

Ion Transport in Rat Antral Mucosa *in vitro*: General Characteristics

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Abstract. Although numerous studies have shown the existence of various types of ion conductance in antral part of gastric fundus mucosa epithelia of amphibian, practically no data are available on ion conductance in higher animal species. Present experiments were undertaken to check the possible existence of ion conductance in rat gastric antral mucosa and to investigate its general electrophysiological characteristics. Antral epithelia were isolated from adult Sprague Dawley rats. The tissues were mounted to a modified Ussing-type chamber and continually perfused with identical Krebs-Henseleit bicarbonate buffer on both sides. Antrum generated a transepithelial electrical potential difference ($V_t = -10 \pm 2.6$ mV) and short-circuit current ($I_{sc} = 76 \pm 15 \mu\text{A} \cdot \text{cm}^{-2}$) with a transepithelial electrical resistance ($R_t = 135 \pm 16.8 \text{ Ohm} \cdot \text{cm}^2$). Ion replacement experiments showed that it is mainly Na^+ transport that contributes to V_t and I_{sc} as evidenced by a) Na^+ and/or Cl^- removal, b) the effects of amiloride a sodium channel blocker, on the apical (secretory) surface, c) the effects of the $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ inhibitor ouabain on the basolateral (nutrient) side of the epithelium. Microelectrode experiments confirmed the existence of Na^+ and/or Cl^- conductance of the apical cell membrane. Antral mucosa also showed a gradual and time-dependent increase in sensitivity to amiloride (10^{-5} mol/l). Maximum inhibition of V_t and I_{sc} by amiloride in dose-dependent manner was detected after 1–2 h. This amiloride-sensitive sodium transport (maximal level $31.5 \pm 5.9 \mu\text{A} \cdot \text{cm}^{-2}$) represented approximately 50% of the whole transepithelial ion conductance. Results of experiments with ouabain (10^{-4} mol/l) suggest the presence of functional $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ and/or $\text{Na}^+ - \text{ATPase}$ in the basolateral cell membranes. Which signals trigger this epithelial ion transport, which hormones are responsible for its regulation and what is the physiological significance of this ion conductance remains to be elucidated.

Key words: Rat gastric antrum — Ion replacements — Na^+ conductance — Amiloride — Ouabain

Introduction

The stomach of both amphibians and mammals consists of two main regions, the fundus and the antrum. Properties of the antral mucosa are of considerable clinical and physiological significance. It is the part of the stomach which most often is the site of peptic ulcer damage (Oi et al. 1969; Kolbasa et al. 1988). This region is located between the acid-secreting, electrically tight epithelium of the gastric fundic mucosa and the alkali-secreting, electrically leaky epithelium of the small intestine. The antral mucosa could therefore be considered neither a leaky nor a tight epithelium. This tissue is characterized by a single layer of columnar mucous cells interrupted by the presence of antral glands and a small population of endocrine G-cells responsible for gastrin secretion (Ito 1987). Peripheral administration of calcitonin gene-related peptide (Ren et al. 1992), as a modulator of antral endocrine cells and cholinergic neurons, was shown to inhibit basal and stimulated gastric acid secretion in G-cells in rats (Lenz et al. 1985a,b), dogs (Pappas et al. 1986), and humans (Kraenzlin et al. 1985). Antral cells are electrically coupled, at least in amphibians (Flemström and Sachs 1975; Soybel et al. 1987).

Studies on electrical and transport characteristics, performed predominantly on isolated antral mucosa of *Necturus maculosus*, have shown that surface epithelial cells actively absorb Na^+ and secrete Cl^- and HCO_3^- (Flemström and Sachs 1975). It was also found that the first step in net mucosa to serosa transepithelial Na^+ transport was translocation of Na^+ across amiloride-sensitive Na^+ channels located in the apical cell membranes (Flemström and Sachs 1975). Data obtained by the use of microelectrode techniques indicated that the passive permeability properties of the gastric surface epithelium largely reflected those of paracellular pathway (Machen et al. 1978; Ashley et al. 1985; Soybel et al. 1987). This pathway contributes 90–95% of the conductance across the tissue whereas the pathway across the cell membrane only 5–10% (Spencey et al. 1975; Soybel et al. 1987).

In our previous experiments on *Necturus* antrum, a gradual spontaneous activation of an amiloride-inhibitable Na^+ conductance was observed during incubation of the tissue in artificial Ringer solutions (Bakoš and Frömter 1994). As no similar information has been available for any other animal species, we decided to check the possible existence of ion conductance in mammals. The present study provides data on the presence and basal electrophysiological characteristics of ion transport in isolated rat gastric antral mucosa.

Materials and Methods

Animals

Adult Sprague Dawley rats of both sexes obtained from Charles River Europe (Sulzfeld, Germany) were used throughout the experiments. The animals were maintained in a

controlled environment (22–24°C) with a 12-hour light/12-hour dark photoperiod and free access to food and water.

