Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

Giuliano Meyer, Carlo Rossetti, Guido Bottà, Rosalba Vanni, Dario Cremaschi

Enterocyte maturation and aminoacid transport in the distal ileum analysed by electrophysiological techniques

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **75** (1983), n.6, p. 393–398.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1983_8_75_6_393_0>

L'utilizzo e la stampa di questo documento digitale è consentito liberamente per motivi di ricerca e studio. Non è consentito l'utilizzo dello stesso per motivi commerciali. Tutte le copie di questo documento devono riportare questo avvertimento.

Articolo digitalizzato nel quadro del programma bdim (Biblioteca Digitale Italiana di Matematica) SIMAI & UMI
http://www.bdim.eu/

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1983.

Fisiologia. — *Enterocyte maturation and aminoacid transport in the distal ileum analysed by electrophysiological techniques* (*). Nota di GIULIANO MEYER, CARLO ROSSETTI, GUIDO BOTTÀ, ROSALBA VANNI e DARIO CREMASCHI, presentata (**) dal Corrisp. V. CAPRARO.

RIASSUNTO. — Sono stati misurati il potenziale di membrana apicale e le attività cellulari di Na^+ e K^+ negli enterociti dell'ileo distale di hamster a diverse altezze lungo i villi. Nel primo terzo di villo a partire dalla base (giovani enterociti) si è osservato che il potenziale di membrana e l'attività del K^+ crescono fino a raggiungere un valore massimo che permane stabile negli altri due terzi di villo (enterociti maturi). L'attività del Na^+ non cambia ai differenti livelli. La somma delle attività cellulari del Na^+ e del K^+ è pari a quella esterna negli enterociti giovani, ma è più elevata di circa 30 mM nei maturi. Solo nell'ultimo terzo di villo (apice) si verifica un effetto significativo degli aminoacidi sul potenziale di membrana.

INTRODUCTION

Measurements of apical membrane potential of enterocytes are generally obtained by randomly puncturing villus surface. This is a correct procedure only if a single cell population is present in the villus, which is an unlikely assumption as it is well known that enterocytes differentiate from crypt bottom to villus tip.

The aim of this paper is to analyse whether electrophysiological changes go together with enterocyte maturation. Preliminary indications of possible modifications were obtained by Tsuchiya, Okada and Inouye (1980) in embryonic cultured intestinal villi of rat and by Cremaschi, James, Meyer, Peacock and Smith (1982) in a stretched preparation of rabbit ileum " in vitro ". The present study is carried out on an unstretched " in vitro " preparation of hamster intestinal villi which represents an improved technique with respect to the one used by Cremaschi *et al.* (1982).

MATERIALS AND METHODS

Hamsters (120 g) were a gift from Lepetit Industries, Milan. They were anaesthetized by intramuscular injection of pentobarbitone sodium (10 mg-

(*) Lavoro eseguito nel Dipartimento di Fisiologia e Biochimica Generali della Università degli Studi di Milano, Via Celoria 26 - Milano 20133. Finanziamento del Consiglio Nazionale delle Ricerche, Roma.

(**) Nella seduta del 10 dicembre 1983.

100^{-1} g body weight), cut open and a 10 cm length of distal ileum removed. The external muscle layer was dissected and a 1.5 cm piece of the mucosa mounted in a double vertical chamber of plexiglass, epithelium upwards (exposed area: 9 × 3 mm). A round headed, plastic coated pin was fitted permanently to the lower chamber so as to make the epithelium protrude above the level of the window present between the two chambers and to present man villi horizontally for micro-electrode impalement. Bicarbonate saline (Krebs and Henseleit, 1932) gassed with 95% O_2 and 5% CO_2 was used to perfuse both the serosal and mucosal sides of the preparation (8.4 ml min^{-1} on both sides) at a temperature of 30°C. Horizontal villi were observed with an MS stereomicroscope (E. Leitz Wetzlar, GMBH, D-6330 Germany) connected through a Grundig telecamera (model FA 703) to a TV monitor (Grundig A.G., Furth, D-8510, Austria). Measurements of distances from villus tip to the points of micro-electrode impalements were made directly on the TV monitor at a final magnification of × 130. Micro-electrodes were dipped in Rotring black ink (art. 595617) to improve visualization of the tip. Details on conventional and selective micro-electrode construction and use were as reported by Cremaschi, Meyer and Rossetti (1983).

