Biochimica et Biophysica Acta, 1027 (1990) 257–263
Elsevier

BBAMEM 74954

Water handling in the human distal colon in vitro: role of Na^+, Cl^- and HCO_3^-

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(Received 6 February 1990)
(Revised manuscript received 28 May 1990)

Key words: Norepinephrine; cyclic AMP; Amiloride; Absorption; Secretion

The minute by minute net water movement (J_w) was measured, in the human distal colon in vitro, simultaneously with the transepithelial potential difference (PD) and short circuit current (SCC) with the following results: (1) An absorptive J_w ($+0.36 \pm 0.04$ $\mu l/(min \cdot cm^2)$) was observed, in 21 cases, when the colon was mounted between two identical standard salines (Na^+ 140, Cl^- 110, HCO_3^- 25 mequiv./l) and in the presence of a hydrostatic pressure gradient (ΔP) of 13 cm of H_2O (mucosal side positive). (2) This absorptive J_w was a linear function of the applied ΔP or the imposed osmotic transepithelial gradient ($P_{hydr} = 0.22 \pm 0.03$ cm/s; $P_{osm} = 0.0020 \pm 0.0005$ cm/s; $n = 6$). (3) A fraction of this J_w was independent of the presence of any hydrostatic, osmotic or chemical gradient while associated with a serosal side positive and partially amiloride sensitive PD (11.3 ± 1.8 mV). (4) Both J_w and PD were dependent on the presence of Na^+ in the incubating media. (5) Replacement of Cl^- by SO_4^{2-} did not change the absorptive J_w, but increased the observed PD and the transepithelial resistance. (6) HCO_3^- removal strongly reduced the SCC and PD together with an important increase in J_w. Unexpectedly, other 9 colon fragments spontaneously showed a secretory J_w when mounted between two identical standard salines (-0.55 ± 0.11 $\mu l/(min \cdot cm^2)$). In these experiments it was observed that: (7) The tissue moved water against the imposed ΔP (13 cm of H_2O), while the associated PD ($+11.9 \pm 2.1$ mV) was similar to the one observed in absorptive fragments. (8) As in the case of absorptive preparations, PD, SCC and the transport associated J_w fell to zero in the absence of Na^+. (9) When SO_4^{2-} replaced Cl^-, secretory J_w reversed to absorptive J_w, together with an increase in PD and resistance. In both absorptive and secretory preparations it was finally observed that: (10) norepinephrine ($5 \cdot 10^{-6}$ M) decreased SCC and increased the absorptive J_w in a tightly parallel manner (half-times for each response: SCC = 11.4 ± 2.1 min; $J_w = 11.4 \pm 2.0$ min, $n = 4$) and (11) 8-Br cyclic AMP (10^{-3} M) increased SCC while simultaneously decreasing the absorptive J_w. It is concluded that the observed J_w in the distal human colon in vitro results from the complex addition of osmotic, hydrostatic and transport associated driving forces. The transport-associated J_w has absorptive and secretory components. The secretory component could be associated with two different mechanisms: bicarbonate secretion or Cl^- secretion. This last mechanism, mediated by cyclic AMP, would be stimulated in spontaneously secretory tissues.

Introduction

Most previous works on human colon in vitro [1–6] have been centered on electrophysiological measurements (transepithelial potential difference (PD), and short circuit current (SCC)) associated with the study of isotopic ions movements [6]. These studies had shown that Na^+ absorption occurs due to both electrogenic Na^+ absorption [5] and electroneutral Na^+/Cl^- co-transport [6]. Bicarbonate and chloride secretion have also been described [4,6] and, more recently, the membrane conductance in proximal and distal segments was studied with microelectrodes, nystatin, ion chemical blockers and Cl^- replacement [7].

The human colon receives a daily volume load of some 2000 ml from the ileum while the fecal excretion is only 50 ml/day [8]. Nevertheless no systematic study has been done on water handling in this organ in vitro and many important points are not yet clarified: (1) What is the real coupling between ions and water in the large intestine? (2) What is the role of hydrostatic and osmotic gradients in the observed water movement? (3)

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What are the mechanisms controlling the switching between water absorption and secretion? To give an initial answer to the previously listed questions, we have now used an experimental approach, previously employed in other epithelial barriers [9,10], that allows the minute by minute recording of the net water movement across the tissue. These measurements were associated with PD and SCC determinations, in different experimental conditions.

