 min. However, in one case, in which H secretion was blocked by CO_2-free incubation this incubation time was only 30 min. With CO_2 in the incubation medium the mean SCC decreased after luminal application of Solutrast solution from -5.2 ± 1.2 to a new steady state of -4.0 ± 0.9 $\mu A/cm^2$ and without CO_2 from -1.0 ± 1.3 to -0.6 ± 0.5 $\mu A/cm^2$. Inhibition of H secretion by serosal SITS, ACZL or CO_2-free incubation reduced SCC to 0.0 ± 0.1, 0.0 ± 0.2 and 0.4 ± 1.3 $\mu A/cm^2$, respectively.

After the application of serosal ouabain and luminal Solutrast the cellular Na and K concentrations of the different epithelial cell types were altered in the same manner as already described for ouabain alone. Solutrast does not appear to cause significant alteration of luminal membrane permeabilities. Under steady-state conditions of H secretion, regardless of whether the secretion rate was relatively high (CO_2 containing Ringer) or low (CO_2-free incubation), no Solutrast uptake could be detected in any cell type. Figure 11 shows typical results obtained in surface cells after inhibition of H secretion by ACZL. Whereas I reflecting the uptake of Solutrast was observed in Cl-rich CA-cells, no I was detectable in granular and Cl-poor CA-cells. Similar results were also obtained after blocking H secretion by CO_2-free incubation. Figure 12 shows typical results obtained in most of the surface

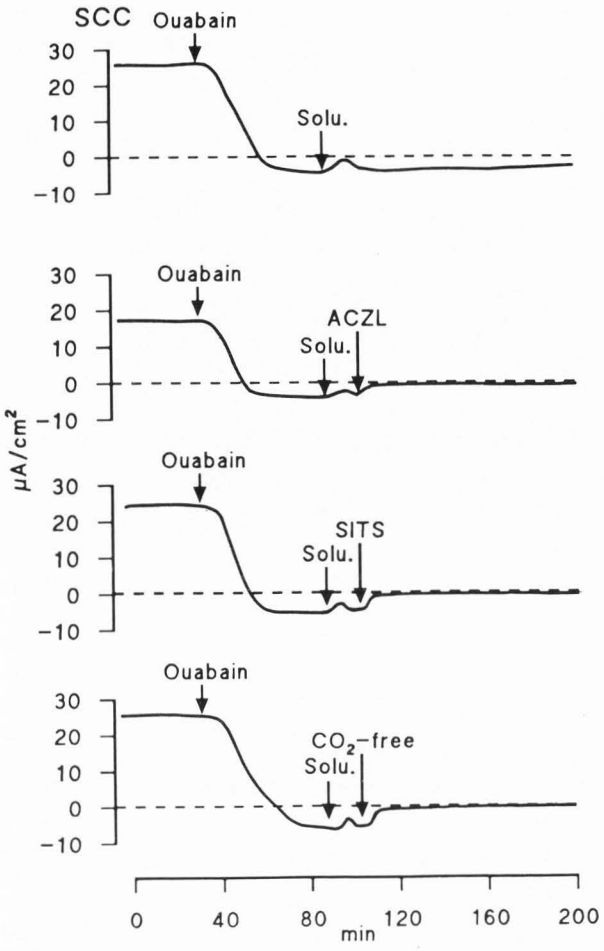


Figure 10. Times courses of short circuit current (SCC) after incubation of the luminal side with Solutrast (Solu.). Solutrast was applied after serosal ouabain (10^{-4}M), without (first panel) and before inhibition of H secretion by serosal ACZL ($5x10^{-4}$M), serosal SITS (10^{-4}M) and incubation of the epithelium with CO_2-free Ringer.

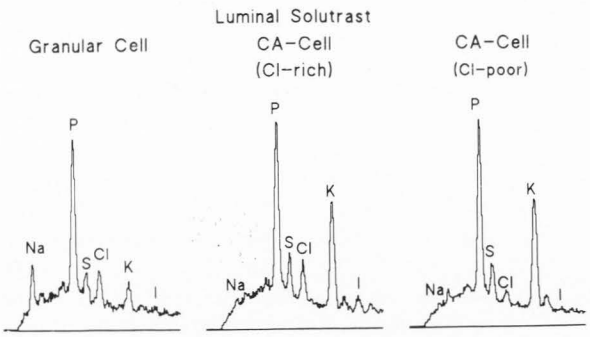


Figure 11. Energy dispersive X-ray spectra obtained in granular, Cl-rich and Cl-poor surface CA-cells after application of Solutrast to the luminal and ACZL ($5x10^{-4}$M) and ouabain (10^{-4}M) to the serosal side. Protocol of application see Figure 10.