Table 1. Composition of bathing solutions

[mmol/l]	Apical			Basolateral		
	Basal	Na ⁺ =0	Cl ⁻ =0	Basal	Na ⁺ =0	Cl ⁻ =0
			ISE SO ₄ ²⁻			ISE SO ₄ ²⁻
Na ⁺	136.1			136.1		
K ⁺	4.7	4.7		4.7	4.7	
Ca ⁺⁺	1.0	1.0		1.0	1.0	
Mg ⁺⁺	1.1	1.1		1.1	1.1	
Cl ⁻	118.9			118.9		
SO ₄ ²⁻			110.0			110.0
HCO ₃ ⁻	25.0		25.0 25.0	25.0		25.0 25.0
H ₂ PO ₄ ⁻	1.1		1.1 1.1	1.1		1.1 1.1
HEPES	10.0	10.0	10.0 10.0	10.0	10.0	10.0 10.0
Glucose	5.6	5.6	5.6 5.6	5.6	5.6	5.6 5.6
NMDG-Cl		135.0		135.0		
Isethionic acid			110.0			110.0
Ca-D-gluconate			1.0 1.0			1.0 1.0
D-gluconic acid			4.7 4.7			4.7 4.7

Tissue preparation

The animals were killed by decapitation. The abdominal cavity was opened immediately and a ring of antral tissue was removed. Antral specimens were opened along the greater curvature and rinsed in ice-cold (2–4°C) oxygenated modified Krebs-Henseleit bicarbonate buffer [hereinafter "Ringer"; (in mmol/l): 110.0 NaCl; 4.7 KCl; 1.0 CaCl₂; 1.13 MgCl₂; 1.15 NaH₂PO₄; 25.0 NaHCO₃; 10.0 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES); and 5.6 glucose] (Table 1). Buffer pH was adjusted to 7.4. Antral mucosa-submucosa was dissected in a continuous sheet and the outer muscle layer was stripped off. Isolated epithelium was then mounted (mucosal surface up) between two Ussing-type lucite half chambers. In a round shaped bore between both half chambers the antrum was supported by a perforated stainless grid. To reduce edge leaks the rim of the upper half chamber was coated with a thin layer of Sylgard 185 (Dow Corning). The exposed surface area was 0.2 cm² and the upper and lower chamber volume were 0.6 ml and 1.8 ml respectively. Both half-chambers were continuously perfused with Ringer solution at a slow rate of 2.5 ml/min (upper) or 3 ml/min (lower) and/or at a fast rate of 4.5 ml/min (upper) or 7 ml/min (lower). Higher perfusion rates were used for washing out the substances applied. Bathing solutions were automatically tempered at 37°C, and gassed with 95% O₂ – 5% CO₂. All experiments were performed at room temperature.

The perfusion system allowed rapid solution changes achieved by switching mechanical valves. Measurements were recorded 1–6 hours after the animal had been killed.

Transepithelial electrical measurements

The transepithelial mucosa-serosa electrical potential difference (V_t) was measured by means of two flowing-boundary electrodes with Ag-AgCl pellets (WPI, Germany) connected to both bathing solutions through 3.0 mol/l KCl bridges with the serosal solution grounded. The electrodes were connected to an own-built preamplifier followed by a two-channel pen recorder (TZ 4620, Laboratorní přístroje Praha, Czech Republic). Transepithelial electrical resistance (R_t) was measured by passing transepithelial current pulses (amplitude: 10–20 $\mu\text{A}\cdot\text{cm}^{-2}$; width: 650 ms; interval: 20 s) from a own-built pulse-generator. Pulses were applied through two Pt-ringlets localized above and below the epithelium. The shape and the position of the electrodes were selected to yield uniform current density over the entire preparation. The value of R_t was then calculated from $R_t = \Delta V_t / I$, where ΔV_t is transepithelial voltage deflection, and I is transepithelial current density. All tissue resistances were corrected for the resistance of the subepithelial tissue and solutions. This was done by scraping the epithelium at the end of an experiment and then letting a current pulse pass across the stainless grid and the denuded tissue with the electrodes in their original positions. The average resistance correction was between 30 and 80 $\text{Ohm}\cdot\text{cm}^2$. Both transepithelial short-circuit current (I_{sc}) and amiloride-sensitive short-circuit current (I_{Na}) were calculated from the Ohm's law: $I_{sc} = V_t / R_t$; $I_{Na} = \Delta V_t / R_t$, where ΔV_t ($\Delta V_t = V_t - V'_t$) is the fall in voltage caused by the application of the sodium channel blocker amiloride. V_t is transepithelial voltage before, and V'_t after addition of amiloride.

Microelectrode experiments

The apical cell membrane potential (V_a) was recorded with conventional borosilicate glass pipette microelectrodes. The glass capillaries were pulled on a two-stage microelectrode puller (model PPP CP-01, CFV, Bratislava) and filled with 3.0 mol/l KCl by back injection. Only microelectrodes with low tip potentials and resistances between 20–50 M Ω were used for cell impalements. Criteria for acceptable punctures were a) an abrupt change in electrode voltage, b) a stable baseline for 60–90 s, c) fluctuation of membrane voltage < 2 mV while inside the cell, d) a return to the original baseline potential after removal of the microelectrode from the cell.

Experimental protocol

Tissues were perfused on the both sides with Ringer solution until a steady state was achieved (40–60 min). After each exposure to a test substance, the luminal, serosal and/or both solutions were changed back to control bathing solution. In ion replacement experiments to study the effects of apical (luminal) Na^+ -free solutions on antral transport parameters, 110 mmol/l NaCl and 25 mmol/l NaHCO_3 in the Ringer solution were replaced with 135 mmol/l N-methyl-D-glucamine (NMDG-Cl), or to study the effects of apical and/or apical and basolateral Cl^- -free solutions on antral transport parameters, 110 mmol/l NaCl, 4.7 mmol/l KCl and 1.0 mmol/l CaCl_2 in Ringer were replaced by adequate amounts of sodium isethionate, D-gluconic acid and calcium D-gluconate respectively (Table 1). In these experiments to test epithelial sensitivity to amiloride, from the beginning the luminal bathing solution was periodically (every 10 or 20 min) replaced by the appropriate amiloride test solution (10^{-5} mol/l) for a time allowing to reach maximum epithelial response, and changes of electrophysiological parameters (V_t , I_{sc} , R_t , I_{Na})

were continuously recorded or calculated. All substances tested were dissolved in Ringer solution and were then applied to the perfusion chamber without changing osmolality, pH or volume of the control bathing solution.