Methods

Fragments of human distal colon were obtained from chirugically extirpated organs in patients with cancer. Inmediately after ablation, pieces of apparently non affected regions (from sigmoid and rectum) were washed and placed in a high K^+ saline (see later) at low temperature (4°C). Before the experiments the mucosa and submucosa layers were dissected from the underlying tissues (always at 4°C) and mounted as a diaphragm between two twin barrels lucite chambers (a nylon mesh was placed on the serosal surface). The chambers were then filled with standard saline at 37°C and immersed in a thermostated bath at the same temperature. In these conditions PD slowly increased and then stabilized in about 30 min. No significant differences were observed in PD between experiments started after 30, 130 or 230 min of incubation at low temperature. Considering these observations, in many experiments three different fragments from the same colon were consecutively mounted to test three experimental conditions (three fragments protocol).

The standard saline contained (mM): 114 NaCl, 4.5 KCl, 1.2 $CaCl_2$, 1.2 $MgCl_2$, 25 $NaHCO_3$, 5 glucose, 1.2 K_2HPO_4, 0.2 KH_2PO_4 (pH 7.4 when bubbled with 5% CO_2/95% O_2). High K^+, low Na^+ saline contained (mM): 120 KCl, 10 $NaHCO_3$, 1.2 $MgCl_2$, 1.2 $CaCl_2$, 1.2 K_2HPO_4, 0.2 KH_2PO_4, 25 glucose. In some experiments a Tris-Hepes buffer (Tris-chloride 7.5 mM, Na-Hepes 12.5 mM) replaced $NaHCO_3$ in the standard saline (pH 7.4 when bubbled with O_2). Hypertonic solutions were obtained by adding polyethyleneglycol (PEG, mol. wt. 4000) to the standard saline. Theoretical osmolarity was corrected by applying the corresponding osmotic coefficient (2.5, Ref. 11).

The net transepithelial water transfer (J_w) was minute by minute recorded as previously described in other epithelial barriers [10]. Briefly, the mucosal chamber was a closed one, where the tissue was applied against the nylon mesh by a variable hydrostatic pressure ($\Delta P \geqslant 5$ or more cm of H_2O). When water moved across the tissue, an automatic device injected or sucked water to maintain the volume constant. A signal, proportional to the injected or sucked volume, was minute by minute recorded. 50-nl variations in the absorptive or secretory fluxes could be detected (positive values indicate absorption and negative values secretion).

Voltage electrodes consisted of agar bridges connected to calomel half cells and placed adjacent to the epithelium. The transepithelial PD could be short-circuited through current passing electrodes (Ag-AgCl wires) located at the rear of each half chamber [12]. In experiments designed to evaluate the effects of ionic replacements, the electrical parameters were tested, for each fragment, first in the standard condition, second in the tested one during which J_w was simultaneously measured, and finally in the standard condition again.

The hydraulic (P_{hydr}) and osmotic (P_{osm}) permeability coefficients

The J_w across a membrane in the presence of a hydrostatic (ΔP) or osmotic (Δosm) gradient is described by

$$J_w = L_p(\text{hydr}) \cdot \Delta P$$

and

$$J_w = \sigma \cdot L_p(\text{osm}) \cdot \Delta\text{osm}$$

where L_p(hydr) and L_p(osm) are phenomenological coefficients. σ is the Staverman reflexion coefficient and ΔP and Δosm are expressed in units of pressure. If J_w is measured in mol/(cm^2 · s), P_{hydr} and P_{osm} can be defined:

$$P_{hydr} = L_p(\text{hydr}) \cdot R \cdot T / V_w$$

$$P_{osm} = L_p(\text{osm}) \cdot R \cdot T / V_w$$

where R and T have the usual meanings and V_w is the volume of one mole of water. Both coefficients are expressed in units of cm/s. On these bases P_{hydr} and P_{osm} can be calculated from the slope of the regression line obtained when J_w values are plotted against ΔP or Δosm.

Results

Fig. 1 shows the minute by minute simultaneously recorded J_w and SCC across the human distal colon in vitro. A net absorptive flux ($+0.36 \pm 0.04$ μl/(min · cm^2), mean ± S.E., $n = 21$) was observed in most cases (see later). The tissue was mounted between two identical standard solutions and under a ΔP of 13 cm of H_2O (mucosal side positive). The associated transepithelial PD was, in these conditions, 11.3 ± 1.8 mV (serosal side positive). This absorptive J_w varied in accordance with the applied hydrostatic or osmotic gradients or when the ionic composition of the medium was changed (Fig. 1). Replacement of Na^+ by choline$^+$ on both the mucosal and serosal sides strongly reduced the SCC and