Results and Discussion

Apical membrane potentials along villi

Villus length measured on the monitor display was equal to 544 ± 14 μm (23 villi in 6 animals). Apical membrane potential (V_m) appeared to increase steadily from crypt-villus junction, reaching constant maximal values in the upper two thirds of the villus (fig. 1). Because of the large scatter of the results no single points were significantly different from their immediate neighbour. Thus, for purposes of comparison, a division between two cell populations (young and old enterocytes: Y- and O-enterocytes) was determined empirically. Y-enterocytes (first third of the villus) exhibited a V_m of -32.4 ± 0.7 *m*V (49 impalements), whereas O-enterocytes had a V_m of -36.7 ± 0.4 *m*V (204 impalements). The difference was highly significant ($P < 0.001$). At least the population of old enterocytes was homogeneous as shown by the frequency distribution of V_m values (fig. 2) which resulted normal with a Kolmogorov-Smirnov statistic analysis. A first conclusion is that an electro-physiological study of absorptive cell properties by random impalements seems to be always valid, as random impalements are much more likely to occur in the upper part of villus which, on the basis of the present investigation, corresponds to the homogeneous population of O-enterocytes. A second conclusion is that with the unstretched preparation here used we obtain higher V_m measurements with respect to those obtained with stretched intestines (Cremaschi *et al.*, 1982); so, V_m values of the O-enterocytes are strictly similar to those collected by random impalements (Rose and Schultz, 1971).

Sodium and potassium cell activities along villi

Young and old enterocytes showed no difference in Na^+ activity (31 $\pm$ 3.5 and 31.9 $\pm$ 2.4 *m*M respectively; 20 and 61 impalements) (also see fig. 1).

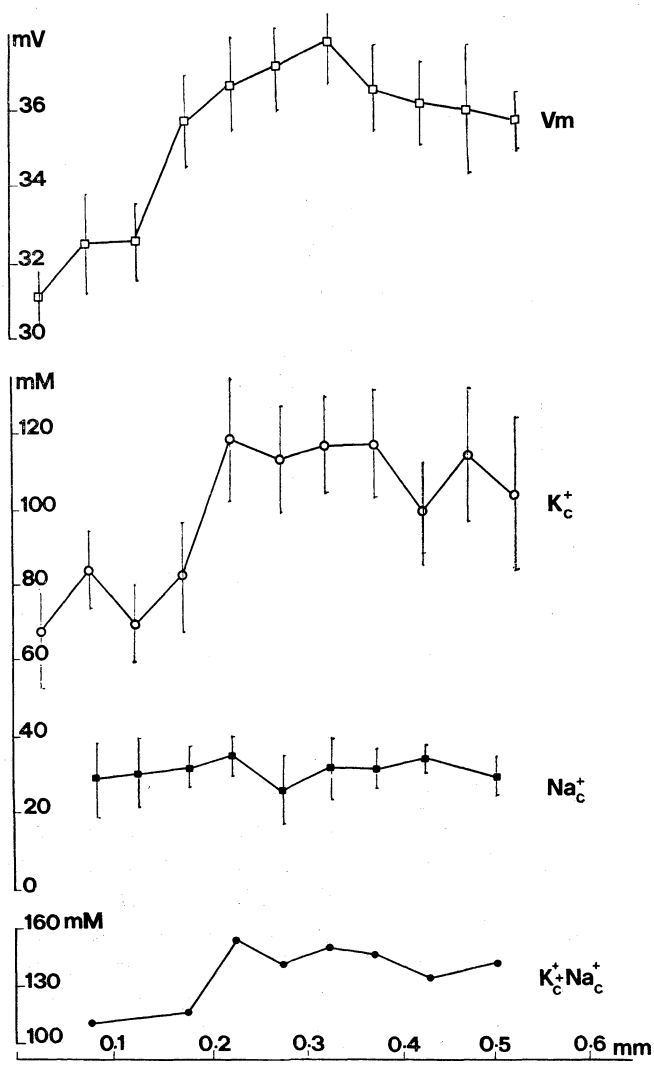

Fig. 1. – Apical membrane potential (V_m), K^+ and Na^+ cell activities (K_c^+ and Na_c^+), sum of K^+ and Na^+ cell activities measured at different distances from crypt-villus junction. Results are reported as means $\pm$ S.E. The mean number of impalements is 23, 11, 8 for the determination of each point of V_m, K_c^+ and Na_c^+ respectively. V_m is reported as *m*V, whereas cell activities as *m*M.