Chemicals

All substances used were purchased from Sigma, Fluka or Merck.

Statistics

Statistical analysis were made by Student's *t*-test for paired data for control and experimental conditions.

Results

1. Basal transport characteristics

Antrum generated a transepithelial electrical potential difference (V_t) and short-circuit current (I_{sc}) with maximal values of -10 ± 2.6 mV and $76 \pm 15 \mu\text{A} \cdot \text{cm}^{-2}$, respectively. Transepithelial electrical resistance (R_t) was $135 \pm 16.8 \text{ Ohm} \cdot \text{cm}^2$ (Fig. 1). Both V_t and I_{sc} showed a gradual and time-dependent increase reaching their maximal levels after 2 hours. Compared to the basal V_t and I_{sc} levels (-3.77 ± 0.54 mV and $39.82 \pm 4.81 \mu\text{A} \cdot \text{cm}^{-2}$), maximal values showed high inter-individual variability (-11 ± 4.65 mV and 69.44 ± 19.16). Levels of transepithelial electrical resistance were stable throughout (Fig. 1).

2. Effects of ion replacement

The effects of Na^+ -free bathing solution (N-methyl-D-glucamine; NMDG-Cl substitution of Na^+) and the effects of SO_4^{2-} and isethionate substitution of Cl^- are shown in Table 2.

a) Effects of Na^+ replacement

NMDG-Cl substitution of Na^+ in basal Ringer solution on the luminal (apical) epithelial side caused a rapid and deep suppression of both V_t and I_{sc} , with a slight but significant increase of R_t (Table 2; Fig. 2, right). Replacement of sodium in the luminal bathing solution also resulted in an increase of electrical potential across the apical cell membranes (V_a) from -15.8 ± 2.15 to -21.6 ± 2.03 mV (Fig. 2, left). After washout all effects were fully reversible.

b) Effects of Cl^- replacement

In these experiments Cl^- in basal Ringer solution was substituted by isethionate on the luminal side of the mucosa. This substitution resulted in an increase of V_t , I_{sc} and R_t (Table 2; Fig. 3, right), but in a decrease of apical membrane potential V_a from -14.85 ± 1.73 to -6.57 ± 1.79 mV (Table 2; Fig. 3, left). All changes were reversible on return to control NaCl solution.

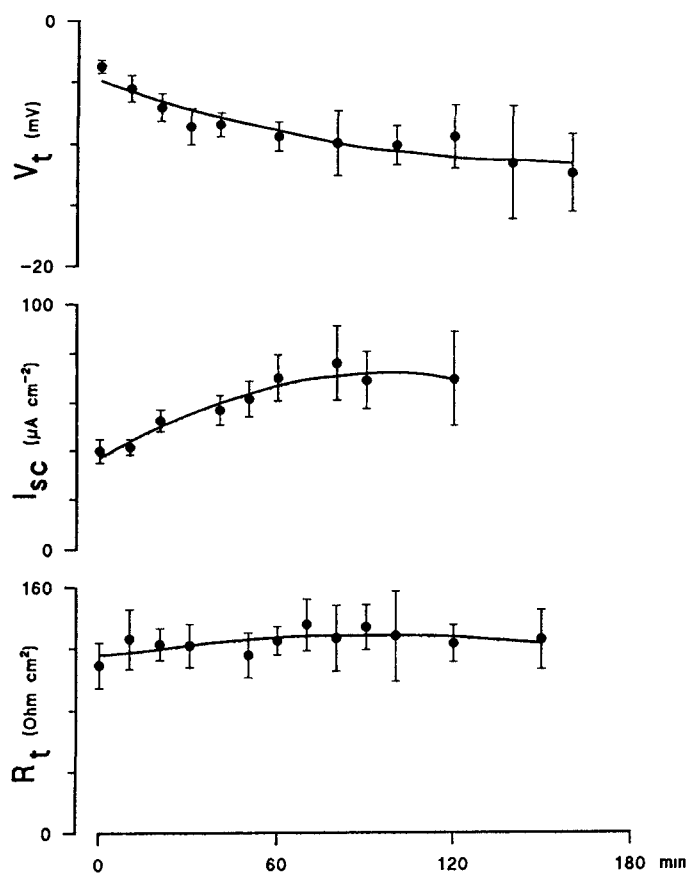


Figure 1. Time courses of transepithelial potential V_t , short-circuit current I_{sc} and electrical resistance R_t of isolated rat antral mucosa. The epithelia were bathed on both sides with identical basal Ringer solution (see Table 1). Means of 10 (V_t), 12 (I_{sc}) and 10 (R_t) values \pm S.E.M.

Replacement of Cl^- by SO_4^{2-} in the control solution on both sides resulted in a biphasic effect. First a rapid increase of both V_t and I_{sc} occurred with no significant changes of R_t (Table 2; Fig. 4). During the second phase of the epithelial response, which lasted approximately 20 min, V_t and I_{sc} gradually returned to levels near or below those observed under control conditions (Fig. 4).

3. Inhibitors of Na^+ transport

Specific inhibitors were applied to reveal whether sodium transport processes take place realized on the membrane and/or paracellular level.

