Biochimica et Biophysica Acta, 688 (1982) 271–274
Elsevier Biomedical Press

BBA Report

BBA 70039

MEMBRANE POTENTIALS OF DIFFERENTIATING ENTEROCYTES

D. CREMASCHI [a], P.S. JAMES [b], G. MEYER [a], M.A. PEACOCK [b] and M.W. SMITH [b]

Istituto di Fisiologia Generale e di Chimica Biologia, Università di Milano, Via Mangiagalli 32, Milano (Italy) and A.R.C. Institute of Animal Physiology, Babraham, Cambridge CB2 4AT (U.K.)

(Received November 9th, 1981)

Key words: Membrane potential; Differentiation; Enterocyte; (Rabbit)

A positional analysis of enterocyte membrane potential has been carried out using in vitro preparations of rabbit distal ileum. Young enterocytes were found to possess a microvillar membrane potential significantly less than that seen in older enterocytes. The length of enterocyte microvilli was also found to be significantly less in younger enterocytes. It is suggested that developmental changes in membrane potential, occurring during the early stages of enterocyte differentiation, probably reflect a changed permeability to ions associated with the establishment of a fully developed microvillar membrane. Other explanations for the observed findings are also considered.

It has until recently been common practice to average random estimates of enterocyte membrane potential obtained in vitro using isolated sheets of intestinal mucosa. The implicit assumption is that membrane potential is unaffected by enterocyte differentiation or that estimates of membrane potential derive from a single population of fully differentiated enterocytes. Earlier attempts to show the presence of electrically different sub-populations of enterocytes proved negative, values of membrane potential from both small and large intestine fitting frequency distribution curves which were essentially normal [1–3]. Recently, however, it has been reported that enterocytes found at the tip of cultured villi possess membrane potentials 5-times larger than those found at the villus base [4]. The question arises as to whether these findings, obtained with cultured villi, accurately reflect the normal process of cell differentiation seen in vivo. The present experiments were designed to provide more information on this point.

Rabbits weighing approx. 3 kg were killed by a blow on the head. A piece of distal ileum was then removed and rinsed in bicarbonate saline [5] before being mounted in an apparatus suitable for recording microvillar membrane potentials [1,6]. The tissue was superfused throughout the experiment by bicarbonate saline gassed with a O_2/CO_2 mixture (95:5, v/v) at a constant temperature of 30°C unless stated otherwise. Impalements took place under visual control, the final view of individual villi being displayed on a TV monitor at a magnification of ×300. Tips of filled microelectrodes were dipped in Rotring ink (Art, 595617) to make their position visible at the site of cell impalement. Distances were measured from the tips of villi to the site of microelectrode impalement. Separate measurements on histological sections of rabbit ileal villi showed their length to be 510 ± 9 μm (mean ± S.E. of 146 measurements).

Cell renewal time was measured in a further series of [^{3}H]thymidine injection experiments. From this it was possible to calculate the age of enterocytes at any position on the villus. Enterocyte age was in turn related to the degree of structural differentiation present, judged in this instance from measurements of microvillus length carried out on longitudinal sections of glutaralde-

hyde-fixed, osmium-treated, villi viewed in a Philips 400 electron microscope.

It has already been reported that the membrane potential of rabbit distal ileum measures -36 ± 0.5 mV ($n = 185$) at a temperature of 37°C [1]. Similar experiments were undertaken initially to test whether this work could be repeated. The mean membrane potential (V_m) recorded in the present work from a series of impalements directed randomly at the mucosa was -53.1 ± 1.1 mV ($n = 30$). The difference between this value and that reported previously is almost certainly attributable to the 2-fold difference in K^+ concentration used in these two sets of experiments (9.9 mM [1]; 4.7 mM [5]). It was concluded from these results that the general technique was being used in a satisfactory way, but it was also decided at this stage to carry out all future experiments at 30°C to minimise tissue deterioration in vitro. The mean V_m measured randomly using rabbit distal ileum at 30°C was -33.9 ± 0.7 mV ($n = 130$), a value significantly less than that measured at 37°C.

The subsequent analysis of these data in terms of enterocyte position upon the villus showed no gradient in V_m (-33.5 ± 0.9 mV from 87 impalements carried out 0–200 μm from the villus tip; -34.7 ± 1.3 mV from 43 impalements carried out 200–400 μm from the villus tip). These results are completely different from those reported previously using cultured pieces of newborn rat intestine [4].

