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Intestinal Iodide Secretion and its Dependence Upon Mucosal I^- Permeability

A. Ondarra, A. Ilundain and J. Larralde

Departamento de Fisiologia Animal
Facultad de Farmacia
Universidad de Navarra
31008-Pamplona

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Iodide secretion across different regions of rat small intestine has been investigated *in vitro* using the standard Wilson-Wiseman technique. Net I^- secretion was observed along the entire small intestine, being significantly higher in the central region.

Anaerobic conditions, ouabain (2 mM) and Na^+ free Ringer solution prevented net I^- secretion, whilts, both theophylline (1 mM) and carbachol (0,1 mM) enhanced the observed basal intestinal I^- secretion. Furthermore, Ca^{2+}-deprived bathing solutions significantly reduced intestinal I^- secretion.

Epithelial I^- uptake from both mucosal and serosal sides was measured by using a Ussing-type chamber technique. The initial rate of I^--uptake across the mucosal membrane was significantly higher in the central region than in the proximal part of rat small intestine. No significant differences were observed in the rate of I^--uptake from the serosal side.

These studies suggest that mucosal I^- permeability might determine the direction of net I^- intestinal transport and that cytosolic Ca^{2+} may be a physiological regulator of intestinal I^- transport.

Key words: Ca^{2+}, Carbachol, Ouabain, Secretagogues, Theophylline.

Secretagogues such as teophylline, choleragen, A23187, act upon mammalian small intestine to change it from a tissue which absorbs fluid, Na^+ and Cl^- into one where fluid and electrolytes are secreted into the luminal, or mucosal bathing solution. However the processes involved in this phenomenon have not been well characterized. Some workers (8, 9) consider that net intestinal secretion results from inhibition of an absorptive process, as well as from stimulation of a secretory one involving an increase in Cl^- conductance across the luminal border. These two ion transport processes are thought to take place in different intestinal cell populations, the former (the inhibition of the coupled absorption of NaCl), in the villus portion of the epithelium and the latter in the crypts.

The observations made by other investigators (13, 14, 17) however, are inconsistent with the view that secretion results from inhibition of coupled NaCl uptake across the brush border, and that only an increase in Cl^- conductance across the

mucosal border of the enterocytes would be responsible for the intestinal secretion of fluid and electrolytes.

Several years ago ACLAN and ILLMAN (2) reported that the central region of rat small intestine transported iodide against a concentration gradient from the serosal to the mucosal bathing solution, whilts net iodide secretion was very small and even negligible in the rest of the small intestine. Therefore as different regions of the intestine appear to differ in their I^- transport characteristics, rat small intestine seems to be a suitable preparation to study processes involved in intestinal electrolytes secretion.

Materials and Methods

Animals Ringer solutions. Wistar-strain rats (130-150 g) of either sex were anesthetized with subcutaneous injections of 13 % urethane (1 ml × 100 g^{-1}). The Ringer's solution contained, in mM: 140 NaCl, 10 $KHCO_3$, 0,4 KH_2PO_4, 2.4 K_2HPO_4, 1.2 $CaCl_2$, 1.2 $MgCl_2$; 3 μM IK and 0.1 μCi/ml Na ^{125}I. Calcium-deprived bathing solutions were made by omitting $CaCl_2$ and replacing it with mannitol. Na^+-free Ringer's solution was obtained by replacement of NaCl by choline Cl.

Transport measurements. The whole rat small intestine beginning from the duodenal end was removed and rinsed with ice-cold standard Ringer's.

The intestine was divided into three portions —1, 2 and 3—, of approximately equal size (numbered from the duodenal end). Two everted sacs were prepared from each portion according to the procedure of WILSON and WISEMAN (19).

