Segmental heterogeneity of swelling-induced Cl⁻ transport in rat small intestine

Abstract The effect of cell swelling induced by hypotonic media was studied in segments of rat small intestine. In the Ussing chamber, exposure to a hypotonic medium caused a decrease in short-circuit current ($I_{sc}$) and potential difference ($V_{ms}$) in the jejunum, whereas the ileum responded with an increase in $I_{sc}$ and $V_{ms}$. The transition from one pattern to the other was located about in the middle of the small intestine. Tissue conductance decreased in both segments, probably due to a reduction of paracellular shunt conductance induced by the cell swelling. Voltage scanning experiments revealed that the observed decrease in total tissue conductance in the ileum was caused solely by a decrease in local conductance in the villus region while the crypt conductance did not change, suggesting that the decrease in paracellular conductance of the crypts is compensated by an increase in cellular conductance. The response in both segments was dependent on the presence of Cl⁻ and was blocked by the Cl⁻ channel blocker 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB). It was not affected by the neurotoxin tetrodotoxin. In the jejunum the swelling-induced decrease in $I_{sc}$ was reduced in the presence of the cyclooxygenase inhibitor, indomethacin, or the lipoygenase inhibitor, nordihydroguaiaretic acid. In the ileum the Cl⁻ secretion induced by hypotonicity was blocked by the K⁺ channel blocker quinine and was reversed into a decrease in $I_{sc}$ when serosal Ca²⁺ was zero. We conclude that the observed volume regulatory changes are initiated in the jejunum by an eicosanoid-mediated opening of basolateral Cl⁻ channels and in the ileum by a Ca²⁺-mediated opening of K⁺ channels which enhances apical Cl⁻ efflux.

Key words Absorption · Cl⁻ channels · Hypotonic stress · Rat small intestine · Secretion · Voltage scanning · Volume regulation

Introduction

Cell swelling is a well-known activator of Cl⁻ currents in many epithelia leading to the opening of volume-sensitive Cl⁻ channels [37]. The Cl⁻ channels stimulated by an increase in cell volume do not represent a homogeneous population. In the colonic tumour cell line, T₄₂, cell swelling leads to the activation of the intermediate-conductance outward-rectifier Cl⁻ channel [33] and of Cl⁻ channels with a very low (below 1 pS) single-channel conductance [13], whereas in the rat colon cell swelling activates a 20- to 30-pS Cl⁻ channel with a linear current-voltage relationship [6]. The localization of the swelling-induced Cl⁻ channels is not uniform in epithelia. In Necturus small intestine [9] and in T₄₂ cells [25], a volume-sensitive Cl⁻ conductance is present in the apical membrane leading to an enhanced Cl⁻ secretion upon cell swelling. In contrast, in Madin Darby canine kidney (MDCK) cells [32] and in the rat colon [6], the volume-sensitive Cl⁻ conductance resides in the basolateral membrane. Opening of volume-sensitive Cl⁻ channels, activated in parallel with volume-sensitive K⁺ channels (for review see [14]), allows an efflux of KCl out of the cell in order to normalize the increased cell volume after cell swelling, i.e. they mediate a regulatory volume decrease.
In addition to the role of these channels in epithelial cell volume homeostasis, volume-sensitive ion channels may also play a role in transepithelial electrolyte transport. For example, the basolateral volume-sensitive Cl\(^-\) channel in the rat colon is not only stimulated during cell swelling induced by hypotonic media [6], but also during cell swelling induced by stimulation of NaCl absorption by short-chain fatty acids [5] or by the proabsorptive hormone somatostatin [3]. Opening of this channel provides a pathway for basolateral exit of Cl\(^-\) ions and thereby contributes to the stimulation of Cl\(^-\) absorption. In the small intestine, volume-sensitive basolateral K\(^+\) channels are well known to be activated during Na\(^+\)-coupled sugar and amino acid absorption [19, 20, 31]. Although cell swelling has been shown to be followed by a regulatory volume decrease, which is sensitive to K\(^+\) and Cl\(^-\) channel blockers in small intestinal epithelial cells from guinea-pig [21], there is no information available about the distribution of the putative volume-sensitive Cl\(^-\) conductance in the small intestine. In the present study, which was performed in order to investigate the response of the isolated rat small intestine to a hypertonic medium, it turned out that the small intestine exhibits a pronounced segmental heterogeneity (see e.g. [27]) between jejunum and ileum.

Materials and methods

Solutions

Most of the experiments were carried out in a 107 mmol/l NaCl Parsons solution containing (mmol/l): NaCl 107, KCl 4.5, NaHCO\(_3\) 25, Na\(_2\)HPO\(_4\) 1.8, NaH\(_2\)PO\(_4\) 0.2, CaCl\(_2\) 1.25, MgSO\(_4\) 1 and glucose 12. The solution was gassed with carbon dioxide (5% CO\(_2\) in 95% O\(_2\)) and kept at a temperature of 37°C; pH was 7.4. In order to induce cell swelling, the 107 mmol/l NaCl Parsons solution was first replaced by a 57 mmol/l NaCl/100 mmol/l mannitol Parsons solution (isotonic) and then replaced by a 57 mmol/l NaCl Parsons solution (hypotonic) in order to avoid any change in the extracellular Cl\(^-\) concentration during induction of cell swelling. For the Cl\(^-\) free solution, NaCl was replaced by Na gluconate (NaGluc); the Ca\(^{2+}\) concentration in this solution was elevated to 5.8 mmol/l in order to compensate the Ca\(^{2+}\)-buffering properties of gluconate [15]. The Ca\(^{2+}\)-free Parsons solution was prepared by omitting CaCl\(_2\) without addition of a chelator.

