MODULATION OF CHLORIDE CHANNELS IN PRIMARY CULTURED CELLS FROM RECTAL GLAND OF SQUALUS ACANTHIAS

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Chloride channels in the apical membrane of the rectal gland cells are believed to be responsible for the secretion of NaCl in spiny dogfish, Squalus acanthias (Greger et al., Pflugers Arch. 409:114-121, 1987 and 409:122-125, 1987). Increase of intracellular cAMP level by hormones such as vasoactive intestinal peptide (VIP) enhances Cl^- secretion (Greger et al., Pflugers Arch. 403:446-448, 1985). Two types of Cl^- channels, a large (50 pS) and a small (11 pS) channel, have been identified in the apical membrane. The cDNA from dogfish rectal gland that encodes for the Cl^- channel has 72% homology with the human cystic fibrosis transmembrane conductance regulator (Marshall et al., J. Biol. Chem. 266:22749-22754, 1991).

The present study attempts to determine the effects of agents known to suppress or stimulate Cl^- secretion in the intact gland, on the chloride channels recorded in single dogfish rectal gland cells.

Single cells from 6-15 day old primary cultures of rectal gland (Valentich, Forrest, Am. J. Physiol. 260:C813-823, 1991) were used in the present study. Chloride currents in single rectal gland cells were studied at room temperature (21°C) using the whole-cell clamp technique (Hamill et al., Pfluegers Arch. 391:85-100, 1981). The intracellular solution contained in (mM) 240 KCl or CsCl, 20 HEPES, 0.2 EGTA, 5 Mg_2ATP, 1 $MgCl_2$, 70 TMAO, 300 urea and pH was adjusted to 7.2 with KOH or CsOH; the bath solution contained in (mM) 270 NaCl, 250 urea, 5 $CaCl_2$, 20 HEPES, 3 $MgCl_2$, 1 Glucose and pH was adjusted to 7.2 with NaOH. Modifications of the internal and external solutions are indicated in the figure legends. All the compounds were applied rapidly (< 50 ms), using a multi-barrelled concentration-clamp device.

Based primarily on their electrophysiological characteristics, cells in the primary cultures were classified into two categories. Both cell types were round and contained cytoplasmic granular material. Figure 1 illustrates the voltage-dependence of the current recorded from two different cells in such primary cultures. Figure 1A represents recordings from a cell expressing predominantly inwardly rectifying K^+ current (I_{K1}). This current reversed at -78 mV (A, upper panel), the measured resting potential (-78 mV) of the cell. The percentage of cells expressing the I_{K1} channel was low (about 11 %). Removal of extracellular K^+ in such cells suppressed the inward part of the current and shifted the reversal potential toward more negative potentials (Figure 1A, upper panel). External application of 10 μM forskolin had little or no effect on this current, but 100 μM Ba^{2+} blocked I_{K1} both in the inward and outward directions, leaving only a linear chloride current with a reversal potential of -18 mV in symmetrical Cl^- solutions (Figure 1A).

Approximately 90% of cells in the primary cultures expressed only a linear Cl^- current, with a reversal potential close to zero mV (Fig. 1B). 10 μM forskolin enhanced and 100 μM Ba^{2+} inhibited I_{Cl} significantly (Fig. 1B).

Figure 2 shows the stimulating effect of forskolin on I_{Cl} in a cell dialyzed with an internal solution containing Cs^+ instead of K^+.

The I_{Cl} effects of forskolin were suppressed by increasing the $[EGTA]_i$ and were enhanced by elevation of $[Ca^{2+}]_o$ (Table 1). Forskolin often had a much stronger effect (up to 6 times of control at -110 mV) on I_{Cl} if $[Ca^{2+}]_o$ were