Table 2. Effects of replacement of Na^+ and Cl^- on electric properties of rat antrum

	V_t (mV)	I_{sc} ($\mu\text{A} \cdot \text{cm}^{-2}$)	R_t ($\text{Ohm} \cdot \text{cm}^2$)	n
NaCl	-6.43 ± 0.55	79.98 ± 6.56	81.0 ± 5.03	(5)
NMDG-Cl (A)	$-0.95 \pm 0.49^*$	$12.48 \pm 4.95^*$	88.5 ± 4.30	
NaCl	-6.06 ± 0.61	72.05 ± 6.55	84.0 ± 3.40	
NaCl	-4.63 ± 0.53	56.86 ± 5.86	79.7 ± 2.28	(17)
Na-ISE (A)	$-12.23 \pm 0.71^*$	$133.42 \pm 7.42^*$	$92.2 \pm 2.55^*$	
NaCl	-4.83 ± 0.49	56.28 ± 5.26	82.7 ± 2.94	
NaCl	-6.25 ± 1.72	43.95 ± 6.59	134.2 ± 28.1	(6)
Na-ISE (A+B)	$-11.48 \pm 1.71^*$	$75.52 \pm 2.26^*$	149.2 ± 18.7	
NaCl	-4.66 ± 1.39	37.69 ± 7.99	120.0 ± 13.5	
NaCl	-5.78 ± 1.01	49.64 ± 4.86	114.4 ± 14.2	(13)
Na_2SO_4 (A+B)	$-10.75 \pm 1.09^*$	$102.27 \pm 5.66^*$	105.4 ± 8.9	
NaCl	-5.75 ± 1.06	47.71 ± 4.53	114.3 ± 16.2	

The mean values \pm S.E.M. of V_t , I_{sc} and R_t are given in control conditions (NaCl) and at maximal response to ion replacements. Ion substitution was performed on the (A) – apical side or both (A+B) – apical+basolateral sides of the epithelium. NaCl in basal Ringer solution was substituted either by NMDG-Cl (N-methyl-D-glucamine) or Na-isethionate and Na_2SO_4 . The experiments were performed in open circuit conditions; I_{sc} was calculated from V_t and R_t . Significance level: * – $P < 0.001$

a) Effects of amiloride

In an initial series of experiments, the sodium channel blocker amiloride (10^{-5} mol/l) was added to either the apical or basolateral side of rat gastric antrum. When introduced from the apical side, there was a 40–50% inhibition of V_t and I_{sc} (Table 3; Fig. 5) with a parallel slight increase of R_t (not shown). The effect of amiloride was fully reversible. When applied to the basolateral side of the epithelium either in above mentioned concentrations or in higher amounts (10^{-3} mol/l) this sodium channel blocker was without any effect.

b) Development of apical amiloride-sensitive Na^+ conductance

The response of the rat antrum-generated sodium transport to amiloride (10^{-5} mol/l) included a gradual and time-dependent increase in epithelial sensitivity to the sodium channel blocker (Fig. 6). First, only a small sensitivity to the blocker was detected. Maximum inhibition of transepithelial electrical potential and short-circuit current by amiloride was reached after 1–2 hours in average. In parallel with the gradual increase of epithelial sensitivity to amiloride, a rise in the level of amiloride-sensitive short-circuit current I_{Na} was also observed (Fig. 7). Amiloride inhibited epithelial sodium conductance in a dose dependent manner (from 10^{-8}

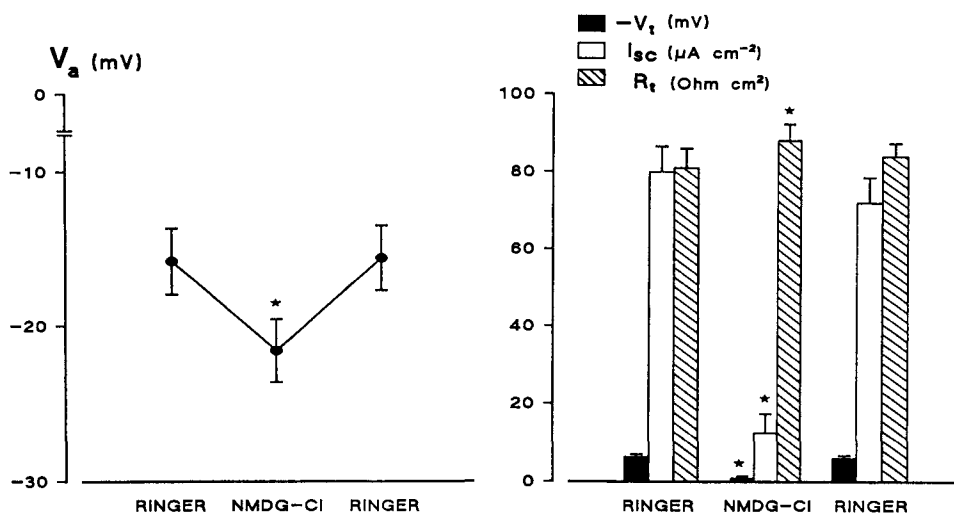


Figure 2. Effect of apical Na^+ -free Ringer solution (NMDG-Cl N-methyl-D-glucamine substitution of NaCl, see Table 1) on the levels of V_t , I_{sc} , R_t and apical membrane potential, V_a in rat antral mucosa. The asterisk indicates statistically significant differences vs. basal Ringer bathing solution. Significance level * - $P < 0.001$. Means of 5 values \pm S.E.M.