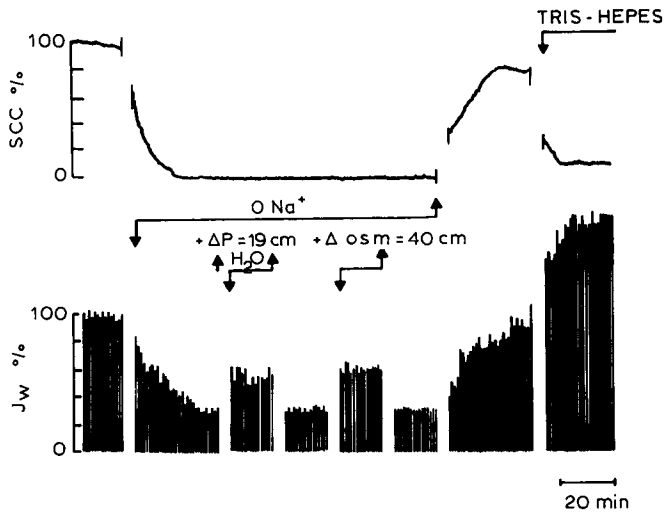

Fig. 1. Short circuit current (SCC) and absorptive net water flux (J_w) simultaneously measured in the human colon mounted between two identical standard salines and under a hydrostatic pressure (ΔP) gradient (mucosal side positive) of 13 cm of H_2O. Effects of Na^+ removal, changes in ΔP (+19 cm of H_2O), transepithelial osmotic gradient (+40 mosM, serosal PEG) and HCO_3^- removal.

the net absorptive J_w. This effect was fully reversible. PD and SCC were partially sensitive to mucosal amiloride 10^{-4} M (mean inhibition 25 ± 8%, $n = 3$).

The hydraulic and osmotic permeabilities

Fig. 2 represents the observed J_w as a function of the applied hydrostatic or osmotic transepithelial gradients. In both cases linear correlations were obtained and P_{hydr} (0.22 ± 0.03 cm/s, mean + S.E., $n = 6$) and P_{osm} (0.0028 ± 0.0005 cm/s, mean + S.E., $n = 6$) were calculated in each case from the slope of the regression line (see Methods). The intercept represents, in the case of ΔP vs. J_w, the J_w observable in the absence of any osmotic, chemical or hydrostatic gradient, and probably indicates the absorptive J_w associated with the ionic active transport.

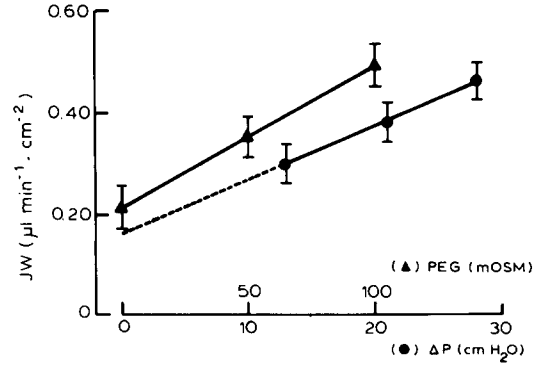

Fig. 2. Net absorptive water flux (J_w) as a function of the applied osmotic or hydrostatic gradients.

TABLE I

Transepithelial PD, SCC, resistance (R) and J_w in the human (absorptive) distal colon

Three different media were tested: standard buffer, no-sodium and no-chloride (see Methods). Means ± S.E. ($n = 6$). The hydrostatic component of J_w (0.20 µl/(min·cm²)) was deduced.

	Standard	No-sodium	No-chloride
PD (mV)	8.4 ± 1.4	0.5 ± 1.6	13.8 ± 3.9 * (ΔPD = 5.4 ± 2.1)
SCC (µA/cm²)	83 ± 10	1.3 ± 6.7	87 ± 12
R (ohm·cm²)	101 ± 16	-	159 ± 27 * (ΔR = 58 ± 18)
J_w (µl/(min·cm²))	0.16 ± 0.03	0.04 ± 0.13	0.17 ± 0.05

* $P < 0.05$, t-test for paired data.

The role of Na^+, Cl^- and HCO_3^- ions in the observed J_w

To understand the role of Na^+, Cl^- and HCO_3^- in the observed J_w, the 'three fragments protocol', as described in Methods, was employed. Three different conditions were tested in a first experimental series (all ionic changes were simultaneously and symmetrically made on both sides of the tissue): (1) The standard saline; (2) NaCl was replaced by choline-Cl and other Na^+ salts by K^+ salts; (3) Cl^- salts were replaced by SO_4^{2-} salts. The obtained results are presented in Table I. It can be observed that: (1) Removal of Na^+ made PD and SCC not significantly different from zero together with a strong reduction in J_w. (2) Replacement of Cl^- by SO_4^{2-} did not change significantly the absorptive J_w, but induced a significant increase in PD and transepithelial resistance.