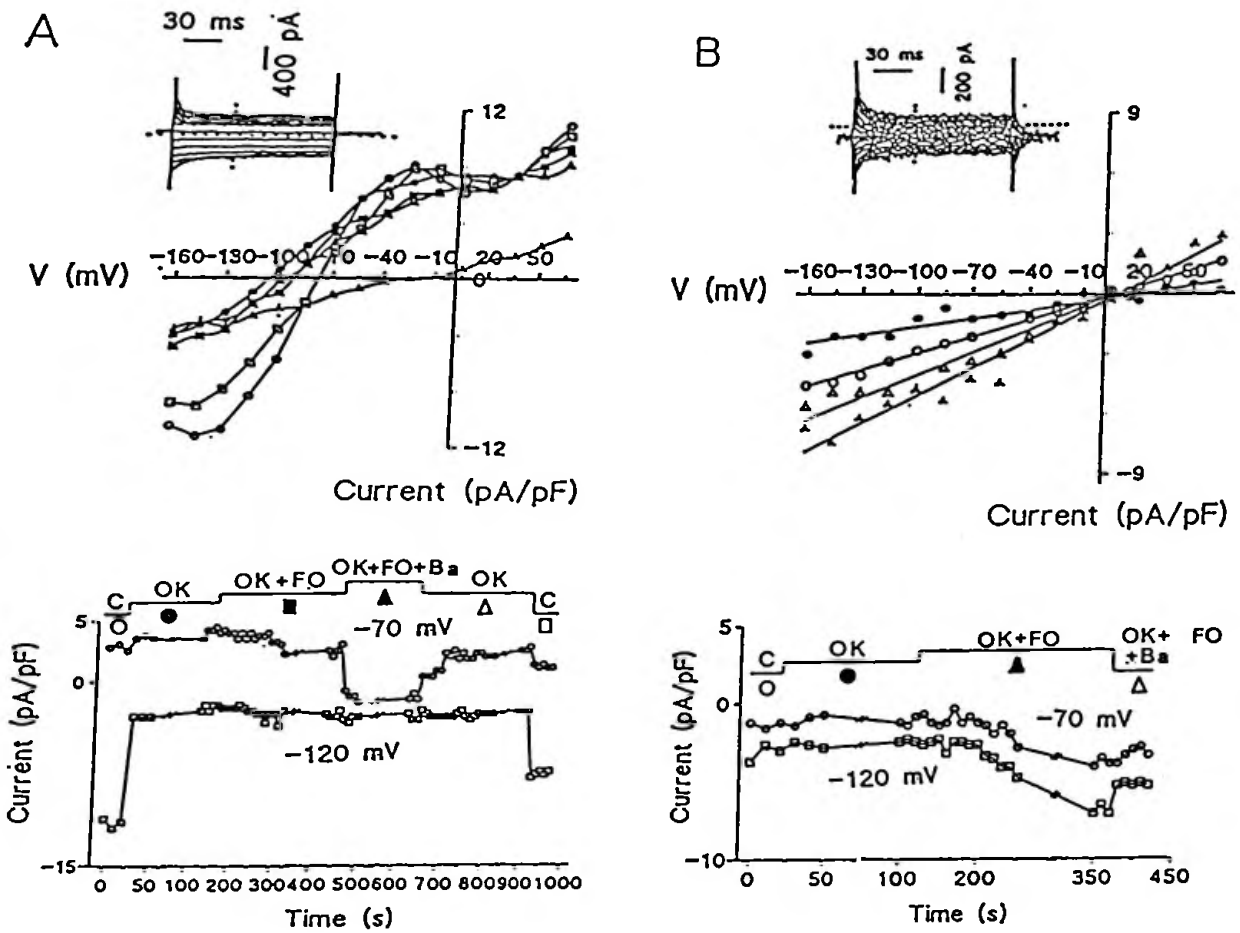

Figure 1. Potassium (I_{K1}) and chloride (I_{Cl}) currents recorded in isolated cultured cells. **A.** Voltage-dependence of inwardly rectifying membrane current (primarily I_{K1}) in a cell bathed first in control solution with 5 mM K^+ (O, C), and then in various K^+ free test solutions, either unmodified (●, OK) or with addition of 10 μM forskolin (■, OK+FO) or both 10 μM forskolin and 100 μM Ba^{2+} (▲, OK+FO+Ba). The experiment was terminated by washout with solutions containing first no K^+ (△, OK) and then 5 mM K^+ (□, C). The lower panel shows the time course of the above effects on I_{K1} activated by a pulse from -70 to -120 mV. Cell capacitance 41pF. **B.** Current-voltage relation of a cell expressing Cl^- channels only. As in panel A the cell was exposed first to control solution with 5 mM K^+ (O, C), and then to K^+free solution (●, OK) to which was added 10 μM forskolin (▲, OK+FO) and, later, also 100 μM Ba^{2+} (△, OK+FO+Ba). The lower panel shows the time course of the effects. The cell in panel B was chosen for illustration purposes in spite of its small Cl^- current (Cf. Table 10) in order to match the protocol used in panel A. Cell capacitance 55 pF. Insets in A and B show tracings of I_{K1} and I_{Cl} recorded with K^+ in internal and external solutions. The dashed horizontal line represents the 0 mV level. The pipette 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 external solution was K^+ free except during the intervals labeled with a C.

increased from 5 to 50 mM. It should be noted that if the intracellular solutions contained 20 mM EGTA the stimulating effect of forskolin on I_{Cl} was abolished, but if $[Ca^{2+}]_o$ was increased to 50 mM forskolin once again enhanced I_{Cl} even with 20 mM EGTA in the internal solution.