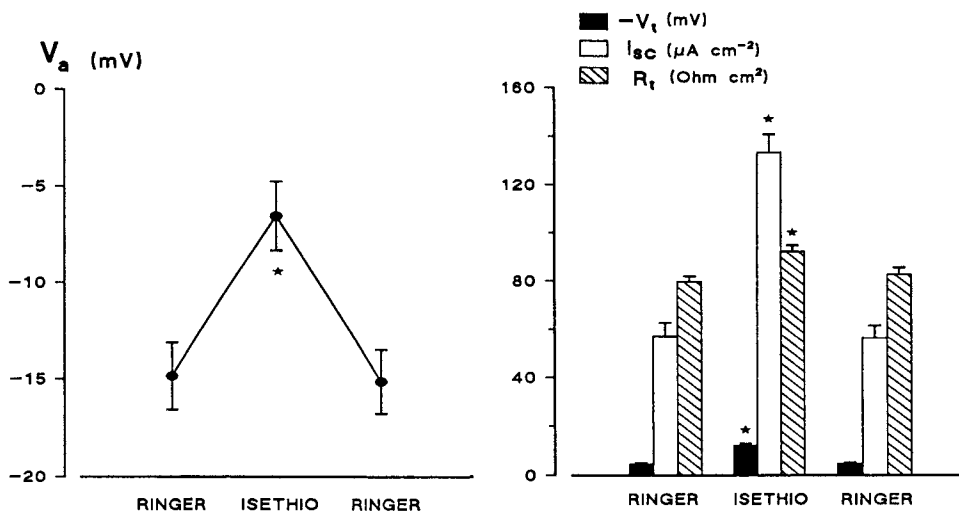


Figure 3. Effect of apical Cl^- -free Ringer solution (ISETHIO isethionate substitution of NaCl, see Table 1) on the levels of V_t , I_{sc} , R_t and apical membrane potential, V_a in rat antral mucosa. The asterisk indicates statistically significant differences vs. basal Ringer bathing solution. Significance level * - $P < 0.001$. Means of 7 (V_a) and 17 (V_t , I_{sc} , R_t) values \pm S.E.M.

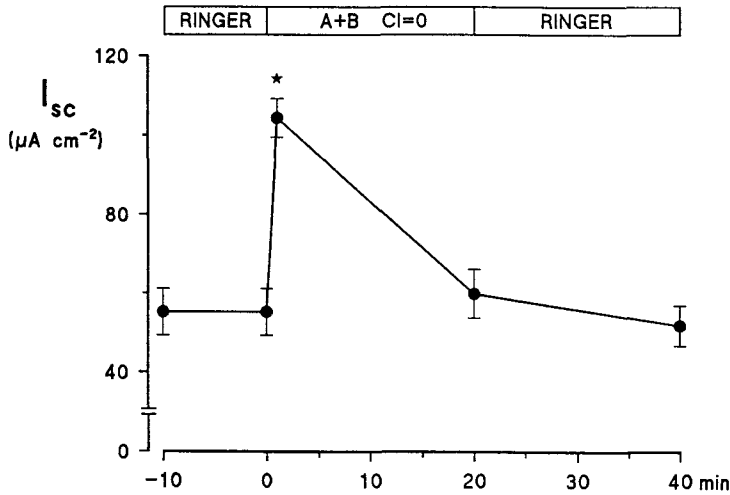


Figure 4. Effect of Cl^{-} -free Ringer solution (Na_2SO_4 substitution of $NaCl$; see Table 1) on the level of transepithelial short-circuit current, I_{sc} in rat antral mucosa. Chloride-free solution was applied to both apical+basolateral (A+B) sides of epithelium. The asterisk indicates statistically significant differences vs. basal Ringer bathing solution. Significance level: * - $P < 0.001$. Means of 9 values \pm S.E.M.

Table 3. Effect of amiloride on transepithelial V_t , I_{sc} and R_t of rat antrum

	V_t (mV)	I_{sc} ($\mu A.cm^{-2}$)	R_t (Ohm.cm ²)	n
Control	-9.34 ± 1.08	65.04 ± 4.09	138.8 ± 10.02	(15)
Amiloride	$-4.86 \pm 0.66^*$	$32.70 \pm 2.95^*$	145.2 ± 11.64	
Control	-9.11 ± 1.03	60.18 ± 4.17	148.2 ± 10.82	

The mean values \pm S.E.M. of V_t , I_{sc} and R_t are given. Amiloride was added to the luminal side in a concentration of 10^{-5} mol/l. The preceding controls were recorded immediately before the addition of amiloride. The test values were obtained at the maximal amiloride action. Significance level: * - $P < 0.001$.

mol/l to 10^{-4} mol/l). Epithelial responses to 10^{-4} mol/l and 10^{-5} mol/l of the substance were effectively the same (Fig. 6).

c) Effects of ouabain

To test the presence and involvement of $Na^{+}-K^{+}$ -ATPase in the ion transport pathway, the inhibitor of this transport enzyme, ouabain (10^{-4} mol/l), was added to either the luminal or basolateral side of isolated rat antral mucosa. Application to

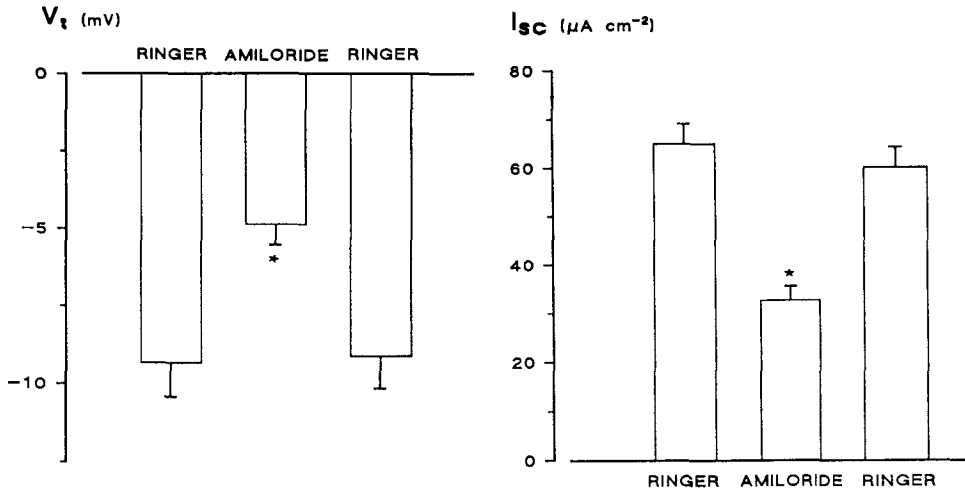


Figure 5. Effect of sodium channel blocker amiloride (10^{-5} mol/l) on transepithelial potential, V_t and short-circuit current, I_{sc} across isolated rat antral mucosa. Amiloride was applied to Ringer solution bathing the apical side of the epithelium after equilibration of transport parameters. The asterisk indicates statistically significant differences vs basal Ringer bathing solution. Significance level: * - $P < 0.001$. Means of 15 values \pm S.E.M.