In a second series employing the 'three fragments protocol', the following experimental situations were compared: (1) the standard saline; (2) Tris-Hepes replaced bicarbonate-buffer, and (3) SO_4^{2-} salts replaced Cl^- salts in Tris-Hepes buffers. The obtained results are summarized in Table II. It can be observed that: (1) in the absence of HCO_3^- both PD and SCC were strongly

TABLE II

Transepithelial PD, SCC, resistance (R) and J_w in the human (absorptive) distal colon

Three different media were tested: standard buffer, Tris-Hepes replacing $NaHCO_3$ and Tris-Hepes replaced $NaHCO_3$ plus SO_4^{2-} replacing Cl^- in both the mucosal and serosal baths. Means ± S.E. ($n = 6$).

	Standard	No-bicarbonate	No-chloride, no-bicarbonate
PD (mV)	9.4 ± 3.4	2.9 ± 1.6	9.8 ± 2.9
SCC (µA/cm²)	78 ± 17	24 ± 5	58 ± 11
R (ohm·cm²)	120 ± 14	122 ± 28	169 ± 15 * (ΔR = 49 ± 15)
J_w (µl/(min·cm²))	0.15 ± 0.05	0.37 ± 0.13	0.25 ± 0.03 * (ΔJ_w = 0.10 ± 0.04)

* $P < 0.05$, t-test for paired data.

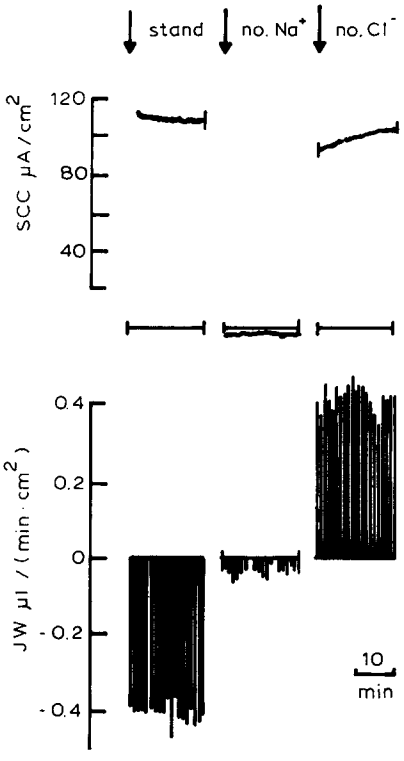

Fig. 3. Simultaneous record of J_w and SCC in a spontaneously secretory colon. Effects of replacement of Na^+ by choline$^+$ and Cl^- by SO_4^{2-}.

reduced, together with an important increase in J_w and no change in transepithelial resistance, and (2) in the absence of both HCO_3^- and Cl^- J_w was higher than in the standard condition, together with an increase in the transepithelial resistance.

Spontaneous secretory J_w in the human distal colon in vitro

Previous presented results can be understood, as it will be discussed later, if the net absorptive J_w is presented as the algebraic addition of a major absorptive J_w and a minor secretory J_w. Nevertheless some preparations showed, when mounted between two identical standard salines, a spontaneous net secretory J_w. It must be remarked that in this case the tissue was moving water against the imposed hydrostatic gradient (13 cm of H_2O). Furthermore, when three fragments from the same organ were consecutively mounted, they consistently showed absorptive or secretory J_w values. Fig. 3 presents the simultaneous recorded J_w and SCC in a secretory experiment. The mean observed PD was 11.9 ± 2.1 mV and the J_w -0.55 ± 0.11 $\mu l/(min \cdot cm^2)$ ($n = 9$). Interesting enough the observed PD and SCC were similar to the PD and SCC seen in absorptive tissues and the only way to differentiate both situations was the simultaneous measurement of J_w.

The 'three fragments protocol' was also employed with the secretory tissues. The tested conditions were:

TABLE III

Transepithelial PD, SCC, resistance (R) and J_w in the human (secretory) distal colon

Three different media were tested: standard buffer, no-sodium and no-chloride (see Methods). Means ± S.E. ($n = 6$). The hydrostatic component of J_w (0.20 $\mu l/(min \cdot cm^2)$) was deduced.

	Standard	No-sodium	No-chloride
PD (mV)	9.8 ± 1.9	−0.7 ± 1.2	17.6 ± 2.6 * (ΔPD = 7.8 ± 2.1)
SCC ($\mu A/cm^2$)	110 ±17	−3.9 ±11.1	104 ±22
R (ohm·cm^2)	89 ±11	-	169 ±14 * (ΔR = 80 ± 18)
J_w ($\mu l/(min \cdot cm^2)$)	−0.60 ± 0.06	0.04 ± 0.09	0.22 ± 0.05

* $P < 0.01$, t-test for paired data.

