CIRCADIAN RHYTHM EXPRESSED BY THE SHORT CIRCUIT CURRENT IN THE OPERCULAR EPITHELIUM OF KILLIFISH, Fundulus heteroclitus.

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The inner lining of the killifish opercular epithelium has a high density of chloride cells, may be separated as a single epithelial sheet and when mounted in an Ussing-style chamber secretes chloride, thus the opercular epithelium has been purposed as a model for studying gill salt secretion (Karnaky, Degnan and Zadunaisky, Science 195:203-205, 1977). This preparation has been very useful in the study of ion transport regulation (Zadunaisky, Fish Physiology, Vol. Xb:129-176, 1984). Ion transport across the opercular epithelium is the result of active chloride transport with the net chloride movement approximating the short circuit current (Degnan, Karnaky and Zadunaisky, J. Physiol. 271:155-191, 1977), while sodium moves by the paracellular pathway (Degnan and Zadunaisky, J. Membrane Biol. 55:175-185, 1980). The short circuit current is related to the chloride cell density (Karnaky, Degnan, Garretson and Zadunaisky, Am. J. Physiol. 246:R770-R775, 1984). Throughout the years a wide range of control values have been reported for the sea water adapted killifish opercular epithelium ranging from 61.7 $\pm$ 5.4 $\mu A/cm^2$ to 209.9 $\pm$ 35.5 $\mu A/cm^2$ (Table 1, Degnan, J. Exp. Zool. 238:141-146, 1986 and Table 2, Degnan, and Zadunaisky, Am. J. Physiol. 238:R231-R239, 1980, respectively). While wide variation of reported control steady state values for the opercular epithelium may be the result of different laboratory settings, annual and daily variations have been noted (Degnan, Karnaky and Zadunaisky, J. Physiol. 271:155-191, 1977; Ericksson, Mayer-Gostan and Wistrand, Acta Physiol. Scand. 125:55-66, 1985). In this report, data is presented showing that some of the observed variation in the *in vitro* opercular epithelium is circadian in origin.

Killifish, Fundulus heteroclitus, were collected locally near Salsbury Cove, ME and maintained in running seawater aquaria on a natural light cycle for at least two weeks prior to experimentation during the months of July, August and September. The killifish were fed at random times of the day with Marinemix to avoid possible entrainment to a feeding rhythm, however the random feedings were weighted toward the afternoon and feeding was never done during the dark cycle. The killifish were killed by pithing and the the operculi dissected from the fish. In Teleost Ringer, the inner lining of the operculum was gently teased from the underlying connective tissue and bone, then pinned onto a Sylgard disk and placed in an Ussing chamber (for chamber description and full dissection methods, see Degnan, Karnaky and Zadunaisky, J. Physiol. 271:155-191, 1977). Both sides of the opercular epithelium were bathed with Teleost Ringer (Scheide and Zadunaisky, Am. J. Physiol. 260:R27-R32, 1988) at room temperature. Standard voltage clamp procedures were used with a University of Iowa Voltage Clamp. Values reported represent steady state values and are defined as the current value at which the current does not change. Values were analyzed with ANOVA and a Students 't' test was used to establish significant differences ($P<0.05$).

Opercular epithelia values were consistently higher in the afternoon. During the Summer 1986, the morning values of the I_{sc} were 92.8 $\pm$ 8.9 $\mu A/cm^2$ while the afternoon I_{sc} values were 135.4 $\pm$ 17.8 $\mu A/cm^2$ ($P<0.05$, $n=21$). These values represent the steady state values of days when morning

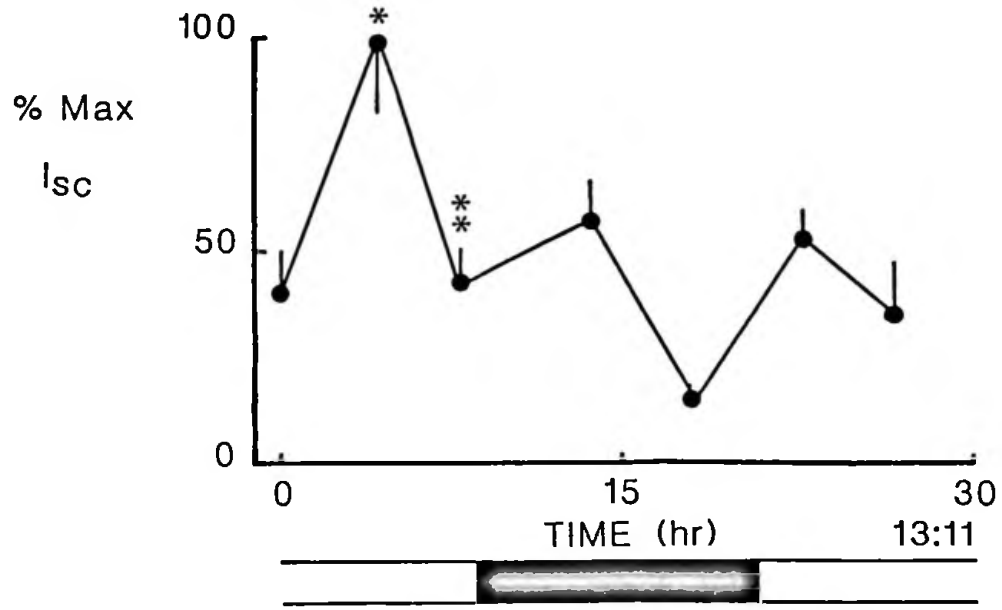

Figure 1. Circadian rhythm of the opercular I_{sc} over a 24 hr cycle. Values were normalized to the high mean value (96.8 ± 17.7 $\mu A/cm^2$) and represent the time of sacrifice mean with the vertical bars representing standard error of the mean (* $P<0.05$, ** $P<0.01$ from the previous value, n=4 for each point).

and afternoon experiments were performed. The G_t increased between morning and afternoon from 12.8 ± 0.9 to 16.4 ± 1.5 mS/cm^2 ($P<0.05$). Ambient temperature influences can not be discounted since daily temperature records were not maintained during this period.

The steady state I_{sc} undergoes a circadian variation with a peak in the middle of the light cycle and a nadir near the end of the dark phase (ANOVA, $F = 21.5$, $P<0.01$, Figure 1). Ambient temperature variance was controlled between 19-21°C. Aquarium water temperature over the period ranged from 12.5 to 14°C and salinity remained constant at 34%.. These observations did not control for animal disturbance since the fish were maintained in a single tank. The peak in the afternoon is consistent with the earlier observations.

The circadian rhythm observed in the chloride current of Fundulus heteroclitus is probably the result of hormonal and environmental factors. Several hormonal factors, when injected, have been observed to influence chloride cell number or function. Injections of prolactin reduce chloride secretion in the seawater adapted killifish (Mayer-Gostan and Zadunaisky, Bull. Mt. Desert Island Biol. Lab. 18:106-107, 1978). Prolactin varies circadially in Fundulus grandis with the acclimation temperature also influencing this rhythm (Spieler, Meier and Noeske, Nature 271:469-471, 1978). Cortisol injections in freshwater adapted fish increase chloride cell number (Foskett, Logsdon, Turner, Machen and Bern, J. Exp. Biol. 93:209-224, 1981). The basis of the circadian I_{sc} rhythm of the killifish opercular epithelium has not been fully explored, however the daily variation should be taken into account when using this tissue as an experimental model for the gill.

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