INHIBITORY EFFECTS OF cAMP ON INWARDLY RECTIFYING K^+ CURRENTS IN FRESHLY ISOLATED CELLS FROM RECTAL GLAND OF SQUALUS ACANTHIAS

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There is evidence for the presence of adenosine receptors on the membrane of the ventral aorta (Evans, J. Comp.. Physiol. 162:179-183, 1992) and the rectal gland cells (Kelley et al., J. Clin. Invest. 85:1629-1636, 1990) of the dogfish shark, Squalus acanthias. It has also been found that cAMP regulates the cardiac Na^+ channel by interacting with the cAMP receptors on the surface of the membrane in rat, guinea pig and frog ventricular myocytes (Sorbera and Morad, Science 253:1286-1289, 1991). Here, we report that inwardly rectifying potassium currents (I_{K1}) were significantly inhibited by external application of cAMP or forskolin in the shark rectal gland.

Single cells freshly isolated from the dogfish rectal gland (Xiao and Morad, MDIBL Bulletin, 1994) were used in the present study. Inwardly rectifying potassium currents were studied using the whole-cell clamp technique (Hamill et al., Pfluegers Arch. 391:85-100, 1981). The intracellular solution contained (in mM) 240 KCl, 20 HEPES, 0.2 EGTA, 5 Mg_2ATP, 1 $MgCl_2$, 70 TMAO, 300 urea and pH was adjusted to 7.2 with KOH; the bath solution contained (in mM) 270 NaCl, 250 urea, 5 KCl, 5 $CaCl_2$, 20 HEPES, 3 $MgCl_2$, 1 Glucose and pH was adjusted to 7.2 with NaOH. All compounds were dissolved in the bath solution and were applied rapidly by a multi-barrelled concentration-clamp device. Experiments were carried out at room temperature (21-23°C).

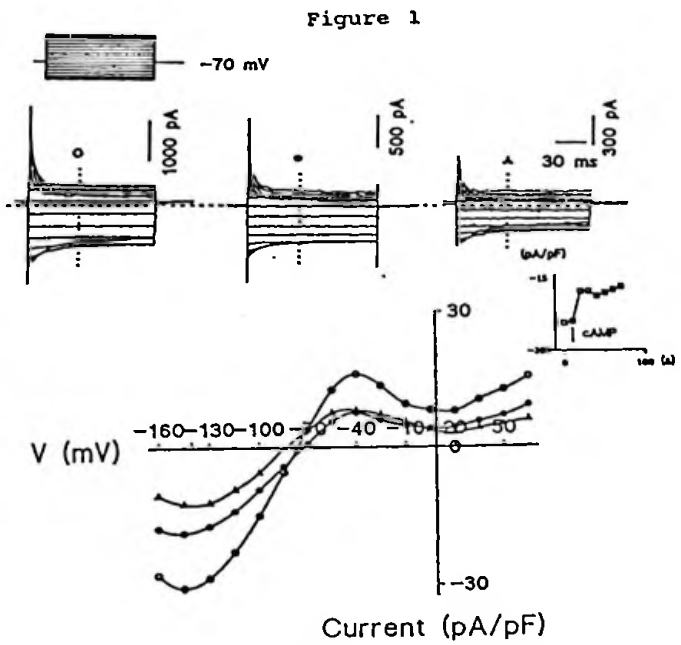

Figure 1. Inhibitory effect of cAMP on inwardly rectifying K^+ currents in a rectal gland cell. Upper panel, tracings of I_{K1} for Control (O), 1 mM cAMP (●) and the difference-current (control minus cAMP treated, ▲). Currents were elicited by 200 ms pulses from a holding potential of -70 mV with 15 mV step increments to -160 and 65 mV at 5 s interval. Currents were measured at the point of dotted vertical lines. The dashed horizontal line represents the 0 mV level. Resting potential -77 mV and cell capacitance 31 pF. Lower panel, current-voltage relationships for control (O), 1 mM cAMP (●) and the different current between the control and cAMP treated. The middle panel shows the onset of the cAMP inhibitory effect on I_{K1} activated by 200 ms pulses from a holding potential of -70 mV to -110 mV applied at 10 s intervals.

Figure 1 shows current recordings from a cell with predominant inwardly rectifying K^+ current. This kind of current was significantly inhibited by external application of 1 mM cAMP. The inhibition was accompanied by a small shift (4 mV) of the reversal potential toward more positive potentials. The onset of the inhibitory effect of cAMP on I_{K1} was rapid, taking place within 10 s (see inset Fig. 1). In cells which express primarily the chloride current (I_{Cl}), cAMP had hardly any effects on the amplitude of the current (not shown). External application of 10 μM forskolin also inhibited I_{K1} significantly.

Table 1 summarizes the inhibitory effects of cAMP and forskolin on I_{K1} in freshly isolated rectal gland cells. The inhibition was in the range of 20 to 70% of control for both cAMP and forskolin. Although cAMP may directly regulate the I_{K1} channel in rectal gland cells, the finding that forskolin also suppresses the current effectively, may suggest that cAMP diffuses rapidly into the cell to induce its effect intracellularly. More critical experiments using flash photolysis of "caged"-cAMP will be required to differentiate between the intracellular or extracellular site of acting cAMP.

Table 1. Effects of cAMP and forskolin on I_{K1} in freshly isolated cells

	n	Current density at -115 mV (pA/pF) Control	Treated	Inhibition (%)	Current density at 35 mV (pA/pF) Control	Treated	Inhibition (%)
cAMP	10	-16.1±2.3	-8.6±1.0	46.6±5.2	11.9±2.5	9.7±2.8	27.6±9.9
FO	4	-33.5±15.0	-17.3±8.1	48.4±4.9	13.6±7.2	4.3±1.7	69.4±20.7

Values in this table are the mean ± SEM. n, the number of cells; Treated, 1 mM cAMP or 10 μM forskolin (FO) was applied extracellularly. Inwardly rectifying K^+ currents were elicited by 200 ms pulses from a holding potential of -70 mV to -115 or 35 mV every 5 s. The amplitude of currents was measured in the middle portion of traces and current density (pA/pF) was calculated by dividing the amplitude of currents by cell membrane capacitance.

The regulation of I_{K1} in rectal gland may be a critical step in the process leading to secretion of Cl^- in the rectal gland. cAMP-induced suppression of I_{K1}, would result in the required depolarization, a probable step in signaling Cl^- secretion. Irrespective of the exact role of the I_{K1} channel, it is clear that this large conductance channel is present in the rectal gland cells and seems also to be rapidly regulated by a cAMP.

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