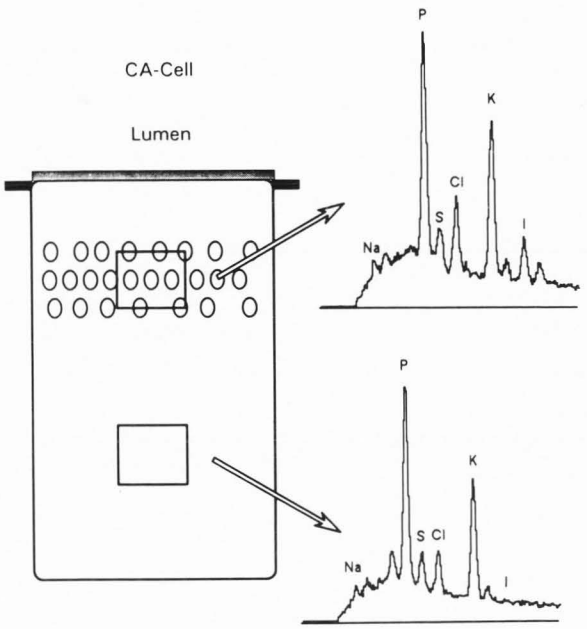


Figure 12. Energy dispersive X-ray spectra obtained in the apical and basal region of a Cl-rich surface CA-cell after application of Solutrast to the luminal and ACZL ($5x10^{-4}$M) and ouabain (10^{-4}M) to the serosal side. Protocol of application see Figure 10.

CA-cells after blocking H secretion. Whereas the spectrum obtained in the apical region exhibits a prominent I-Lα-peak, no I signal is present in the spectrum from the basal region of the cell.

Figure 13 demonstrates by cellular I the uptake of Solutrast into apical parts of surface CA-cells (filled symbols) and into CA-cells without visible access to the luminal surface (open symbols).

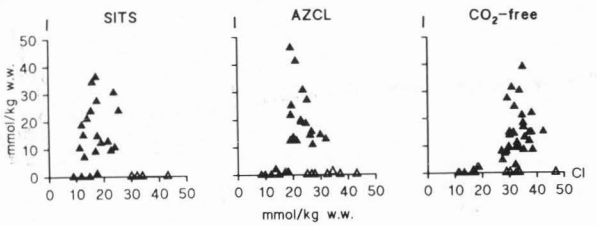


Figure 13. Relationship of I and Cl concentrations of CA-cells. The I containing contrast medium Solutrast was applied luminally after serosal ouabain and 10 min before inhibition of H secretion with serosal SITS (10^{-4}M), ACZL ($5x10^{-4}$M) or CO_2-free incubation. Solid and open triangles represent data from CA-cells with and without contact to the luminal surface.

Since the data obtained after inhibtion of H secretion by CO_2-free were the same, regardless whether Solutrast incubation lasted 30 or 90 min, they were pooled. Under all three conditions, the basally located CA-cells exhibit a

relatively high Cl concentration (35.5 ± 7.9 mmol/kg w.w., n = 16), but no substantial uptake of Solutrast (I concentration 0.1 ± 0.1 mmol/kg w.w., n = 15). In the apical region of surface CA-cells, in which variable amounts of I and therefore of Solutrast were taken up, Cl concentrations differed somewhat under the three experimental conditions: 28.8 ± 7.9 (n = 40) after CO_2-free incubation, 20.6 ± 5.9 (n = 24) after ACZL and 16.9 ± 4.5 mmol/kg w.w. (n = 27) after SITS. The Cl concentration in the basal regions of surface CA-cells showed a similar behavior: 25.6 ± 3.1 (n = 7) after CO_2-free incubation, 20.8 ± 2.7 (n = 10) after serosal ACZL and 13.1 ± 5.7 mmol/kg w.w. (n = 10) after serosal SITS. Since ACZL has been shown to inhibit the serosal anion exchanger directly in H secreting renal cortical collecting duct cells (Zeidel et al. 1986; Seifter et al. 1988) the chain of events already discussed for the action of SITS might lead to the decrease in Cl concentration after ACZL. At least under the action of CO_2-free incubation and ACZL, surface CA-cells seems to comprise two distinct cell populations, one with high Cl concentration and high Solutrast uptake (high I concentrations), the other with low Cl concentration and no Solutrast uptake (almost zero I concentrations). Although after SITS surface CA-cells with low Cl concentration and no Solutrast uptake were also found, no clear discrimination of two distinct cell populations could be made since all cells had relatively low Cl concentrations under this condition. The mean I concentration in Cl-rich surface CA-cells was 15.0 ± 8.1 after CO_2-free incubation and 20.4 ± 10.1 after ACZL. The ratio of Cl-rich to Cl-poor surface CA-cells was 3.4:1 after CO_2-free incubation, 2.4:1 after ACZL. From the observation that only Cl-rich surface CA-cells exhibit an endocytotic activity the same conclusion can be drawn as from the Br uptake measurements: Cl-rich surface CA-cells seem to be involved in H secretion and Cl-poor surface cells seem to be responsible for HCO_3 secretion and Cl absorption. Since there was no difference of Solutrast uptake into surface CA-cells after 30 and 90 min of CO_2-free incubation, it might be concluded that endocytosis is already complete after 30 min.

