COMPARISON OF WINTER FLOUNDER (PSEUDOPLEURONECTES AMERICANUS) AND GOLDFISH (CARASSIUS AURATUS) INTESTINE.

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Using the same experimental setup, we have compared the electrical characteristics of winter flounder intestine (experiments performed in Maine) with goldfish intestine as measured in Amsterdam. Stripped sheets of intestine were mounted in a lucite chamber (Siegenbeek van Heukelom et al, J.Membr.Biol. 63:31,1981). Ringer-Agar bridges connected to AgAgCl electrodes were used as potential sensing electrodes and AgAgCl electrodes as current sending electrodes ($\pm$10 µA and $\pm$100 µA). Cell impalements were done with 3 M KCl filled micropipettes, pulled in Amsterdam (tip resistance 15-40 MΩ), using a Leitz micromanipulator. Transepithelial and cell potential differences were amplified (WPI-M4A) and recorded on a multipenrecorder (kindly loaned to us by Dr. Greger). Usually the mucosal side was grounded. Mucosal and serosal compartments were continuously perfused. Composition of saline was as described earlier (Thompson & Kleinzeller, MDIBL Bull 23:38,1983 for winter flounder with the following modifications: Ca = 2.5 mM, K = 5.7 mM and mannitol = 28 mM, temp. 17°C and Bakker & Groot, Am.J.Physiol. 246:G213,1984 for goldfish). All solutions were checked by their osmolarity. Results are given as means $\pm$ SEM. Between brackets, n is given as number of measurements, number of sheets, number of fish or as number of sheets and number of fish.

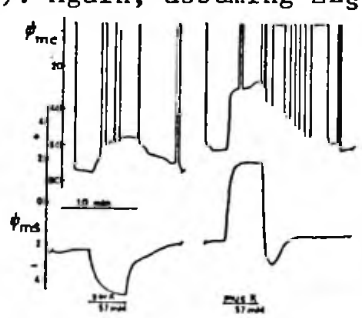
RESULTS

1) Estimation of values of R_{ms}, R_m, R_1, R_s, E_m and E_s from conventional microelectrode measurements and ion substitutions (K-Na in mucosal compartment).

Measurements of electrical characteristics can be summarized as follow: ψ_{ms}= -3.2$\pm$0.3, range: +0.5/-8 mV (57,37), ψ_{mc}= -64.9$\pm$0.8, range: -40/-82 mV (96,40, 26), R_{ms}= 19.3$\pm$0.6, range 11/36 Ωcm^2 (57,37), f_r= 0.31$\pm$0.01, range: 0.11/0.44 (32, 26). The three resistances in a conventional equivalent circuit for leaky tissue were calculated as described by Groot et al (In: Intestinal Transport.(ed) Gilles & Gilles-Baillien, Springer 1984). The ratio $\Delta\psi_{mc}/\Delta\psi_{ms}$ (= 12.3$\pm$2.3 (6,5,4)) was determined by measuring simultaneously the change in ψ_{mc} and ψ_{ms} upon a mucosal change of the potassium concentration from 5.7 to 57 mM. Thus from R_{ms} ($=R_1(R_m+R_s)/R_m+R_s+R_1$), f_r ($=R_m/(R_m+R_s)$) and $\Delta\psi_{mc}/\Delta\psi_{ms}$ ($=(R_s+R_1)/R_1$) we can calculate: R_1=20.5 Ωcm^2, R_m=101 Ωcm^2, R_s=225 Ωcm^2. With these values for the R's and an estimation for E_1= 1.9 mV (Bakker, in press, 1st Int.Conf.C.P.B.1984, Liege) E_m and E_s can be calculated from ψ_{mc} and ψ_{ms}: E_m= -72 mV and E_s= -48 mV.

2) The effect of an increase in solution K concentration.

Mucosal replacement of 51 mM Na with K induces, in the steady state, a change of ψ_{mc} of 40.6$\pm$1.1 mV and of ψ_{ms} (corrected for the change in diffusion potential across the Ringer-Agar bridge) of 6.7$\pm$0.5 mV (16,10,8). Again, assuming ΔE_s = 0 and no change in R's, the change in electromotive force of the mucosal membrane due to a 10x change in K can be calculated: ΔE_m= 56 mV. This indicates that the mucosal membrane behaves nearly as a K selective electrode. This in accordance with the calculated value of E_m= -72 mV, which is nearly identical with E_K = -77 mV (Smith et al, MDIBL Bull 20:96,1980). Serosal replacement of 51 mM Na with K induces a slower and smaller depolarization. In the steady state $\Delta\psi_{ms}$ = -3.2$\pm$0.3, $\Delta\psi_{sc}$ = 15$\pm$2 mV (10,9,6). Assuming ΔE_m = 0 and no change in R's we can estimate ΔE_s= 40 mV. From these experi-



Effect of serosal and mucosal 57 mM K on ψ_{mc} and ψ_{ms}.