In order to study deeper parts of the villus it was necessary to stretch preparations of intestine over plastic wedges as described previously [6]. Results obtained using this type of preparation are shown, together with a diagrammatic representation of a villus for purposes of orientation, in Fig. 1. The V_m of villus tip enterocytes, -24.5 ± 1.0 mV, was actually less than that found around the middle portion of the villus, though this difference was not statistically significant. No gradient in V_m could be detected down to a depth of 400 μm from the tip of the villus. This finding confirmed results obtained in the present work using unstretched pieces of intestine. Measurements of V_m lower down the villus towards the crypt-villus junction were, however, significantly less than values found nearer the tip (V_m values of -20.7 ± 0.6 and -25.9 ± 0.4 mV for enterocytes located 400 to 500 and 0 to 400 μm from the tip; $t = 5.39$, $P < 0.001$). This 5 mV difference, though significant, is an order of magnitude less than that reported for cultured rat villi [4]. The enterocytes of newborn animals are known to be structurally very different from those found in older animals [7,8] and the newborn intestine shows a pattern of functional development quite different from that seen in the adult [9]. These differences probably account for the discrepancy in results mentioned above.

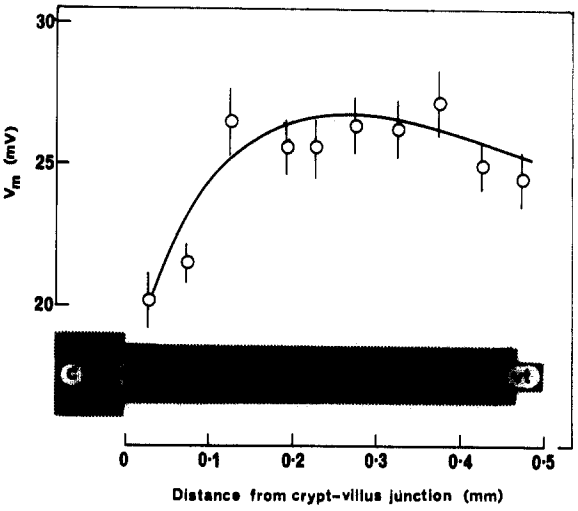

Fig. 1. Relation between microvillar membrane potential and enterocyte position on rabbit intestinal villus. Pieces of rabbit distal ileum were presented for microelectrode impalement in vitro at 30°C in bicarbonate saline gassed with a O_2/CO_2 mixture (95:5, v/v). Points give mean values of $V_m \pm$S.E. of from 43 to 81 separate determinations. The hatched area represents a typical villus from which recordings were made. c, crypt; cvj, crypt-villus junction; v, villus proper; vt, villus tip.

Cell membrane potential depends both on the passive distribution of ions across the plasma membrane and the asymmetric movement of cations through the Na^+ pump. This latter effect has been estimated, under normal circumstances, to contribute up to 7 mV to the measured V_m of rat duodenal enterocytes [10]. The V_m gradient seen for rabbit ileal enterocytes could reflect changes in Na^+ pump activity or passive leak properties occurring during normal development. To try to distinguish these possibilities it was decided to estimate enterocyte age at different points on the villus and compare the microvillar structure of

these cells with their capacity to maintain different values of V_m.

Enterocyte age was determined by the intraperitoneal injection of tritiated thymidine ($1\,\mu Ci \cdot g^{-1}$) into a group of rabbits. These rabbits were then killed 24, 48, 72 and 96 h later and sections of ileum processed for autoradiography. The location of labelled enterocytes on rabbit intestinal villi is plotted against time in Fig. 2. Radioactive enterocytes first appeared on villi 24 h after the injection of thymidine. These enterocytes were extruded from the tips of villi some 72 h later. The length of microvilli in the brush border membranes of these differently aged cells was also determined using thin sections of rabbit ileum viewed under an electron microscope. These results are plotted, along with V_m values determined in cells of similar ages, in Fig. 3.

Cells at the base of intestinal crypts have microvilli of average length 0.28 μm. These microvilli increase in length at a constant rate during the following 60 h period of development (rate of microvillus assembly: 0.031 $\mu m \cdot h^{-1}$). Microvillus length then remains constant, or decreases slightly, until the enterocyte is shed into the intestinal lumen some 40 h later. The membrane potential, like microvillus length, remains virtually unchanged as the enterocyte migrates along the upper half of the villus. The rise in V_m, seen to occur as enterocytes leave the crypt-villus junction, occurs at a time when microvillus assembly is taking place. It is tempting to conclude that it is the growth of the microvillus membrane, rather than an increase in Na^+-pump activity, which is responsible for the increase in V_m. Such an increase could result from a reduction in the passive permeability to ions brought about by stabilization of a rigid microvillar membrane during development.