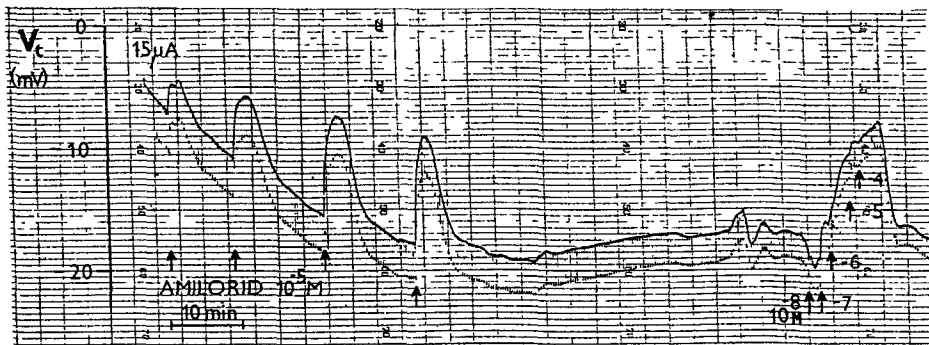


Figure 6. Original record of a time course of transepithelial potential difference, V_t with a gradual and time- and dose-dependent increase of epithelial response to 10^{-5} mol/l amiloride in rat antral mucosa in the period of equilibration. Arrows indicate amiloride application to the Ringer solution bathing apical epithelial side. To determine electrical resistance of the tissue 15 μA current pulses were applied transepithellally.

the bathing solution at the basolateral side induced two types of responses. In epithelia with higher levels of basal transepithelial potential and short-circuit current,

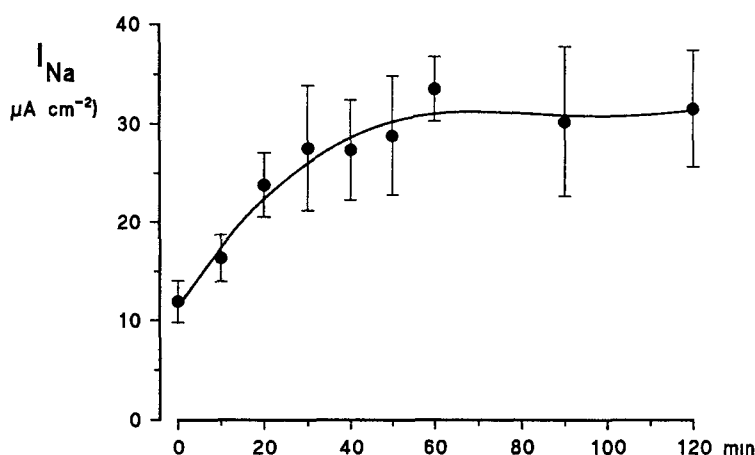


Figure 7. Time course of the development of amiloride-sensitive short-circuit current, I_{Na} in isolated rat antral mucosa. Means of 9 values \pm S.E.M.

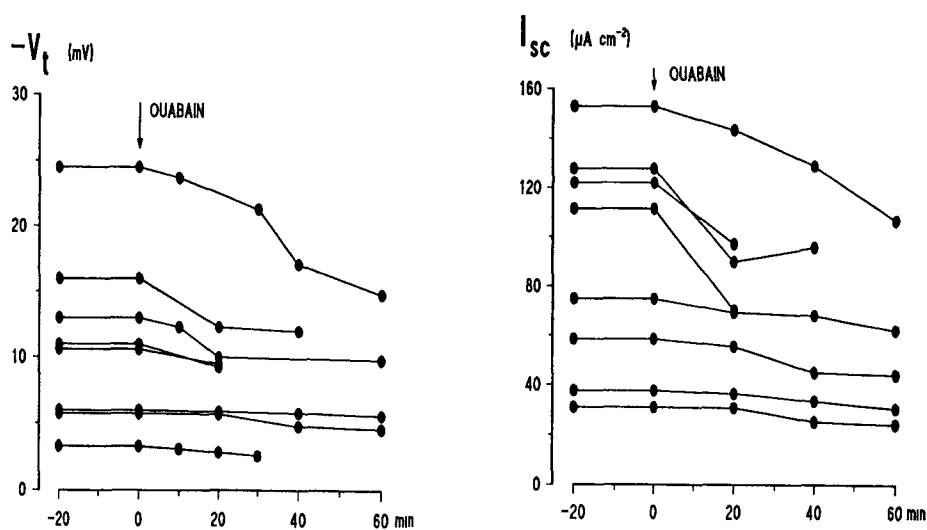


Figure 8. Time course of the effect of ouabain (10^{-4} mol/l) on transepithelial potential, V_t and short-circuit current, I_{sc} in isolated rat antral mucosa. Ouabain was applied to the Ringer solution bathing the basolateral side of the epithelium after equilibration of transport parameters (time = 0 min).

ouabain inhibited both V_t and I_{sc} (Fig. 8). On the other hand, in epithelia with smaller initial levels of the measured transport parameters, ouabain effectively had no effect. In all measurements transepithelial electrical resistance was unchanged.

or slightly increased (not shown). In the same concentrations, ouabain failed to affect the electrical transport parameters when added to the solution bathing the luminal epithelial side.