Ringer's solution (1 ml) was placed within the sacs. After 45 min incubation in 10 ml mucosal bathing solution, maintained at 37 °C and gassed with 95 % O_2: 5 % CO_2, the sacs were carefully blotted, weighed, opened and weighed again. The amount of radioactivity present in the original Ringer's solution, and in the serosal and mucosal fluids at the end of the experiment, was measured in a γ-scintillation counter. Tissue was homogenized in 0.1 N nitric acid and the radioactivity of the supernatant was also measured.

Secretion is expressed as total nmol of I^- disappeared from the serosal fluid per gram of wet tissue.

When studying I^- absorption only $^{125}I^-$ was present in the mucosal bathing solution, the absorption being expressed as total nmols gained by the serosal solution per gram of tissue.

Epithelial I^--uptake measurements. The intestine was opened along its mesenteric border, rinsed with ice-cold Ringer's and mounted as a flat sheet in Ussing type chambers. The bathing solutions on the mucosal and serosal surfaces of the tissues were maintained at 37 °C using a circulating water-bath as described previously (14) and gassed with 95 % O_2/ 5 % CO_2. At 20 or 25 minutes after mounting tissues, 3 μM IK and 0,1 μCi/ml $^{125}I^-$ were added either to the mucosal bathing solutions to measure mucosal I^- uptake, or to the serosal solution to measure serosal I^- uptake. Thirty minutes after mounting the tissue, the tissues were punched out and their radioactivity was extracted and assayed as previously described. Wet weights were obtained prior to extractions.

Ouabain, theophylline, carbachol and 2,4-α-dinitrophenol were obtained from the radioachemical Centre, Amersham.

The significance of differences between mean values were analyzed using Student's t-test. Results are shown as means ± S.E.

Results

Iodide transport by everted sacs of rat small intestine. The total amount of iodide disappeared from the serosal side of everted sacs, prepared from different regions of rat small intestine, was determined after 45 minutes of incubation. The results (table I) show that the entire rat small intestine secreted iodide. However significant differences were observed among the different regions of the small intestine. Maximal net iodide secretion took place in the central region, and no significant differences were observed between the secretion of iodide across either the initial or distal part of the small intestine. The opposite was observed when tissue iodide content was evaluated. The total amount of iodide within the tissue was lower in the central region than in the rest of the small intestine.

Table I. *Iodide transport by everted sacs of rat small intestine.*

Sacs were incubated for 45 min at 37 °C in Ringer's solution containing 3 μM IK, labelled with $^{125}I^-$ and gassed with 95 % O_2 + 5 % CO_2. The results are the means ± S.E. of twelve experiments. Intestinal segments were numbered from the duodenal end. Significant test determined by Student's-t-test.

	nmol I^- × g^{-1} w.w.	
Segment	Secretion	Tissue content
1	1.68 ± 0.15	2.03 ± 0.09
2	3.76 ± 0.13 *	1.77 ± 0.08 *
3	1.52 ± 0.15	2.28 ± 0.19

Central region vs. rest of intestine: * $p < 0.001$.

Iodide absorption across proximal, central and distal region of rat small intestine. To study iodide absorption by everted sacs of rat small intestine, $^{125}I^-$ was only present in the mucosal bathing solution. The results obtained in these experiments were opposite to those observed when net iodide secretion was evaluated. Thus, iodide absorption was lower in the central region (2.5 ± 0.08 nmol I^- g^{-1} w.w.) than in, either the proximal (4.0 ± 0.1 nmol g^{-1} w.w.) or distal part (3.7 ± 0.1 nmol g^{-1}) of the small intestine.

Epithelial I^- uptake. The findings so far discussed seem to support the view that a less efficient absorptive mechanism for iodide could be responsible for the greater iodide secretion found in the central region of rat small intestine.

However, a lower iodide absorption would also be expected in the central region, if the mucosal iodide permeability in this part of the intestine were higher than in the rest of the small intestine. Higher mucosal iodide conductance would induce higher backflux of iodide from within the tissue into the mucosal side. Increased iodide backflux would be indistinguishable operationally from a low iodide absorption, except that with low influx the initial rate of iodide uptake should be lower in the central region than in the rest of the small intestine.