(1) the standard saline, (2) no sodium in the media, and (3) SO_4^{2-} ions replaced Cl^- ions. It was observed that (Table III); (1) as in the case of absorptive preparations, PD, SCC and J_w fell to zero in the absence of Na^+, and (2) when SO_4^{2-} replaced Cl^- ions J_w reversed from secretion to absorption, together with an increase in PD and transepithelial resistance.

The effect of norepinephrine and cyclic AMP on J_w and SCC

It has been previously reported that epinephrine elicits a distinct decrease in SCC in the human colon in vitro, with no change in the unidirectional Na^+ and Cl^- fluxes [6]. We have now tested the effects of norepinephrine ($5 \cdot 10^{-6}$ M) on the simultaneously determined J_w and SCC. It can be observed in Fig. 4 (mean curve for four experiments) that the decrease in

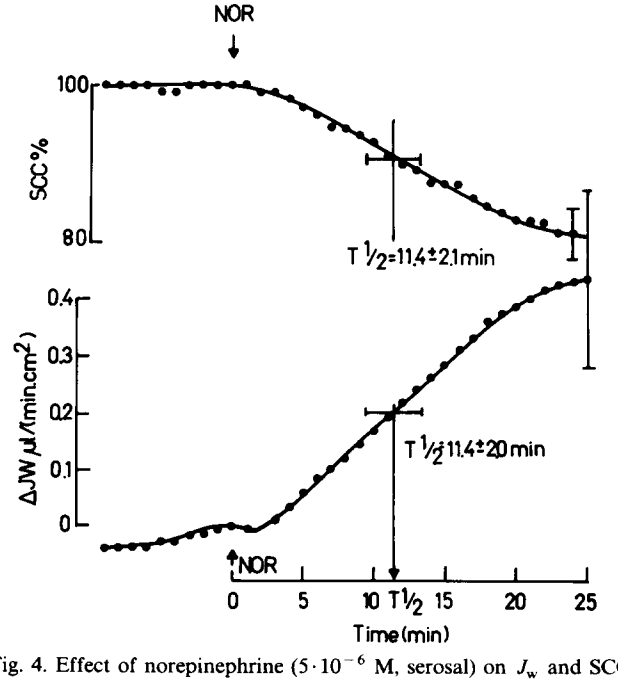

Fig. 4. Effect of norepinephrine ($5 \cdot 10^{-6}$ M, serosal) on J_w and SCC simultaneously recorded in four experiments (SCC: % of the control value).

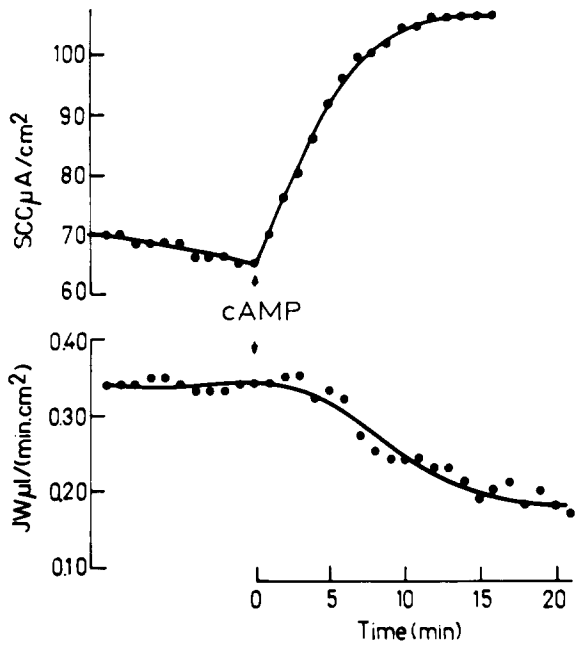

Fig. 5. Effects of 8-Br cyclic AMP (cAMP, $1 \cdot 10^{-3}$ M, serosal) in the simultaneously recorded J_w and SCC in a representative experiment.

SCC was clearly in parallel with an increase in J_w. The mean half-times for both processes were, respectively, 11.4 ± 2.1 min and 11.4 ± 2.0 min. Two of the tested tissues were initially secretory ones, and norepinephrine reversed this situation. The mean observed variations in J_w, PD, SCC and resistance are presented in Table IV

It has also been reported that theophylline, a cyclic AMP mediated secretagogue, increases SCC and induces Cl^- secretion in the distal human colon in vitro [6]. We have now simultaneously studied SCC and J_w under the action of a potent analog of cyclic AMP: 8-Br cyclic AMP, 10^{-3} M. Fig. 5 shows a typical record in which the effect of the nucleotide on SCC and J_w was simultaneously tested. It can be observed that the increase in SCC was now paralleled with a decrease in the absorptive J_w (mean values in Table IV). These results were accompanied by a significant increase in transepithelial resistance (Table IV).