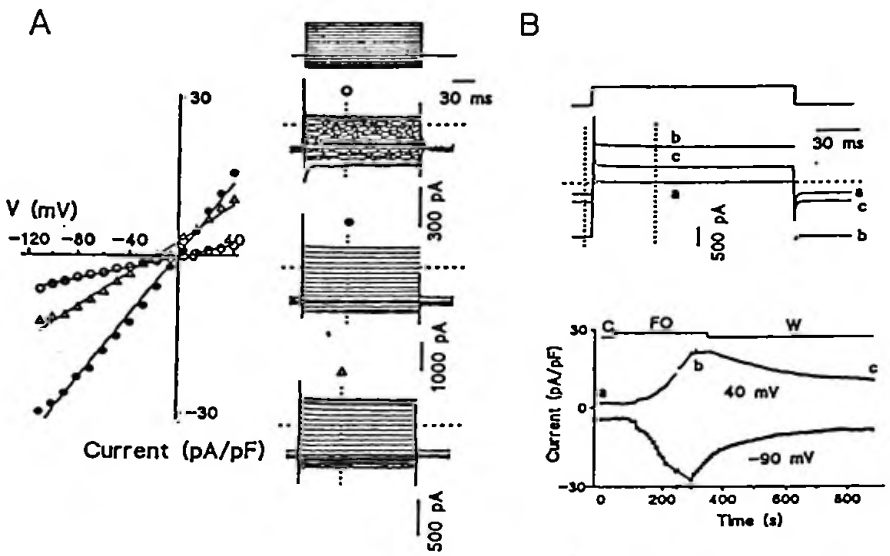

Figure 2. Enhancing effects of forskolin on chloride currents (I_{Cl}) in a single rectal gland cell dialyzed with CsCl based solution A. Superimposed original whole-cell currents and I-V relations elicited by 200 ms pulses from a holding potential of -70 mV in 10 mV increments (range -110 to 40 mV) at 5 s intervals. Control (O), Forskolin (●), wash (△), cell capacitance 52 pF. Dashed horizontal lines represent 0 mV level. B. Upper panel, currents recorded from the same cell as in A by a 200 ms voltage step applied from a holding potential of -90 mV to 40 mV at 5 s intervals. Lower panel, time course of the effect of forskolin on I_{Cl}. The points where the continuous line is interrupted were used for measurements of I-V currents. C, control; FO, 10 μM forskolin; W, washout. a, b and c correspond to the original current recordings in the upper panel. The pipette solution contained (in mM) 240 CsCl, 20 HEPES, 0.2 EGTA, 5 Mg_2ATP, 1 $MgCl_2$, 70 TMAO, 300 urea and pH was adjusted to 7.2 with KOH.

Table 1. Stimulating effect of forskolin on I_{Cl} in primary cultured cells

n	Internal EGTA(mM)	External Ca^{2+}(mM)	Current density at -110 mV (pA/pF) C	FO	Increase (%)	Current density at 40 mV (pA/pF) C	FO	Increase (%)
6	0.2	5	-13.3±3.9	-22.3±3.5[a]	68±16	4.2±0.8	9.7±1.2[b]	131±15
5	20	5	-6.1±1.7[c]	-5.7±2.1	-7±37	6.0±1.4	6.9±3.9	15±23
2	0.2	50	-11.2±6.0	-42.6±19.7	280±46	2.4±0.1	22.1±4.4	821±20
4	20	50	-3.7±1.2	-9.8±5.5	170±56	2.9±0.7	11.8±8.1	307±68

Values are the mean ± SEM. n, the number of cells; C, control; FO, forskolin, 10 μM was applied extracellularly. Chloride currents were elicited by 200 ms pulses from a holding potential of -70 mV to -110 or 40 mV every 5 s. [a] and [b], $p < 0.05$, significant difference between control and treatment of forskolin; [c], $p < 0.05$, significant difference between low and high EGTA. All experiments were done with K^+ free in both internal and external solutions.