To find out whether the differences in net iodide transport, observed along rat small intestine, were due to differences either in the efficiency of the absorptive process or in mucosal iodide permeability, we examined mucosal iodide uptake in both proximal and central part of rat small intestine. We also measured epithelial iodide uptake from the serosal side. The initial rate of mucosal iodide uptake was higher in the central region than in the proximal part of rat small intestine (fig. 1). No significant differences were observed in epithelial iodide uptake from the serosal side.

Effect of metabolic inhibitors on intestinal I^- secretion. As ACLAND and ILLMAN reported (2), I^- transport was negligible under anaerobic conditions, or in the presence of 2,4-α-Dinitrophenol (table II).

Effect of Ringer Na^+ replacement and ouabain on intestinal I^- secretion. Table III summarizes the effects of replace-

Table II. *Effect of anoxia and 2,4-α-Dinitrophenol (10^{-4}M), on net iodide secretion across the central region of rat small intestine.*
2,4-α-Dinitrophenol was present in both, serosal and mucosal bathing solutions, from the beginning of the experimental period. Values are mean ± S.E., in parenthesis the number of animals studied.

Experimental conditions	nmol $I^- \times g^{-1}$ w.w.	
	Secretion	Tissue content
Control	3.76 ± 0.13 (25)	1.77 ± 0.08 (25)
Anaerobic (95 % N_2 + 5 % CO_2)	0.01 ± 0.01 (6) *	2.35 ± 0.05 (6) *
2,4-α-dinitrophenol (0.1 mM)	0.63 ± 0.12 (6) *	2.42 ± 0.15 (6) *

Significance levels obtained by using Student's t-test. Test vs. control: * $p < 0.001$.

ment of Ringer Na^+ on intestinal I^- secretion. Removal of Na^+ from the mucosal solution abolished net iodide secretion. When Na^+ was absent from the serosal bathing solution net iodide secretion was partially inhibited. This latter finding would suggest that I^- entry across the serosal border might be also linked to Na^+ as has been suggested for Cl^- (3, 11, 12). In the absence of Na^+ from both bathing solutions net I^- transport was reversed. As can be seen the effect of either mucosal or serosal Na^+ replacement were additive and equal to that found when Na^+ was omitted from both bathing solutions.

Ouabain (table IV) also, inhibited intestinal I^- secretion, which suggests that secretion is dependent on the function of the Na^+-pump tissue, which is known to be relatively ouabain resistant in rat small intestine.

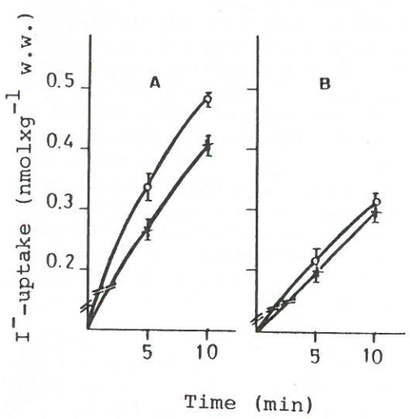

Fig. 1. *I^--uptake across either the mucosal (A) or serosal (B) membrane, of either the proximal (X) or central (o) region of rat small intestinal, as a function of time.*
Values are means ± S.E. of 8 independent determinations.

Table III. *Effects of replacement of Na^+ in Ringer's solution with choline on net I^- secretion across the central region of rat small intestine.*
Na^+ was absent from either mucosal (m), or serosal (s) or from both (m + s) bathing solutions. Values are the means ± S.E. of ten independent observations.

Secretion (nmol $I^- \times g^{-1}$ w.w.)			
Control	m	s	m + s
3.8±0.3	0.76±0.6 *	2.3±0.1 *	—0.77±0.2 *

Significant test determined by Student's t-test. Test vs. control: * $p < 0.001$.