Discussion

Osmotically and hydraulically drived J_w in the human distal colon

J_w was, in the human distal colon in vitro, a linear function of the applied transepithelial or osmotic gradients. The observed values were similar to those reported in the rate caecum [13], and P_{hydr} and P_{osm} values would indicate that hydrostatic pressure was 100-times more effective than the osmotic gradient to move water across the human colon in vitro. It must be, however, considered that our osmotic measurements were probably affected by the 'sweeping away' and 'solute polarization' phenomena [14], associated with the presence of unstirred layers [15].

It is generally accepted that hydrostatic pressure, in the range employed in this study (24.4 cm $H_2O = 1$ mosM), does not move water transcellularly [16]. In the case of the osmotic gradients or transport associated fluxes, water can probably be moved either between or through the cells [17]. We have recently studied the relative contribution of paracellular and transcellular movements to water transfer in the human amnion [12]. Further experiments are necessary to clarify the situation in the human colon.

Transport associated J_w in the human distal colon

Present observations on the transport associated J_w can be accomodated in the frame of previous reports on ionic movements in the human colon [6], and the proposed mechanisms are shown in Fig. 6. In the upper cell (A) an electrogenic and amiloride sensitive entry of Na^+ in the mucosal border is coupled with the Na^+/K^+-ATPase present in the serosal membrane [2,4]. Cl^- moves across a low resistance paracellular path [7]. The non-electrogenic entry of Na^+ plus Cl^- ions [18] is proposed in the second cell (B). Both mechanisms (A and B) result in NaCl reabsorption. This salt movement will drive a net absorptive J_w that we have represented as partially moving transcellularly and partially paracellularly. The importance of paracellular vs. transcellular routes in the transport associated net water movement remains an open question.

TABLE IV

Effects of norepinephrine and 8-Br cyclic AMP on transepithelial PD, SCC, resistance and J_w in the human distal colon

t-test for 'before–after' conditions (mean ± S.E., $n = 6$).

	ΔJ_w ($\mu l \cdot min^{-1} \cdot cm^{-2}$)	ΔPD (mV)	ΔSCC ($\mu A \cdot cm^{-2}$)	ΔR ($\Omega \cdot cm^2$)
Norepinephrine ($5 \cdot 10^{-6}$ M)	$+0.43 \pm 0.13$	-0.90 ± 0.37	-13.4 ± 5.2	-1.0 ± 5.7
P	< 0.025	< 0.05	< 0.05	n.s.
8-Br cyclic AMP ($1 \cdot 10^{-3}$ M)	-0.12 ± 0.03	$+1.43 \pm 0.48$	$+2.26 \pm 3.40$	$+24.7 \pm 7.4$ *
P	< 0.01	< 0.05	n.s.	< 0.025

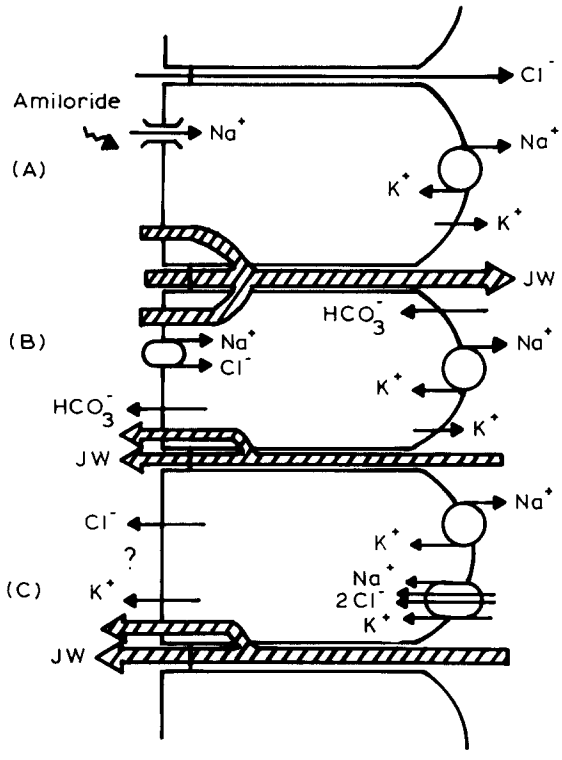

Fig. 6. Proposed model, based on present and previous results, to understand water handling in the human distal colon in vitro.

The absorptive J_w was, in our experimental conditions, dependent on the presence of Na^+ in the incubation media. This was not unexpected if Na^+ transport is driving water reabsorption. Replacement of Cl^- by SO_4^{2-} did not change significantly the absorptive J_w and SCC. Nevertheless, the membrane PD and resistance went up in this situation, indicating that SO_4^{2-} had a lower permeability than Cl^-, as it was also observed in other tissues.