Discussion

This study has provided general electrophysiological characterization of transepithelial ion transport in isolated rat gastric antral mucosa with the evidence on the existence of amiloride-sensitive Na^+ conductance. This ion conductance was found to be very similar to that observed in *Necturus* antral mucosa.

It has previously been established that the isolated gastric antrum of *Necturus maculosus* actively transports Na^+ and Cl^- ions. Sodium ions are transported from the luminal to the serosal epithelial side and chloride ions in the opposite direction (Flemström and Sachs 1975). The same authors postulated that only Na^+ transport contributed to the epithelium-generated transepithelial electrical potential difference and short-circuit current, which were -5.5 mV and $7 \mu\text{A}\cdot\text{cm}^{-2}$, respectively, whereas transepithelial electrical resistance was about $800 \text{ Ohm}\cdot\text{cm}^2$. Microelectrode studies of this epithelium determined the values of both apical and basolateral cell membranes potentials, which were approx. -43 mV and -48 mV, respectively (Grady and Cheung 1983). This epithelium was classified as a moderately-leaky tissue. In these experiments isolated rat gastric antrum generated practically the same electrical potential (approx. -10 mV) as measured for *Necturus* antrum. Differences were observed in the values of transepithelial electrical resistance which was lower for rat (approx. $150 \text{ Ohm}\cdot\text{cm}^2$). This in turn resulted in a higher short-circuit current (approx. $70 \mu\text{A}\cdot\text{cm}^{-2}$). Preliminary microelectrode studies of the epithelium showed also lower apical and basolateral cell membrane potentials (approx. -25 mV and -29 mV). According to the values of electrical resistance, the rat antrum could be considered a leaky epithelium.

Our results obtained with NMDG-Cl substituted for sodium suggest a significant contribution of sodium ions to antrum-generated transepithelial electrical potential and short-circuit current, and the importance of these ions in the process of ion transport across antral epithelium. These results are comparable to those obtained by Flemström and Sachs (1975) and Rutten et al. (1990) for *Necturus* antrum. The obtained data only differ in the magnitude of the amiloride effect, which is higher in *Necturus*.

Luminal Cl^- removal, with isethionate substitution, had a stimulatory effect on all transepithelial transport parameters measured: a decrease of potentials was observed across apical cell membranes. On the other hand, chloride replacement on both sides of the epithelium for SO_4^{2-} resulted in a biphasic effect: a rapid increase of both V_t and I_{sc} followed by a slow decline to reach values similar to those

before the ion substitution. All these steps were accompanied by unchanged tissue resistance. In the case of *Necturus*, $\text{Cl}^- - \text{SO}_4^{2-}$ substitution was reported not to affect the transepithelial potential, resistance or short-circuit current (Flemström and Sachs 1975). However, it is not clear, whether the above mentioned biphasic effect with an initial increase was detected by other authors, or whether they only mentioned the values in the maximum. At present, it is not possible to explain the reason of the initial stimulation of the parameters. We suggest that it could be the result of a sudden ion redistribution with a secondary developed majority of sodium ions in the bathing solution and/or other ion permeation dependent processes. It is well recognized that gastric mucosa may transport Cl^- into the lumen (Machen and McLennan 1980; Machen and Zeuthen 1982). *In vitro* studies in both mammalian and amphibian preparations have suggested that part of this nonacidic secretion originates from the gastric surface epithelium rather than from the acid-secreting oxyntic glands (Forte and Machen 1975). The mechanisms by which gastric surface cells might accumulate and secrete Cl^- into the lumen are not understood. Microelectrode studies have shown that intracellular Cl^- activity is not altered during prolonged removal of chloride from luminal perfusate (Schettino and Curci 1985). Intracellular levels of chloride were found to be regulated mainly by processes located in the basolateral membrane that accumulate Cl^- from the serosal solution into the cell (Machen and Zeuthen 1982). In addition, it was suggested that mechanisms of Cl^- transport by gastric surface cells were energy-dependent but might be coupled to transport of other ions (e.g. Na^+ or K^+) that directly contribute to short-circuit current. Recently, Soybel et al. (1993) documented the importance of basolateral $\text{Na}^+ - \text{K}^+ - \text{Cl}^-$ cotransport in regulating cell Cl^- levels in surface cells of the gastric antrum in *Necturus*. From these and our results it may be suggested that similarly as in *Necturus*, Cl^- transport in rat antrum does not directly contribute to the electrical characteristics of the tissue.

The present results obtained with the application of the highly selective sodium channel blocker amiloride indicate that about 50% of all sodium ions transported across the apical cell membranes of the antral epithelium penetrate the tissue via apically localized amiloride-sensitive sodium channels. Amiloride is known to inhibit sodium transport across various gastrointestinal (e.g. colon, fundus, intestine) but also renal (e.g. proximal tubule) and other (abdominal frog skin) epithelia. While in the frog skin amiloride inhibition reaches practically 100% (e.g. Benos 1982) of the control value, the degree of inhibition in other epithelia varies between 80–90% (Gögelein and Greger 1986; Moran et al. 1988; Bridges et al. 1989). Studies on *Necturus* antrum showed about 70% inhibition by amiloride (Flemström and Sachs 1975).

