INTRACELLULAR RECORDINGS IN THE CHLORIDE CELL-RICH OPERCULAR EPITHELIUM OF FUNDULUS HETEROCLITUS

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The epithelium lining the operculum of the killifish (Fundulus heteroclitus) is a model for the study of gill ion regulation in sea water teleosts (Zadunaisky, Fish Physiology, Vol. Xb:129-176, Academic Press, 1984). The high density of chloride cells, the net outward secretion of chloride and the correlation of cell density with net chloride transport, permits study of chloride cell properties and extrapolation to the chloride cells of the gill.

In order to substantiate a model for the chloride secretion of the chloride cell recently proposed (Zadunaisky, Fish Physiology, Vol. Xb:129-176, Academic Press, 1984) intracellular electrical potentials and ionic activities must be measured. The heterogeneous cell population of the operculum does not help in obtaining intracellular electrical recordings, however the large size, the high density (greater than 10^5 cells/cm^2) and the response to specific stimulants or inhibitors of chloride cell secretion should permit identification of the origin of the observed cellular potential differences.

In this report, results of the impalement of 53 cells in 12 tissues with conventional intracellular microelectrodes are presented.

Dissection of the opercular epithelium has been described in detail (see Degnan et al., J. Physiol., 271:155-199, 1977). The epithelium was mounted as a flat sheet in a lucite chamber with an aperature of 0.178 cm^2, sea water side up, and perfused with Ringer's (Degnan and Zadunaisky, J. Memb. Biol. 55:175-185, 1980). Cellular impalements were performed blindly with conventional electrodes (filled with 2.7 M KCl, resistance ranging between 50 to 120 Mohm). The region of the opercular epithelium that was preferably used for impalement was a highly pigmented zone that contains a greater density of chloride cells. All measurements were made after the transepithelial potential and the tissue resistance were at a steady state value (after 20 minutes). Intracellular potential differences were referred to the apical (V_a) or the basolateral (V_b) side of the cell and transepithelial p. d. (V_t), short circuit current (I_{sc}) and transepithelial resistance (R_t) were measured with calomel electrodes connected through Ringer-agar bridges. An automatic voltage clamp unit and a DC pulse generator delivering 4 to 20 uA of current were used to measure the fractional resistance (R_a/R_t). The criteria for successful impalements were 1) stability of the cell membrane p.d. for at least 60 seconds with less than 1 mV variation 2) stable p.d. deflections in response to current pulses (R_a/R_t) 3) no change in microelectrode tip potential or little change in microelectrode resistance.

A typical recording is shown in Figure 1a, upon cellular impalement the V_a stabilizes rapidly as does the fractional resistance. With the reference changed (from apical to basolateral side), the cell p. d. increases