Bicarbonate secretion has been described in the human colon in vivo [19] and it has also been postulated in the human colon studied in vitro [6]. We have added this mechanism in cell B, coupled to a HCO_3^- entry in the basolateral membrane. This hypothetical mechanism allows to explain the effects of HCO_3^- removal in our experiments: a reduction in SCC together with an increase in the absorptive J_w. This mechanism, as previously proposed [6], would not be Cl^- dependent.

Hence, it can be concluded that the net absorptive J_w observed in most experiments results from a major absorbing fraction, coupled to NaCl absorption, and from a minor secretory fraction, coupled to bicarbonate secretion. Both mechanisms need Na^+ to be operative (SCC and J_w fell to zero in the absence of this ion).

According to the previous model, the observed J_w must be purely absorptive in the absence of bicarbonate and Cl^- in the media. From the observed short circuit current (58 $\mu A/cm^2$) and net water flux (0.25 $\mu l/(min \cdot cm^2)$), the ionic concentration of the transported fluid can be calculated about 136 mequiv./l. This would indicate that NaCl reabsorption was not far from isotonicity in this experimental condition. Nevertheless, there is considerable evidence in the literature indicating that the colon is able to absorb against an osmotic gradient [20,21].

Bicarbonate secretion and the action of norepinephrine

Epinephrine reduces SCC in the human colon in vitro together with no changes in Na^+ or Cl^- unidirectional fluxes [6]. These results have been interpreted as due to an alteration in the transport of another ion, most probably to an inhibition of bicarbonate secretion [6]. We have now observed that norepinephrine induced a decrease in SCC tightly paralleled (Fig. 4) by an increase in the absorptive J_w. These results are also compatible with an inhibitory effect on bicarbonate secretion. From data presented in Table IV we can calculate the amount of water coupled to HCO_3^- secretion, if we accept that the increase in the net absorptive J_w was due to a reduction in the secretory component of the net water movement. An increase in J_w of 0.43 $\mu l \cdot min^{-1} \cdot cm^{-2}$ was coupled with a reduction of 13.4 $\mu A/cm^2$ in SCC. This would indicate an ionic concentration of 16 mequiv./l, which is not significantly different from the bicarbonate concentration here employed.

The action of cyclic AMP and the presence of a net secretory J_w

It has been previously demonstrated that cyclic AMP [22] and theophylline [6] stimulate Cl^- secretion in the mammalian colon. We have now observed, under the nucleotide action, an increase in the secretory J_w together with an increase in transepithelial PD and SCC (Fig. 5, Table IV).

The situation described in C (Fig. 6), also based in previous information available on the human colon [23,6], can be useful to understand both the action of cyclic AMP and the spontaneously secretory J_w observed in some experiments: A non electrogenic $Na^+/K^+/2Cl^-$ symport in the serosal border [24] is coupled with Cl^- and K^+ secretion in the mucosal border. This system is similar to the one described under K^+ adaptation in the rat colon [18,25], and is dependent on the present of Cl^- ions and sensitive to cyclic AMP stimulation [22].

The observation, in some tissues, of a spontaneously net secretory J_w was a rather unexpected result. It must be remarked here that PD and SCC were similar in absorptive and secretory fragments. We propose a working hypothesis to understand these observations: Cl^- secretion, via the cyclase system, would be stimulated in these patients. This is supported by the fact that secretory fragments switched to absorptive ones in the absence of Cl^- as well as by the effects of cyclic AMP

(Table IV, Figs. 3 and 5). Cl^- secretion would be operating at a low level in net absorptive fragments.

The role of the paracellular and submucosal hydraulic conductances in the observed phenomena

In the previous sections it has been considered that both paracellular and submucosal hydraulic conductances were constant in the different tested conditions. Nevertheless, there is experimental evidence showing that this is not always the case. Ionic substitution could affect paracellular hydraulic conductivity. It has been observed, however, in the rat colon, that HCO_3^- or Cl^- or Na^+ substitution do not change the magnitude of the J_w induced by applying a hydrostatic pressure up to 30 cm of water on the mucosal side [26]. This situation can not probably be compared with the case in which the hydrostatic pressure is applied on the serosal surface [27].

Naftalin and Simmons [28] have found that theophylline and cyclic AMP raised Cl^- conductance relative to Na^+ conductance in the small intestine. We have observed a decrease in the absorptive J_w under the nucleotide action. This could then be interpreted (alternatively to an increase in transcellular secretion) as due to an enhanced reflux of NaCl via the increased paracellular conductance in this situation. This hypothesis is, however, difficult to conciliate with the simultaneously observed increase in SCC (Fig. 5).