Table IV. *Effect of ouabain on net iodide secretion across the central region of rat small intestine.*
Ouabain was present in both mucosal and serosal solution from the beginning of the experimental period. Values are the means ± S.E. In parenthesis the number of animals studied.

Ouabain (mM)	nmol $I^- \times g^{-1}$ w.w.	
	Secretion	Tissue content
0	3.60 ± 0.01 (16)	1.70 ± 0.06 (16)
2	0.57 * ± 0.28 (8)	2.67 * ± 0.17 (8)
4	0.35 * ± 0.12 (8)	3.15 * ± 0.18 (8)

Test vs. control: * $p < 0.001$.

Effect of secretagogues and Ca^{++} on I^- secretion by everted sacs of rat small intestine. Table V shows that both theophylline (1 mM) and carbachol (0.1 mM) further increased I^- secretion across the central region of rat small intestine. These results are consistent with their known effects on Cl^- secretion across mammalian small intestine. Tissue I^- content was decreased in the presence of either theophylline or carbachol as could be expected if these agents increased mucosal anion conductance.

A role for calcium as regulator of intestinal electrolyte transport has recently been suggested (4-7, 10, 15, 16, 18) and DONOWITZ and ASARKOF (6) have shown that Cl^- secretion was abolished when Ca^{++} is omitted from the tissue bathing solutions.

The basal intestinal I^- secretion is significantly reduced when Ca^{++} was absent from the bathing solutions, suggesting that intestinal I^- transport might also be regulated by Ca^{++} (table V).

Discussion

The results presented in this study (table I) show that iodide was transported against a concentration gradient from the serosal to the mucosal bathing solutions, across the whole rat small intestine. However, net iodide secretion was observed to occur preferently in the central region of the small intestine, which is consistent with the previously reported experimental findings (15). As ACLAND and ILLMAN (1, 2) showed intestinal iodide secretion is an aerobic process, since it is prevented by metabolic inhibitors, such as 2,4-α-Dinitrophenol, and anaerobic conditions (table II). The secretory process was further ouabain-inhibitable (table IV) and Na^+-dependent (table III), suggesting that the Na^+ gradient might be

Table V. *Effect of teophylline (1 mM), carbachol (0.1 mM) and Ca^{2+}-deprived bathing solutions on iodide secretion across the central region of rat small intestine.*
The sacs were incubated as described in table I. The agents were present in both bathing solutions. Values are the means ± S.E. In parenthesis the number of independent observations.

Experimental conditions	nmol $I^- \times g^{-1}$ w.w.	
	Secretion	Tissue content
No addition	3.7 ± 0.1 (40)	1.8 ± 0.08 (40)
Theophylline (1 mM)	4.7 ± 0.2* (15)	1.4 ± 0.05* (15)
Carbachol (0.1 mM)	5.0 ± 0.2* (10)	—
Ca^{+2}-free Ringer solution	2.0 ± 0.2* (15)	2.5 ± 0.20* (15)

Significance levels obtained by using Student's t-test. Test vs. control: * $p < 0.001$.

the driving force for net iodide secretion.

It is interesting to note that the amount of iodide remaining within the tissue at the end of the experimental period was inversely related to its rate of secretion i.e. the highest iodide secretion and the lowest I^- tissue content were observed in the central region of the small intestine, whilts low I^- secretion and high I^- tissue content were found in the rest of the small intestine.

In the case of intestinal Cl^- transport, two NaCl linked transport processes had been involved (3, 11, 12), one located in the brush-border and the other, at the basolateral membrane. The former is thought to be involved in the so called neutral NaCl absorption and the latter has been suggested as being involved in electrogenic chloride secretion. FIELD (8, 9) proposed that Cl^- secretion is triggered when i) the coupled NaCl absorption mechanism is inhibited at the villus level and ii) the secreting mechanism is stimulated by increasing Cl^- conductance across the mucosal membrane at the crypts region. On the other hand, NAFTALIN *et al.* (13, 14, 17) defended the view that the NaCl linked brush-border uptake process is not impaired when Cl^- secretion is triggered, but that an increase in Cl^- conductance across the epithelial brush-border is responsible for intestinal Cl^- secretion.