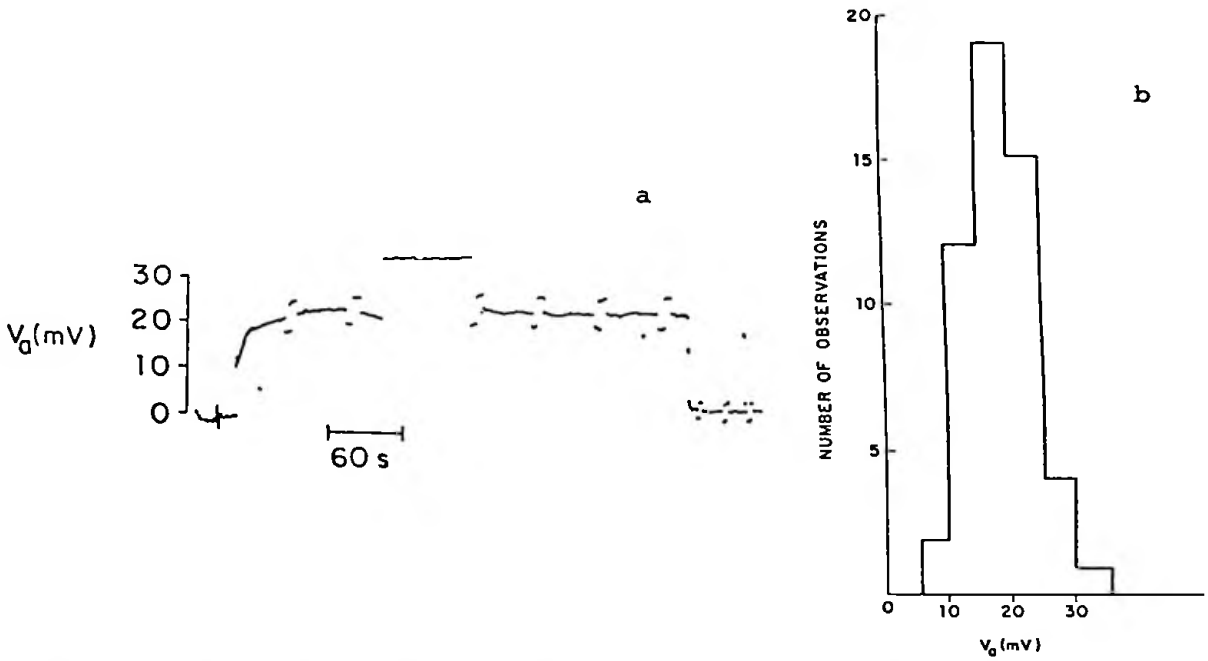

Figure 1. a) Typical intracellular p. d. recording of an individual cell of the operculum epithelium, V_a (referred to the apical side), plotted versus time. The deflections in the p. d. line were produced by transepithelial current pulses. b) Frequency distribution of successful cellular p. d. measurements, referred to the apical side, from 12 tissues.

by V_t and returns to V_a when the electrode reference was changed back to the apical side. After cellular impalement, the electrode value returned to baseline. In Figure 1b a histogram is presented of the values of V_a for the 53 cells. The values are all distributed around a single maximum, indicating that we recorded from a single population of cells. Table 1 presents the mean intracellular observations V_a and R_a/R_t, with the corresponding mean opercular epithelial values for V_t, R_t, and I_{sc}.

Table 1. Electrical parameters obtained in the chloride cells of the opercular epithelium (F. heteroclitus) obtained under open circuit conditions (I_{sc} was calculated). V_t: transepithelial p. d. as referred to the serosal side; R_t: transepithelial resistance; I_{sc}: calculated short circuit current; V_a: intracellular p. d. referred to the apical side; R_a/R_t: fractional resistance

V_t (mV)	R_t (ohm cm^2)	I_{sc} (uA cm^{-2})	V_a (mV)	R_a/R_t
-11.6 ± 0.6	121.6 ± 7.3	63.5 ± 3.0	-18.7 ± 0.7	0.79 ± 0.02
n = 12	n = 12	n = 12	n = 53	n = 53

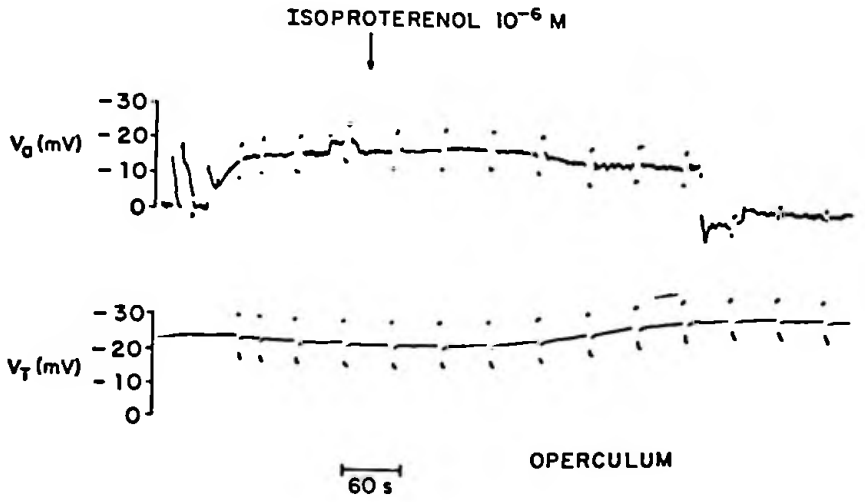

Figure 2. Effect of the serosal perfusion with 10^{-6} M isoproterenol on the cellular potential referred to the apical bath (V_a) and on the transepithelial p. d. (V_t), as a function of time.

In order to confirm if the cells impaled were chloride cells , isoproterenol was added to the serosal side (10^{-6} M). This beta agonist produces an increase in chloride secretion by the epithelium resulting in an increase in the I_{sc} and V_t (Degnan and Zadunaisky, J. Physiol. 294:483-495, 1979). If the impaled cells were chloride cells, isoproterenol exposure should result in cellular depolarization and a decrease in the fractional resistance at the expense of the apical membrane resistance. As is shown in Figure 2, the addition of 10^{-6} M isoproterenol to the serosal solution during a micropuncture resulted in depolarization of V_a in parallel with an increase of V_t and a decrease of R_a/R_t (from 0.75 to 0.65 during isoproterenol) indicating that an increase in apical membrane conductance (of chloride) in response to the stimulant. These experiments show that the impaled cells are sensitive to isoproterenol and consequently give support to the conclusion that the cells impaled were chloride cells. Further studies including chloride intracellular activities should permit the construction of a cellular model for the chloride cell.