Ahsan et al. [29] have observed an α_2-adrenergic dependent increase in rabbit ileal water flow due to enhanced submucosal hydraulic conductance, which was accompanied by a small decrease in tissue resistance. If this effect can be observed in colon, it might provide an alternative explanation to the one proposed here for the adrenaline dependent increase in J_w. However, it must be difficult to understand the parallel reduction in short circuit current (Fig. 4).

In summary, it can be accepted that the observed net water movement in the human distal colon results from a transport associated absorptive J_w, a transport associated secretory J_w, an osmotically drived J_w and a hydrostatically drived J_w. The secretory J_w would be associated with two different ions: HCO_3^- secretion or Cl^- secretion. This complex situation can be, however, understood on the bases of previous available information on ionic movements in the mammalian colon. Future experiments will clarify the different ionic mechanisms and whether the corresponding absorptive or secretory J_w can be correlated with specific anatomic structures.

Acknowledgements

This study was supported by grants from Consejo Nacional de Investigaciones Cientificas y Tecnicas (CONICET), Universidad de Buenos Aires and Fundacion Roemmers, Argentina. This work was performed in the frame of a cooperative program (CONICET-INSERM).

References

1 Archampong, E.Q., Harris, J. and Clark, C.G. (1972) Gut 13, 880–886.
2 Grady, C.F. Duhamel, R.C. and Moore, E.W. (1970) Gastroenterology 59, 585–588.
3 Rask-Madsen, J. and Hjelt, H. (1977) Scand. J. Gastroenterol. 12, 1–6.
4 Hawker, P.C., Mashiter, K.E. and Turnberg, L.A. (1978) Gastroenterology 74, 1241–1247.
5 Wills, N.K., Alles, W.P., Sandle, G.I. and Binder, H.J. (1984) Am. J. Physiol. 247, G749–G757.
6 Sellin, J. and De Soignie, R. (1987) Gastroenterology 93, 441–448.
7 Sandle, G.I. and McGlone, F. (1987) Pflügers Arch. 410, 173–180.
8 Luckhoff, A. and Horster, N. (1984) Korn. Wochenschr. 62, 555–563.
9 Bourguet, J. and Jard, S. (1964) Biochim. Biophys. Acta 88, 442–444.
10 Parisi, M., Ibarra, C. and Porta, M. (1987) Biochim. Biophys. Acta 905, 399–408.
11 Porta, M., Capurro, C. and Parisi, M. (1989) Biochim. Biophys. Acta 980, 220–224.
12 Parisi, M. and Cancela, L. (1988) Kidney Int. 33, 58–63.
13 Capurro, C., Escobar, E., Ibarra, C., Porta, M. and Parisi, M. (1989) Biol. Cell. 66, 145–148.
14 Wright, E.M., Smulders, A.P. and Tormey, J.M. (1972) J. Membr. Biol. 7, 198–219.
15 Van Os, C.H., Widner, G. and Whright, E.M. (1979) J. Membr. Biol. 49, 1–20.
16 Fischbarg, J., Warshawsky, C.R. and Lim, J.J. (1976) Nature 266, 71–73.
17 Carpi-Medina, P., Leon, V., Espidel, J. and Whittembury, G. (1988) J. Membr. Biol. 104, 35–43.
18 Hayslett, J.P., Miketey, N., Binder, H.J. and Aronson, P.S. (1980) Am. J. Physiol. 239, F378–F382.
19 Davis, G.R., Morawsky, S.G. and Santa Ana, C.A. (1983) J. Clin. Invest. 71, 201–207.
20 Harris, J. and Shield, R. (1970) Gut 11, 27–33.
21 Parsons, D. and Paterson, C. (1965) Q. J. Exp. Physiol. 50, 220–230.
22 McCabe, R.D. and Smith, P.L. (1985) Am. J. Physiol. 248, G103–G109.
23 Fondacaro, J.D. (1986) Am. J. Physiol. 250, G1–G8.
24 Heintze, K., Stewart, C.P. and Frizzel, R.A. (1983) Am. J. Physiol. 244, G357–G365.
25 Knauf, H. and Haag, H. (1986) Pflügers Arch. 407, 585–589.
26 Escobar, E., Ibarra, C., Todisco, E. and Parisi, M. (1990) Am. J. Physiol., in press.
27 Wanitschke, R., Nell, G. and Rummel, W. (1977) Arch. Pharmacol. 297, 191–194.
28 Naftalin, R. and Simmons, N. (1979) J. Physiol. 290, 331–350.
29 Ahsan, M., Naftalin, R. and Smith, P. (1988) J. Physiol. 404, 385–404.