The following remarks can be made with respect to the validity of the above method for the assessment of endocytosis by the uptake of the contrast medium Solutrast. For the relatively small but significant decrease of the negative SCC after luminal Solutrast application no concise explanation is apparent. Since, however, a luminal sucrose solution reduced the negative SCC to about the same extent, Solutrast would appear not to have an specific effect on H secretion. From the lack of Solutrast uptake under steady-state conditions of H secretion, it might be concluded that neither the granular nor the CA-cells possess any endocytotic activity under these conditions. This finding conflicts with investigations using FITC-dextran, in which relatively large amounts of this fluid phase marker were found to be taken up from the luminal side into CA-cells (Dixon et al. 1986, 1988). Since FITC-dextran uptake was not accompanied by changes in the luminal membrane area, a constitutive endocytotic pathway involved in membrane shuttling between the luminal membrane of CA-cells and cytosolic vesicles has been proposed (Dixon et al. 1988). There is nothing to suggest that differences in physico-chemical parameters or an inadequate sensitivity for I detection could be responsible for these divergent results. Diffusional hindrances cannot explain the absence of Solutrast uptake since the molecular weight of Solutrast is much lower than that of FITC-dextran. The fact that endocytotic processes are temperature dependent (Dixon et al. 1988) was considered by keeping the temperature of the perfusion solution at 25°C. The uptake of FITC-dextran was about 0.14%/min of CA-cell volume and constant up to 45 min (Dixon et al. 1986). Uptake of Solutrast at a similar rate would have yielded a readily detectable I concentration of more than 40 mmol/kg w.w. in CA-cells. One must conclude that either Solutrast completely inhibits the constitutive pathway of endocytosis, or that FITC is taken up by transport pathways other than endocytosis. Whilst there is no evidence that Solutrast has such an effect, the non-endocytotic uptake of low molecular weight impurities of FITC-dextran (FITC and uncharacterized degradation products of FITC) into yeast vacuoles has been demonstrated (Preston et al. 1987). Moreover, it should be mentioned that the uptake of FITC-dextran into CA-cells of turtle urinary bladder would appear to be far to high to be accounted for by endocytotic vesicles. Morphometric measurements in the vesicle containing apical region of CA-cells revealed a vesicular volume of 6.2% (Stetson and Steinmetz 1983). For an uptake rate of FITC-dextran of about 0.14% CA-cell volume/min this vesicular volume should be fully occupied by FITC-dextran in far less than 45 min. However, the uptake of FITC-dextran was still linear at this time. Considering the fact that the morphometric measurements were performed at relatively low, and the FITC-dextran uptake determinations at high H secretion rates, at which the numbers of cytosolic vesicles should be high and low, respectively, the inconsistency between both results is even more pronounced. Although the discrepancy between morphometric and uptake measurements could be explained by a non-endocytotic uptake of FITC, the nature or existence of such a transport mechanism is completely unproven. In this regard it has also to be mentioned that Graber et al. (1989) using in one experimental set purified FITC-dextran found an even higher endocytosis rate than that obtained with commercial FITC-dextran. Particularly relevant is the question why FITC is only taken up into CA-cells (and not into granular cells). Further experiments are clearly needed to establish whether the uptake of FITC under steady-state condition of H secretion is, infact, endocytotic or whether Solutrast blocks the endocytotic pathway involved in membrane shuttling.

