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Alanine and glucose effects on the intracellular electrical potential of rabbit ileum

The presence of actively transported sugars or amino acids in the solution bathing the mucosal surface of rabbit ileum results in an increase in the transmural electrical potential difference, the short-circuit current and the rate of active Na^+ transport from mucosa to serosa[1,2]. In order to gain further insight into the mechanisms underlying these phenomena we have undertaken an investigation of the effects of sugars and amino acids on the electrical potential profile across the absorptive epithelium, that is, the electrical potential difference across the individual mucosal (brush border) and serosal membranes that bound the cell. The present communication reports some of the findings that have emerged from this investigation. Similar findings on bullfrog small intestine have recently been reported by WHITE AND ARMSTRONG[3].

Rabbits were sacrificed by intravenous injection of pentobarbital. The terminal ileum was excised, opened along the mesenteric border and rinsed in a buffered electrolyte solution. In most cases the serosal musculature was stripped off to prevent spontaneous muscle contraction which would render prolonged micropuncture difficult. The epithelium was mounted mucosal surface up between two lucite half-chambers. Oxygenated buffer solution at 37° was continuously circulated across both surfaces of the tissue. The composition of the electrolyte solution was (in mM): NaCl, 142; $MgCl_2$, 1.2; $CaCl_2$, 0.9; KH_2PO_4, 1.5; K_2HPO_4, 4.2; and pH 7.2.

Micropipettes were drawn from borosilicate glass tubing and filled with 3 M KCl using standard procedures[4]. The microelectrodes had a tip resistance of 5–20 MΩ and a tip potential less than 5 mV. A Brinkman micromanipulator was used to drive the microelectrode through the mucosal surface of the cell. The reference electrode was located in the mucosal solution. The transmucosal potential difference (PD) (mucosal solution to cell interior), designated ψ_{mc}, was measured using a Medistor (Model A-35) amplifier and recorded on one channel of a dual-channel recorder. The transmural PD (mucosal solution to serosal solution), designated ψ_{ms}, was measured using a Keithley 602 electrometer and recorded on the other channel of the recorder. The transserosal PD, ψ_{cs} (cell interior to serosal solution), is defined by the relation $\psi_{cs} = \psi_{ms} - \psi_{mc}$. The time-course of changes in the transmural and intracellular PD's in response to the addition of sugars or amino acids to the solution bathing the mucosal surface were recorded simultaneously.

The criteria for a successful impalement were a sudden jump in negativity of the microelectrode, the attainment of a steady level which persisted for at least 15 sec, a prompt return to the baseline upon retraction of the microelectrode from the cell, and no change in tip resistance. The average intracellular PD of epithelial cells from 15 tissues was 26 ± 4 mV negative with respect to the mucosal solution (ψ_{mc}).

When D-glucose (final concn., 20 mM) was infused for approx. 5 sec into the mucosal solution there was a rapid 1–7-mV increase in ψ_{ms} and a 3–12-mV decrease in the absolute magnitude of ψ_{mc} (*i.e.* the cell interior became less negative with respect to the mucosal solution) as shown in Fig. 1. In some instances the decrease in ψ_{mc} was equal to, and thus could account for, the increase in ψ_{ms}. In 80% of

Abbreviation: PD, potential difference.

the experiments, however, the decrease in ψ_{mc} was larger than the increase in ψ_{ms} by 1–9 mV and therefore there must also have been a decrease in ψ_{cs}, as illustrated in Fig. 2. As the glucose was washed away from the membrane ψ_{mc} and ψ_{ms} returned toward their original value.

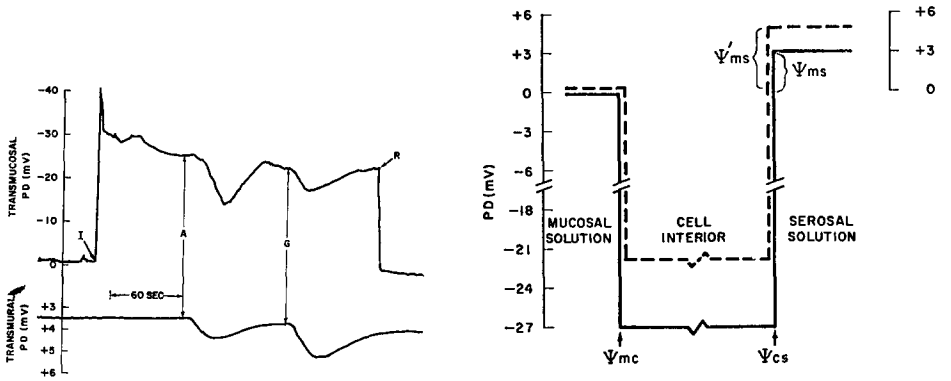

Fig. 1. Effects of L-alanine (A) and D-glucose (G) on the transmucosal (top) and transmural (bottom) PD's in rabbit ileum. Figure traced from the original recording. I signifies time of impalement of cell; A, time of alanine addition; G, time of glucose addition; R, time of retraction.

Fig. 2. The electrical potential profile of epithelial cells of isolated rabbit ileum in the absence (———) and presence (— — —) of glucose or alanine. ψ_{ms} designates the transmural PD in the absence of these solutes and ψ'_{ms} designates the transmural PD in their presence.

The addition of L-alanine (20 mM) to the mucosal solution generally elicited somewhat smaller changes in ψ_{mc} and ψ_{ms} than did glucose. However, in most experiments the decrease in ψ_{mc} was larger than the increase in ψ_{ms} indicating an accompanying decrease in ψ_{cs}. When nonactively transported solutes (*e.g.* 20 mM D-mannitol) were added to the mucosal solution or when actively transported solutes were added to only the serosal solution, there were no significant changes in either ψ_{mc} or ψ_{ms}.

The decreased transmucosal PD is consistent with a stimulated electrogenic entry of Na^+ across the mucosal border. An increase in Na^+ influx has been demonstrated in response to the addition of alanine[5] or 3-*O*-methyl-D-glucose[6] to the solution bathing the mucosal surface of rabbit ileum. The decreased PD across the serosal border is difficult to explain at this time and further investigation of the factors that influence the PD across this boundary is necessary. In particular, the possibility must be considered that the difference between the change in ψ_{ms} and the change in ψ_{mc} is due to the fact that the microelectrode samples a single cell, whereas ψ_{ms} reflects changes occurring in a large population of cells. Nevertheless, these data support the notion suggested by BARRY *et al.*[7] and, more recently, by HOSHI AND KOMATSU[8] that the increase in transmural PD is directly attributable to the transfer mechanisms for sugars and amino acids.

These results are in agreement with those recently reported by WHITE AND ARMSTRONG[9] for bullfrog small intestine, a preparation that in many ways resembles rabbit ileum with respect to the interaction between sugar or amino acid transport and Na^+ transport[9]. These findings are, however, distinctly different from those reported by GILLES-BAILLIEN AND SCHOFFENIELS[10] and WRIGHT[11] for tortoise small

intestine. These investigators found that the increase in transmural PD in the presence of L-alanine or D-glucose can be attributed entirely to an increase in the transserosal PD; the transmucosal PD is unaffected. However, SCHOFFENIELS[12] has reported that L-alanine enters the cell passively at the brush border and that the active transport mechanism for alanine is localized at the serosal border. For the case of rabbit small intestine, Na^+-dependent transport mechanisms located at the brush border appear capable of bringing about alanine and sugar transport against concentration differences. Thus the differences between our results and those reported for tortoise small intestine may be due to species differences with respect to the location of the mechanisms responsible for active amino acid and sugar transport.

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