cAMP-STIMULATED CHLORIDE CURRENT IN XENOPUS OOCYTES EXPRESSING mRNA FROM THE ALKALINE GLAND OF THE LITTLE SKATE RAJA ERINACEA

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The alkaline gland of the male skate secretes a fluid with a pH >9. This fluid presumably neutralizes the acid urine and preserves sperm viability (Maren et al., Comp. Biochem. Physiol. 10:1, 1963). More recent studies by Smith (AJP 248:R346, 1985) employed both transepithelial flux studies and microelectrode measurements which identified active chloride secretion as the principle electrolyte transport event. Therefore, the cellular mechanism of bicarbonate secretion would require a chloride bicarbonate exchange mechanism in parallel with the apical chloride conductance to account for luminal alkalinalization. The purpose of this study was to determine whether this apical membrane conductance could be expressed in Xenopus oocytes, stimulated by cAMP, and whether it is likely to arise from the expression by alkaline gland cells of a protein homologous to the cystic fibrosis transmembrane conductance regulator (CFTR).

The bilobular glands from two skates were excised, and the epithelial layer scraped free with a glass slide, yielding approximately 0.5 g tissue wet weight. PolyA$^+$ mRNA from alkaline gland cells was isolated by poly(dT) chromatography using the FastTrack kit of Invitrogen. A final spin at 500 X g was included to eliminate particulate matter that would interfere with mRNA injection. 50 ng mRNA was injected into Xenopus laevis oocytes and 2-3 days later the transmembrane currents were recorded using double-electrode voltage clamp. Methods are described in more detail by Cunningham et al. (AJP:Cell, 1992, in press).

Currents in mRNA-injected oocytes were initially low (<0.2 μA at $\pm$80 mV) and were similar in magnitude to those observed in uninjected cells. Stimulation of mRNA-injected oocytes with a cAMP cocktail containing 10 μM forskolin, 200 μM 8-(4-chlorophenylthio)-adenosine (3':5'-cyclic monophosphate) (cpt-cAMP), 1 mM 3-isobutyl-1-methyl xanthine (IBMX) produced a 5-7 fold increase in transmembrane currents at $\pm$80mV (n=6). This stimulation was reversible on removal of the cAMP cocktail. Figure 1 illustrates a representative current-voltage (I-V) relation showing the basal and stimulated currents, and Table I summarizes the cAMP-induced changes in membrane potential (V_m) and conductance (G_m) associated with this stimulation. As indicated in Fig. 1, the effects of cAMP were entirely reversible. Currents were usually elevated to a steady level within 2-5 min, and returned to control values approximately 10-15 min following cAMP cocktail washout from the bath. During current stimulation, reduction of bath chloride concentration shifted the I-V relation in the direction expected for stimulation of a chloride conductance pathway (Fig.1). Removal of chloride produced a 14 mV depolarization of the membrane potential and a reduction in membrane conductance (Table I). This represents a 22% inhibition of membrane conductance when bath chloride was reduced from 96 to 13 mM. Much of the non-chloride dependent conductance associated with cAMP stimulation was blocked by addition of 2 mM Ba. A similar phenomenon was associated with the expression of human CFTR mRNA in this system which may be due to either a direct or indirect effect of CFTR on membrane potassium conductance (Cunningham et al., AJP:Cell 1992, in press).

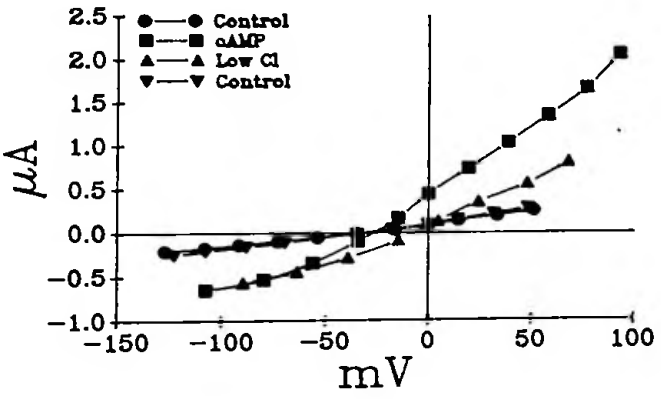

Figure 1. Current-voltage relations of an oocyte injected with 50 ng skate alkaline gland mRNA in the presence and absence (control) of cAMP cocktail or cAMP cocktail + low Cl media (13mM). Currents represent an average of the values obtained from 100 to 200 ms during a 250 ms voltage pulse.

Table 1. Effect of skate alkaline gland mRNA expression on oocyte membrane potential (V_m) and conductance (G_m). Values are mean ± SE (n).

	E_m (mV)	G_m (mV)
Control	-33±4 (6)	2.3±0.4 (6)
cAMP	-25±3 (6)	10.4±1.3 (6)
Low Cl	-11±4 (6)	8.4±1.3 (4)

Northern blot analysis of the polyA$^+$ mRNA isolated from skate alkaline gland was carried out using [^{32}P]-labeled CFTR cDNA isolated from shark rectal gland, a model chloride secreting epithelium (CFTR cDNA was kindly provided by Dr. J. Riordan, Univ. of Toronto). The preliminary results from mRNA blotting suggest that the alkaline gland expresses an elasmobranch homolog of CFTR, which is therefore a reasonable candidate for mediating the cAMP response.

Our findings are consistent with the following conclusions: 1) The expression of skate alkaline gland mRNA in Xenopus oocytes produces a cAMP-stimulated conductance response which is similar to that observed from expression of human CFTR. This is consistent with the idea that a cAMP-regulated chloride conductance in the apical membrane of alkaline gland cells provides the driving force underlying bicarbonate secretion and luminal alkalization. 2) The currents induced in oocytes expressing alkaline gland mRNA are similar to those observed with shark rectal gland mRNA (Sullivan et al., AJP, 260:C664, 1991), and presumably arise from expression of elasmobranch CFTR homologs in both tissues. 3) Skate alkaline gland expresses message that hybridizes with a probe derived from shark rectal gland, suggesting that the species which lie in the same subclass elasmobranchii express homologous CFTR-like proteins. 4) The cAMP- stimulated chloride conductance which is similar to that derived from rectal gland, therefore, probably results from CFTR itself. Thus, CFTR plays a role both in chloride secreting epithelia, such as rectal gland, and by coupling to other transport processes, this conductance can form the basis for bicarbonate secretion.

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