In contrast to the large difference between FITC-dextran and Solutrast uptake studies under steady-state conditions of H secretion, the cellular uptake of Solutrast after inhibition of H secretion is in good agreement with both FITC-dextran uptake measurements and morphological studies. Like FITC-dextran, Solutrast was only taken up into CA-cells (Gluck et al. 1982). Furthermore, I was detected only in apical regions of surface CA-cells in which, according to morphological investigations, the endocytosed vesicles are located (Stetson and Steinmetz 1983). Assuming that endocytosed vesicles have the same I concentration as the luminal perfusion solution, a cellular volume of about 1.5% occupied by endocytosed vesicles can be calculated from the mean I

concentration measured in the apical half of Cl-rich surface CA-cells. Using impedance analysis Dixon et al. (1986) found a decrease in apical membrane area after ACZL of 0.47 cm^2/cm^2 and, by morphometric measurements, a surface/volume ratio of cytosolic vesicles of 2.8 $\pm$ 1.5 cm^2/cm^3. Using these data, and assuming a CA-cell volume of 0.2 $\mu l/cm^2$, about 1% of the CA-cell volume should be occupied by endocytosed vesicles after ACZL. On the other hand, stimulation of H secretion by CO_2 addition resulted in a pronounced, morphometrically determined decrease of vesicular volume from 6.2 to 2.4% in the most apical region (first 5 μm) of α-type CA-cells (Stetson and Steinmetz 1983). Taking into account that CA-cells do not contain vesicles in their basal parts, and that they are usually some three times higher than the 5 μm deep region studied by Stetson and Steinmetz (1983), the decrease in vesicular volume after stimulation of H secretion is more or less equivalent to the cytosolic volume occupied by Solutrast after inhibition of H secretion. The good agreement between these independently calculated endo- or exocytosed volumes can be taken as evidence that Solutrast is a convenient fluid-phase marker for studying endocytotic processes related to inhibition of H secretion.

Acknowledgments

We gratefully acknowledge the invaluable technical assistance provided by Brigitte Wörndl and Ibrahim Öztürk. This work was supported by grants from the Deutsche Forschungsgemeinschaft.

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Discussion with Reviewers

T. von Zglinicki: I don't understand your Fig. 9. What is a volume measured in $\mu l/cm^2$ and how can it be estimated from inspection of micrographs? Aren't there factor(s) missing in the formula's exponent to render it dimensionless?
Authors: The volumes of the different cell types were estimated from the area the cells occupy in cross sections of the epithelium and then referred to an epithelial area of 1 cm^2. Since the H secretion rate measured by the short circuit current was also referred to 1 cm^2 epithelial area, the exponent in the equation of Fig. 9 is dimensionless.

T. von Zglinicki: What means CO_2-free incubation resulting in low H secretion and no I uptake as opposed to blocking H secretion by CO_2-free incubation resulting in I uptake in Cl-rich CA cells.
Authors: To prove whether a constitutive endocytotic pathway (membrane shuttling) can be recognized by the uptake of Solutrast, we applied Solutrast under steady state conditions to low H secreting (CO_2-free incubation) bladder pieces. No Solutrast was endocytosed under these conditions. In contrast, if Solutrast was applied and then H secretion blocked by CO_2-free, incubation Solutrast was endocytosed, indicating a transport related endocytotic pathway.

R.F. Husted: The authors state that the basal portion of granular cells was studied due to the presence of mucin granules near the apical surface which might affect the measured ion concentrations. CA cells, at least under some conditions, may have many vesicles near the apical surface. Does the presence of such vesicles alter measured ion concentrations?
Authors: Assuming that the extracellular content in endocytosed vesicles of H secreting CA-cells is maintained, reduction of H secretion - a situation in which the number of endocytosed vesicles is high - should lead to overall electrolyte concentration changes in the apical part and thereby to electrolyte concentration differences between apical and basal parts of these cells. Measurements in apical and basal parts of Cl-rich surface CA-cells during reduced H secretion have only been performed in some cases after the application of Solutrast solution which contained 10 mM NaCl. Endocytosis of such a solution should not influence cellular Na concentration but should reduce Cl and K concentrations in the apical cell region. However, taking into account that, according to morphometric measurements

the vesicular apical volume is about 6%, the decrease in Cl and K concentrations in this region should account for only about 4 and 6%, respectively. This might then explain that, in the few measurements performed so far, no systematic difference was found for electrolyte concentrations between apical and basal cell regions of Cl-rich surface CA-cells.