The measurement of intracellular Na^+ and K^+ activities in enterocytes throughout differentiation and the use of strophanthidin to inhibit Na^+-pump activity provide further ways to characterize changes in membrane potential seen to occur as enterocytes migrate along the villus. This work is being carried out at the present time.

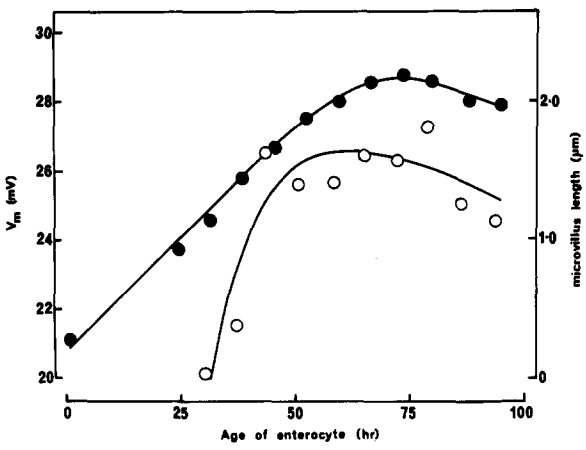

Fig. 3. Effect of age on microvillus length and membrane potential of rabbit ileal enterocytes. The length of enterocyte microvilli, determined under the electron microscope, was related to enterocyte age, estimated from thymidine labelling experiments as described in the text. Values for microvillus length (●——●) give means based on estimates carried out on six rabbits. Values of V_m (○) plotted for comparison are taken from Fig. 1.

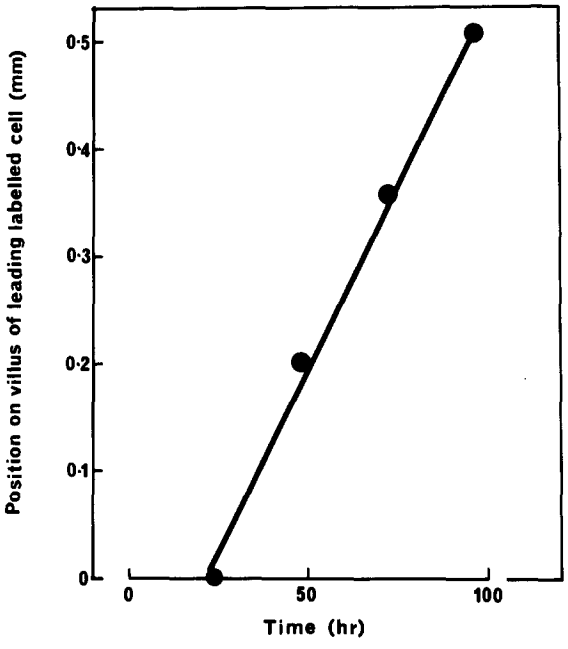

Fig. 2. Enterocyte migration along rabbit intestinal villi. Pieces of rabbit distal ileum, taken from rabbits at known times after the intraperitoneal injection of tritiated thymidine, were processed for autoradiography as described in the text. Values show the highest points reached on the villi by migrating labelled enterocytes. Each value gives the mean of 10 measurements carried out on one or two rabbits.

References

1 Rose, R.C. and Schultz, S.G. (1971) J. Gen. Physiol. 57, 639–663

2 Okada, Y., Sato, T. and Inouye, A. (1975) Biochim. Biophys. Acta 413, 104–115

3 Hénin, S. and Smith, M.W. (1976) J. Physiol. (Lond.) 262, 169–187

4 Tsuchiya, W., Okada, Y. and Inouye, A. (1980) Membrane Biochem. 3, 147–153

5 Krebs, H. and Henseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. 210, 33–66

6 James, P.S. and Smith, M.W. (1981) J. Physiol. (Lond.) 319, 6P–7P

7 Wissig, S.L. and Graney, D.O. (1968) J. Cell Biol. 39, 564–579

8 Rodewald, R. (1976) in Maternofoetal Transmission of Immunoglobulins (Hemmings, W.A., ed.), pp. 137–149, Cambridge University Press, Cambridge

9 Smith, M.W. (1981) Experientia 37, 868–870

10 Okada, Y., Irimajiri, A., Tsuchiya, W. and Inouye, A. (1978) Jap. J. Physiol. 28, 511–525