Conversely K^+ cell activity increased from crypt-villus junction to reach a steady value at a distance of about 200 μm.

Y-enterocytes had a K^+ cell activity of 78.8 ± 5.6 *m*M (37 impalements) and O-enterocytes 113.1 ± 5.3 *m*M (84 impalements); the two values are significantly different ($P < 0.001$).

The sum of Na^+ and K^+ cell activities was lower for Y- than for O-enterocytes. The former was equal to 111 *m*M a value similar to that of $Na^+ + K^+$ activity of the bicarbonate saline (112 *m*M), the latter contained an excess of

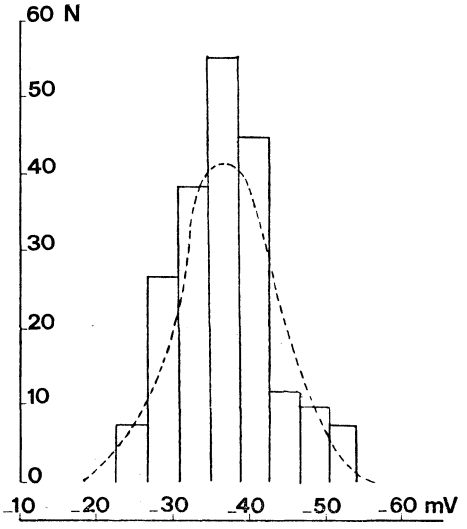

Fig. 2. - Frequency distribution diagram of apical membrane potential measured in O-enterocytes. The broken line indicates the normal frequency distribution. V_m is expressed in *m*V.

cations of approximately 30 *m*M. Since O-enterocytes also have to preserve electroneutrality and osmotic equilibrium, it is necessary to postulate that the ratio of polyvalent to monovalent anions increases as cells develop. This could occur through the synthesis of new macromolecules or with an increase in cell pH. It is noteworthy that K^+ cell activity in its turn controls proteins synthesis (Lubin, 1963, 1976). Thus the changes in membrane potentials, K^+ activity and total cation content could be signals for the subsequent cell maturation. It is important that in the upper two thirds of the villus sucrase-isomaltase activity increases (Cremaschi, James, Meyer, Rossetti and Smith, 1984), microvilli elongate (Cremaschi *et al.*, 1982) absorption (King, Sepulveda and Smith, 1981) and receptor function (Mason, Dallman and Barclay, 1981; Rodewald, Lewis and Kraehenbuhl, 1983) develop.

Effects of alanine along villi

In order to test electrophysiologically the site of Na^+-alanine co-transport along the villus, 30 *m*M alanine was added to the luminal fluid, transepithelial potential difference (V_{ms}) was monitored and villus punctured along its length.

V_{ms} was immediately hyperpolarized: maximal increase (1.9 ± 0.2 *m*V) was reached in about 6 min.

V_m measured within 200 μm from villus tip, was highly depolarized ($P < 0.001$) from 39.7 ± 2.7 to 25.3 ± 1.2 *m*V (20 impalements per each point) (fig. 3); conversely, when it was determined along the second 200 μm from tip villus, it decreased only transiently ($P = 0.05$) from 37.8 ± 1.9 to 31.7 ± 1.3 *m*V (13 impalements per each point).