Short-circuit current (I\(_s\)) measurement

Female SIVZ-50 (Institut für LaborTierkunde, Universität Zürich, Switzerland) were used for the Ussing chamber experiments. The animals weighed between 180 and 220 g. They had free access to water and food until the day of the experiment. Animals were stunned by a blow on the head and killed by exsanguination. A segment of the small intestine was mounted in a modified Ussing chamber [6], bathed with a volume of 4 ml on each side of the mucosa and short-circuited by a voltage clamp [35] with correction for subepithelial resistances. The exposed surface of the tissue was 1 cm\(^2\). I\(_s\) was continuously recorded and tissue conductance (G\(_s\)) was measured every minute. From I\(_s\) and G\(_s\) the potential difference (V\(_m\)) was calculated according to Ohm’s law.

Experimental protocol of I\(_s\) measurements

After an equilibration period of 30 min, the isotonic 107 mmol/l NaCl Parsons solution was replaced on both sides of the chamber with a Parsons solution with a decreased NaCl concentration and in which mannitol was present in order to maintain isotonicity. After additional 30 min, the isotonic mannitol solution was replaced by a solution without mannitol and a decreased NaCl concentration. In the last 30-min period, the electrical parameters were measured again in the isotonic 57 mmol/l NaCl/100 mmol/l mannitol Parsons solution. The osmolarity of the solutions was regularly checked using an osmometer (model V7024, Knauer, Bad Homburg, Germany). The baseline in the electrical parameters was determined as the mean value over 3 min just before a change in osmolarity. The changes in I\(_s\), G\(_s\) or V\(_m\) after a change of the osmolarity were measured, when they had reached a stable plateau; they are presented as the difference from the former baseline in the tables.

Voltage scanning

In order to localize the observed changes in G\(_s\), voltage scanning experiments were performed as described previously [10, 16, 17]. Briefly, female albino Wistar rats (180–220 g) were killed by inhalation of an overdose of ether. Ileal tissues were taken at 70 and 105 cm distal to the pylorus. Since there was no difference in the conductance responses, data from both locations were pooled. Then the lumen was perfused for 2–3 min with a 50 mmol/l dithiothreitol in 107 NaCl mmol/l Parsons solution to dispose the mucus, while the serosal site was submerged in 107 mmol/l NaCl Parsons solution. After rinsing the mucosal site as well as with Parsons solution, the muscular layer of the tissue was stripped as described before [17]. Tissues were then mounted mucosal side up into a horizontal four-electrode Ussing-type chamber (aperture 0.28 cm\(^2\)) [17]. The preparation was visualized with long-distance Nomarski-optics (20×), digitally processed (Argus 10, Hamamatsu, Herrsching, Germany) and displayed on a video monitor. To prevent vertical movement, the epithelium was supported from the serosal side by a fine nylon mesh. G\(_s\) was determined by conventional four-electrode technique (direct current pulses of ±20 μA).

For determining local conductances, a sine-wave alternating current (24 Hz, ±50 μA) was passed across the epithelium. The distribution of this current depends on the local conductance of the epithelial structures. To sense this local current over a defined distance a microelectrode was positioned in the center of a crypt opening and moved back and forth by a piezo-electric driver (20 μm, 0.4 Hz). Signals recorded at the forwards and backwards position of the stepping electrode were collected in a phase-locked loop amplifier which was locked in to the alternating clamp current. Both signals were rectified and subtracted from each other. The final result was about 0.5 μV. Precise and reproducible positioning of the scanning electrode was controlled for every single measurement in each experimental condition. For positioning the scanning electrode above a crypt opening, the electrode was placed above the crypt wall and lowered until barely touching the wall, as indicated by disturbances of the electrical signal. Then the electrode was moved horizontally under visual control to the centre of the crypt opening.

Two conductances were determined directly, G\(_s\) and the local conductance of the crypts (G\(^{c}\)). G\(^{c}\) was calculated from voltage scanning signals, fractional area, G\(_s\), and the conductance of the bathing fluid using equations published previously [17]. The complex morphology of the villus region precluded direct measurement and calculation of the local conductance of this region which consists of three parts at least, horizontal plateau-shaped villus tops, almost vertical villus flanks, and a small area of horizontal surface epithelium around the crypts. For that reason all non-crypt epithelia were pooled as the “villus region” and simply calculated by subtraction, G\(_s\)-G\(^{c}\). Voltage scanning experiments were not performed for the