The finding that net I^--secretion was inversely related to both tissue I^--content and intestinal I^- absorption, could indicate that I-uptake, and hence I^- absorption was impaired in the central region of rat small intestine. However similar results could be expected if secretion resulted from increased brush-border I^- permeability. Thus, increased I^- conductance across the brush border would stimulate backflux of I^- from within the epithelium into the mucosal bathing solution, and the result would be operationally indistinguishable from inhibition of influx into the tissue, as it has been postulated for Cl^- (17); except that with inhibition of influx the rate of mucosal I^--uptake into the tissue would be expected to be lower in the central part than in the rest of the small intestine.

Measurements of epithelial I^--uptake (fig. 1) showed that the initial rate of mucosal I^--uptake was higher in the central region than in the proximal part of rat small intestine. This finding suggests that the high I^- secretion observed in the central region of the small intestine might not result from a less effective iodide absorption mechanism, but from a high mucosal iodide permeability that would account for the low iodide absorption found in the central region, and hence for the high net I^- secretion observed at this level.

Furthermore no significant differences between the two intestinal regions were found when epithelial I^--uptake was measured across the serosal side, suggesting that no secretory mechanism was stimulated at the serosal side of the central part of the intestine.

Altogether, these results are in good agreement with those reported by NAFTALIN and SIMMONS (17) on intestinal Cl^- secretion, supporting the view that electrolytical intestinal secretion would result from increased mucosal anion permeability.

It has recently been suggested that cytosolic free calcium concentration plays an important role in the regulation of intestinal electrolyte transport (4-7, 10, 15, 16, 18). The current study shows that net I^- secretion was significantly inhibited and final tissue I^- content enhanced when the calcium was removed from the bathing solutions, suggesting that the intracellular free calcium linked to extracellular Ca^{2+} might be involved in the regulation of I^- permeability across the mucosal border. Opposite results to those observed by omitting Ca^{2+} from the bathing solutions were found in the presence of carbachol and theophylline

(table V), agents thought to induce intestinal Cl^- secretion by increasing cytosolic free Ca^{2+} concentration (4, 16). All these findings seem to suggest that the mechanisms involved in intestinal I^- transport are those for Cl^- transport across mammalian small intestine. Therefore, I^- transport across rat small intestine appears to be a suitable preparation to investigate the processes involved in intestinal secretion.

In conclusion, the present work shows that mucosal I^- permeability appears to be the main factor in determining the direction of net I^- transport across rat small intestine. Furthermore this parameter might be regulated by cytosolic calcium as has been postulated for Cl^- (4-7, 10, 15, 16, 18). We cannot state as yet, how the transport mechanisms at the mucosal membrane and its cellular regulator(s) are controlled under physiological conditions. Several gastrointestinal chemical messengers have been implicated in the regulation of intestinal electrolyte transport (9). Furthermore, the physiological significance of an intestinal I^- secretion is not at first evident.

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Resumen

Se investiga la secreción de ioduro a través de las diferentes regiones del intestino delgado de rata. En la región central se observa una secreción significativamente mayor. La anaerobiosis, la ouabaína y la ausencia de Na^+ disminuyen la secreción intestinal de I^-, mientras que la teofilina y el carbacol la aumentan. La ausencia de Ca^{++} del medio de incubación la reduce significativamente. La entrada de I^- al tejido a través del borde mucosal del intestino delgado es mayor en la región central que en la distal. No se observan diferencias significativas entre las dos regiones intestinales en relación con la entrada de I^- al tejido desde el lado serosal. Estos resultados sugieren que la permeabilidad de la membrana mucosal al I^- determinan la dirección del paso neto del ión a través del intestino delgado de rata y que la concentración citosólica de Ca^{++} puede ser un regulador fisiológico de este proceso.

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