Table 1 also shows that at lower EGTA concentrations, forskolin had much stronger enhancing effects on I_{Cl} if $[Ca^{2+}]_o$ were raised. The data thus suggest a strong intracellular Ca^{2+}-dependence of modulation of I_{Cl} via the PKA pathway. In this respect it is important to note that we failed to identify significant time-dependent Ca^{2+} currents even at high $[Ca^{2+}]_o$ and -100 mV holding potentials.

50 μM nickel blocked the enhancing effect of forskolin on I_{Cl} in dogfish rectal gland cells (not shown). Ni^{2+}-induced block occurred slowly, requiring about 2 min.

In experiments where extracellular Na^+ was replaced by TEA, I_{Cl} decreased significantly. But neither the voltage dependance nor the reversal potential of I_{Cl} changed significantly upon replacement of Na^+ with TEA.

Table 2 summarizes the modulating effects of several compounds on I_{Cl} in primary cultures of rectal gland cells. Except for VIP all the other compounds listed in the table inhibited I_{Cl} from about 20 to 50%.

Our electrophysiological studies suggest the presence of two cell types in primary cultures of the dogfish rectal gland and of three cell types in freshly isolated gland (Xiao & Morad, MDIBL Bull. 1994). The differences in cell types found between the fresh and cultured cells may provide some explanation for the disparity of the data reported in literature. For example, Greger et al. have found that although the large (50 pS) Cl^- channel in the rectal gland cell could be activated by an increase of intracellular cAMP level, the smaller channel (11 pS) was insensitive to cAMP levels (Pflugers Arch. 403:446-448, 1985; 409:114 and 122, 1987). In sharp contrast, Devor et al reported that in cultured rectal gland cells the small (6 pS) Cl^- channel could be activated by forskolin and cAMP (MDIBL Bulletin, 32:45-47, 1993). Interestingly, Devor et al. did not find the large 50 pS Cl^- channel in their experiment. Thus the differentiation of cells in culture may be an important factor in determining their electrophysiological properties.

Our data suggest that the intracellular free Ca^{2+} concentrations are critical both in maintaining the basal activity of Cl^- channels and in mediating the effects of forskolin on the gland. This conclusion is based upon observations that: 1) Increases in $[EGTA]_i$ not only abolished the enhancing effect of forskolin on I_{Cl}, but also decreased the basal activity of Cl^- channel; 2) Elevation of $[Ca^{2+}]_o$ enhanced the stimulating effects of forskolin on I_{Cl} in either low or high EGTA-dialyzed cells; 3) Ni^{2+} blocked the stimulating effect of forskolin on I_{Cl}. Since it is known that Ni^{2+} blocks Ca^{2+} entry into the cell (Fox et al., J. Physiol. 394:173-200, 1987) and since Ni^{2+} inhibits an increase of intracellular free Ca^{2+} induced by VIP (Kelley and Forest, Am. J. Physiol. in press) it is possible that the inhibition of forskolin-stimulating effect on Cl^- channels is related to the suppression of Ca^{2+} influx into the cell. The molecular mechanism for the Ni^{2+}-induced blocking effect of forskolin-activated I_{Cl} remains unknown, since no evidence for a voltage-dependent Ca^{2+} current could be found in these studies.

Table 2. Chemical and ionic modulation on I_{Cl} in primary cultured cells

n	Compound*	Concentration (mM)	Effect at -110 mV (%)	Effect at 40 mV (%)
4	ATP	1	↓ 23±12	↓ 29±17
3	VIP	0.001	↑ 15±2	↑ 31±5
4	Caffeine	10	↓ 23±10	↓ 21±8
7	Ca^{2+}	50	↓ 50±10	↓ 20±17
6	Ba^{2+}	0.1	↓ 28±5	↓ 42±8
5	Ni^{2+}	0.05	↓ 41±9	↓ 44±14
6	K^+	5	↑ 41±14	↑ 31±23
3	Na^+	0	↓ 35±8	↓ 24±3

Values are the mean ± SEM. Chloride currents (I_{Cl}) were elicited by 200 ms pulses from a holding potential of -70 mV to 40 or -110 mV every 5 s. n is the number of cells; *, the effect of 10 μM forskolin on I_{Cl} is listed in the table 1; VIP, vasoactive intestinal peptide; ↓, inhibition of I_{Cl}; ↑, increase of I_{Cl}. All the recordings (except for the test of 5 mM K^+) were made with K^+ free in the external solution.

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