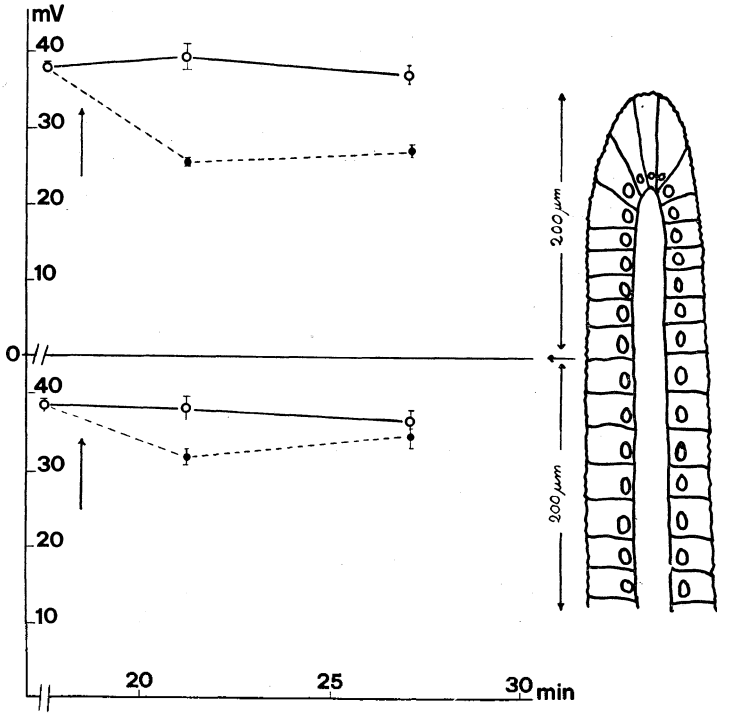

Fig. 3. – Apical membrane potential (V_m) as a function of time in the absence (O) or in the presence (●) of 30 *m*M alanine at different distances from villus tip. V*m* and time are espressed as *m*V and min respectively. Results are reported as means ± S.E. The mean number of impalements is 20 and 13 for the determination of each point of the higher and lower part of the figure respectively.

This is evidence that Na^+ dependent alanine transport is localized in the upper third of the villus height. The small and transient effect on the second third of the villus height could be due either to a residual presence of the Na^+-aminoacid co-transport or to the short-circuit currents generated both by junctional shunts and by enterocyte heterogeneity. These results confirm the data obtained by P. James and M.W. Smith (personal communication) with autoradiographies of labelled alanine uptakes which show that the aminoacid absorption does not extend below the depth of 200 μm from the villus tip.

References

[1] Cremaschi D. James P.S., Meyer G., Peacock M.A. and Smith M W. 1982) – *Membrane potentials of differentiating enterocytes.* « Biochim. Biophys. Acta », *688*, 271-274.

[2] Cremaschi D., James P., Meyer G., Rossetti C. and Smith M.W. (1984) – *Developmental changes in intra-enterocytes cation activities in hamster terminal ileum.* « J. Physiol. (Lond.) », in the press.

[3] Cremaschi D., Meyer G. and Rossetti C. (1983) – *Bicarbonate effects, electromotive forces and potassium effluxes iu rabbit and guinea-pig gal-bladder.* «J. Physiol. (Lond.)», *335*, 51–64.

[4] King I.S., Sepulveda F.V. and Smith M.W. (1981) – *Cellular distribution of neutral and basic aminoacid transport systems in rabbit ileal mucosa.* « J. Physiol. », *319*, 355-368.

[5] Krebs H.A. and Henseleit K. (1932) – *Untersuchungen über die Harnstoffbildung in Tierkörper.* « Hoppe-Seyler's Z. Physiol. Chem. », *210*, 33-66.

[6] Lubin M. (1963) – *A primary reaction in protein synthesis.* « Biochim. Biophys. Acta », *72*, 345-348.

[7] Lubin M. (1967) – *Intracellular potassium and macromolecular synthesis.* « Nature (Lond.) », *213*, 451-453.

[8] Mason D.N., Dallman M. and Barclay A.N. (1981) – *Graft-versus-host disease induces expression of Ia antigen in rat epidermal cells and gut epithelium.* « Nature (Lond.) », *293*, 150-151.

[9] Rodewald R., Lewis D.M. and Kraehenbuhl J.P. (1983) – *Immunoglobulin G receptors of intestinal brush borders from neonatal rats.* « Ciba Found. Symp. », *95*, 287-299.

[10] Rose R.C. and Schultz S.G. (1971) – *Studies on the electrical potential profile across rabbit ileum. Effects o/ sugars and aminoacids on transmural and transmucosal electrical potential differences.* « J. Gen. Physiol. », *57*, 639-663.

[11] Tsuchya W., Okada Y. and Inouye A. (1980) – *Membrane potential measurements in cultured intestinal villi.* « Membr. Biochem. », *3*, 147-153.