Thus, in contrast to other animal species, isolated rat antral mucosa only transports 50% of sodium cellularly. However, our results of ion replacement experiments showed total suppression of transepithelial potential and short-circuit

current after luminal Na^+ removal. The remaining Na^+ conductance, not influenced by amiloride, could use other carriers e.g. the Na^+/H^+ exchange mechanisms. Furthermore, it can be transported through amiloride-insensitive sodium pathways, through nonselective cation channel, or it could penetrate epithelium paracellularly. The possible existence of other ion transport mechanisms in isolated rat antral mucosa and the actual ratio between cellularly and paracellularly transported ions remains to be elucidated.

Similarly as in our previous study on isolated antrum of *Necturus maculosus* (Bakoš and Frömter 1994), a peculiar time course of the development of transepithelial amiloride-sensitive short-circuit current I_{Na} (Na^+ absorption) was observed in the rat. Isolated epithelium of rat antral mucosa exhibited a gradual and time-dependent increase of sensitivity to the sodium channel blocker-amiloride. Immediately after mounting the tissue into the chamber transepithelial electrical potential difference, transepithelial short-circuit current and transepithelial amiloride-sensitive short-circuit current were low, and amiloride produced either small or no response. However, in the course of a few minutes, the response to amiloride began to rise gradually until the parameters measured reached stable values. Maximum inhibition of V_t , I_{sc} and I_{Na} by amiloride was determined after 1–2 h. Similar changes were presumably observed also by other investigators working with *Necturus* antral mucosa who reported that they had to wait for 45 to 90 min until the tissue “stabilized” before the experiments were begun (Rutten et al. 1989, 1991; Soybel et al. 1992, 1993). Gradual increase of Na^+ absorption from near zero was thought to reflect gradual sealing of the tissue in the chamber (Higgins et al. 1975). This explanation can be ruled out, as transepithelial resistance R_t remained constant during the period of V_t , I_{sc} and I_{Na} increase. The increase of Na^+ conductance over time might reflect a protracted action of hormones or transmitters, activation of preexisting Na^+ channels in apical cell membranes, changes in protein synthesis and fresh expression of Na^+ channels, etc. As yet, preliminary experiments on *Necturus* antrum failed to provide for a reasonable explanation of this phenomenon (Bakoš and Frömter, unpublished observation).

As for other tissues, the existence and/or secondary development of amiloride-sensitive Na^+ conductance in rat distal colon (Will et al. 1980; Perrone et al. 1984; Sandle et al. 1984; Edmonds and Mackenzie 1987) and hen intestine (Clauss et al. 1984, 1987) was shown to depend on pretreatment of the epithelium with aldosterone which is known to induce amiloride-sensitive sodium channels. Increased sensitivity to amiloride was also observed when animals were depleted of sodium or were fed a potassium-rich diet (Foster et al. 1985). In the case of *Necturus* gastric fundic mucosa, the development of the sensitivity to sodium channel blocker depended on the incubation time (Kottra et al. in press). Similar results were also obtained by Elbrond and Skadhauge (1992) after long-term incubation of the lower intestine of the hen. However, in our preliminary experiments on rat fundic

mucosa, no response to amiloride was obtained either after hormonal pretreatment or long-lasting incubation.

The last part of the present work was undertaken to evaluate the presence of ouabain-sensitive $\text{Na}^+\text{-K}^+\text{-ATPase}$ and the possible involvement of this transport enzyme in the ion transport processes occurring in isolated rat gastric antrum. The results showed two different epithelial responses to ouabain, a specific inhibitor of the enzyme. Ouabain inhibited the transport parameters studied in epithelia with higher initial levels of V_t and I_{sc} , but not in those with low basal values. We suggest that this might be related to the kind and functional state of transport mechanisms of low- or high-voltage epithelia. It may be speculated that epithelia with a low initial transepithelial electrical potential and current exhibited lower transport levels because of a lack of existence or insufficiency and/or reduced activity of membrane carriers including basolateral membrane-bound $\text{Na}^+\text{-K}^+\text{-ATPase}$ and/or other transport enzymes.

It is well documented that ouabain-sensitive $\text{Na}^+\text{-K}^+\text{-ATPase}$ is an enzyme that catalyses Na/K exchange, maintaining constant gradient of these ions across the plasma membrane of a wide variety of cells (Lechene 1988; Dobrota et al. 1988). However, the existence of other ion-transporting ATPases has also been demonstrated for many tissues. It has been shown that Ca-ATPase activity is responsible for active transport of calcium (Rega 1986), and that H/K-ATPase plays an important role in gastric acid secretion (De Pont et al. 1988). Recently $\text{Na}^+\text{-ATPase}$ has been observed and characterized in various animal tissues (Proverbio et al. 1989; Moretti et al. 1991). It does not require K^+ to work, it is ouabain insensitive, furosemide- and bumetanide-sensitive, cell-volume-dependent, and it seems to play an important role in active cell volume regulation. This ATPase was also detected in rat small intestinal cells.

Compared to other tissue, such as frog skin, rat antrum exhibited much weaker or no response to ouabain. Taking into account that application of K^+ -free bathing solution to the basolateral side of the rat antrum failed to change basal transport parameters (data not shown), the existence of an ouabain-insensitive $\text{Na}^+\text{-ATPase}$ in the rat gastric antrum may be suggested. These findings give support to the hypothesis proposed by Borgatti et al. (1985) on the possibility of a parallel widespread distribution of the $\text{Na}^+\text{-ATPases}$ and the $\text{Na}^+\text{-K}^+\text{-ATPases}$. Whether $\text{Na}^+\text{-ATPase}$ is only present in the low-voltage epithelia and the existence of the response to ouabain in high-voltage tissues confirms the involvement of $\text{Na}^+\text{-K}^+\text{-ATPase}$ remains to be elucidate.

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