R.F. Husted: The authors state that calculated Na influx into CA-cells accounts for 0.5% of the measured I_{SC}. Does calculated Na influx into the granular and basal cells account for the remainder of the I_{SC}?
Authors: Since the Na concentrations of CA-cells after 1-2 h of ouabain increased only from about 10 to 20 mmol/kg w.w., it was expected that cellular Na influx calculated from this concentration change reflects Na influx under control conditions. If a similar calculation is made for granular and basal cells, in which the increase in Na concentration is about 10 times larger and which occupy an volume about 4 times larger in the epithelium, the so calculated Na influx accounts for about 20% of the short circuit current. However, such a calculation drastically underestimates the Na influx into granular and basal cells under control conditions, since it can be anticipated that observed K/Na-exchange in these cells was already complete in a shorter time than the duration of the ouabain experiments.

R.F. Husted: For Fig. 9, do the authors assume that only one cell type is responsible for all H secretion and that all anion flux is via anion exchange? What effect would anion flux via a channel have on the interpretation of these data?
Authors: First of all, it must be stated that the experiments to identify H secreting cells by serosal Br uptake were based on the conclusion of you and your coworkers that HCO_3 generated by the H secreting process leaves the cell via an anion exchanger in the serosal membrane. If the HCO_3 exits the cell by an anion channel this would not, of course, be reflected in an uptake of serosal Br. However, for Cl-rich surface CA-cells it was found that for each H secreted one HCO_3 leaves the cell across the serosal membrane in exchange for one Br (Cl). Since it was further shown that this Cl/Br exchange could be inhibited by reducing H secretion by serosal SITS or lowering the luminal pH to 4.5, we conclude that HCO_3 exit occurs only by serosal anion exchange and that Cl-rich surface CA-cells are solely responsible for H secretion.

J.H. Durham: Graber et al. (Am. J. Physiol., 253:F588, 1987) found that intracellular dye movement between G-cells, or between G- or CA-cells did not occur, but did between surface and basal cells. They concluded on that basis that cell-cell coupling did not occur between cells containing tight-junctions and noted that this was consistent with the lack of gap junctions in that portion of the epithelium. Do the authors believe that the results of the present paper are consistent with those conclusions?
Authors: Since after the application of ouabain drastic differences were found in the electrolyte patterns between granular and most of the basal cells on the one hand (high Na concentration) and surface CA-cells and some basal cells on the other hand (low Na concentration) it has to be concluded that no coupling exists between these cell groups. Whether there are intercellular bridges between granular cells cannot be established from the present results. On the other hand there is obviously a cellular coupling between granular and most of the basal cells since the ouabain-induced Na increase could be prevented in both cell types by blocking luminal Na entry. Beside the fact that such a coupling was not demonstrated by Graber et al., their results are more-or-less consistent with ours.

J.H. Durham: Scheffey et al. (Am. J. Physiol., 261:F963, 1991) found that the magnitude of acidification currents from individual CA-cells matched that of the concomitant transepithelial acidification current if about 80% of the CA-cells (the estimated population of α-CA-cells) of the epithelium were producing that current. They also directly determined that electrogenic alkalinization current was produced by cells other than those producing acid secretion and that the two processes occurred at the same time - i.e., different cell sources for each current. The magnitude of the cell "alkalinization" currents matched most closely that of the transepithelial alkalinization current if about 20% of the CA-cells (the estimated population of ß-CA-cells) were producing that current. These results appear to be consistent with the numbers of the "high" and "low" Cl cells described in the present paper. Could the authors please comment on whether they believe both investigations are consistent?
Authors: From the characteristics of serosal and luminal Br uptakes into Cl-rich and Cl-poor surface CA-cells and from their relation of about 3:1 to each other it was concluded in the present study that Cl-rich and Cl-poor surface CA-cells represent the morphologically determined H secreting α- and HCO_3-secreting ß-CA-cell types, respectively. A similar conclusion was drawn by Scheffey et al. by comparing acidification and alkalinization currents as measured in the total epithelium from the short circuit current with those obtained for localized cells using the vibrating probe. However, it must be mentioned that using the vibrating probe only H secreting cells were identified as CA-cells and that in most cases alkalinization currents were generally distributed across the epithelial surface and could not be attributed to specific cells.

A.T. Marshall: After ouabain treatment you established that amiloride inhibited Na influx from the luminal side and state that this completely prevented ouabain-induced changes in the electrolyte composition of all cell types. Why is there not, under these conditions, still an efflux of K?
Authors: For reasons of electroneutrality K can leave the cell only in exchange for a cation (possibly Na) or if it is accompanied by an anion (possibly Cl). Since almost no alteration in electrolyte concentrations occurred after the successive application of amiloride and ouabain it has to be assumed that cellular uptake of Na as well as exit of Cl are negligible under the given conditions.