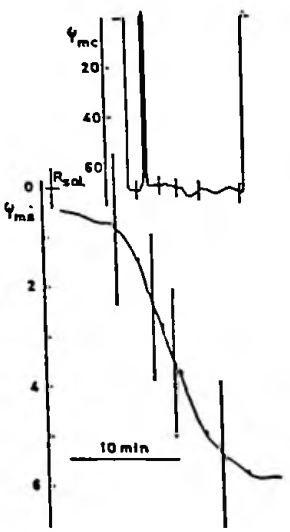
ments we conclude that the basolateral membrane has a conductance for K. This is in contrast with earlier findings of Stewart et al (MDIBL Bull 20:92,1980). The slower development of the potential change may be due to the thicker unstirred layer at the serosal side.

3) Effects of alanine and glucose.

Mucosal replacement of 28 mM mannitol by 28 mM alanine induces a depolarization of ψ_{mc} (5.3±0.6 mV, range 0-14 mV) and a change in ψ_{ms} (1.18±0.06 mV, range 0.8-1.9 mV) (n= 26,7,6). ΔE_m and ΔE_s can be calculated as 11 mV and -9 mV respectively (E_m depolarizes and E_s hyperpolarizes). Qualitatively, the measured potential changes are similar to the goldfish (Albus et al, Pflüg.Arch.398:10,1983). However, they are much smaller and the spread is much larger. Interestingly, several times a change in ψ_{ms} without a change in ψ_{mc} was observed. This indicates that in the winter flounder intestine the chance of impaling a cell that does not transport alanine is greater than in the goldfish. One explanation may be that, in the winter flounder, cell division takes place randomly (Trier and Moxey, Cell Tissue Res.206:379,1980) and young cells probably have no alanine-Na cotransporters. The $\Delta\psi_{ms}$ induced by replacement of mucosal mannitol by 28 mM glucose was very small as reported earlier (Thompson and Kleinzeller, MDIBL Bull 22:54,1982). No reliable changes in ψ_{mc} could be detected. Serosal glucose increases the serosa negativity. This change was so slow, however, that no intracellular correlation could be found.

4) Ion selectivity of the tight junction and the effect of serotonin.

Just after mounting the tissue, the transepithelial potential is small and then, in the next 10 to 40 minutes, a steady ψ_{ms} develops, that may become as great as -8 mV (serosa negative). In 2 tissues we could keep the micropipette in the cell during the increase of ψ_{ms} from ca -1.5 to -4.5 mV. There was no significant change in ψ_{mc}. This suggests that the development of ψ_{ms} is not due to ΔE_m (which should give a $\Delta\psi_{mc}$ of 12x $\Delta\psi_{ms}$) or to ΔE_s (which should give a $\Delta\psi_{mc}$ of 5x $\Delta\psi_{ms}$).



Development of ψ_{ms} after mounting the stripped intestinal sheet. ψ_{mc} was measured simultaneously.

In tissues with a very slowly developing ψ_{ms} we could measure several times in succession the diffusion potential, induced by replacement of half the NaCl at the mucosal side by mannitol. This is a measure of the ion selectivity of the tight junctions. After reaching a steady state of ψ_{ms} the NaCl diffusion potential is -7.7 mV (28,28,20). During the development of ψ_{ms} the diffusion potential increases. This indicates that ψ_{ms} is, at least in part, dependent on the contribution of E_1. It may be postulated then, that the isolation, stripping and mounting of the tissue induces the release of neurohumoral agents that give rise to cAMP in the epithelial cells, thereby reducing the ion selectivity of the paracellular pathway (Krasny et al, MDIBL Bull.22:82,1982. Bakker and Groot Am.J.Physiol.246:G213,1984).

In the goldfish we did observe that serotonin induces a large decrease in the ion selectivity (Groot, in press, 1st Int.Conf.C.P.B.1984, Liege). In winter flounder intestine, however, the effect of serotonin under control conditions was equal to the effect of serotonin after partial mucosal NaCl-mannitol replacement: $\Delta\psi_{ms}$= 1.34±0.27 (9,9,9) and $\Delta\psi_{ms}$=1.35±0.27 (6,6,6) mV respectively. Most of our results are compatible with earlier work on winter flounder. Hoewever, we observed a distinct conductance of the basolateral membrane for K. This study was supported by grants from Netherlands Organization for the Advancement of Pure Research, ZWO and Stichting Dondersfonds to J.G.