A NEW ENZYMATIC TECHNIQUE PROVIDES ISOLATED FRESH CELLS FOR ELECTROPHYSIOLOGICAL STUDIES ON RECTAL GLAND OF SQUALUS ACANTHIAS

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Electrophysiological studies in single rectal gland cells are fairly rare and are carried out with great difficulty (low success rate) primarily on cultured cells. Although a large number of viable cells can be obtained using previously described digestion techniques, such cells are difficult to patch-clamp when used fresh. Here, we describe a rapid (10 min) enzymatic dissociation technique which provides a large number (> 85% viability) of fresh cells for reliable use in electrophysiological studies. The cells isolated by this method appear round in shape, have mostly (more than 90%) cytoplasmic granular appearance, and measure 8 to 15 μm in diameter. Another population of cells (less than 10%) are devoid of granular material and have a clear appearance.

Dogfish ranging in weight between 5-7 kg were paralyzed by pithing of the spinal cord, the abdomen was cut open and the rectal gland artery cannulated *in situ* toward the gland with a PE-50 polyethylene tube and secured with surgical suture. The blood in the rectal gland was flushed out with about 25 ml of Ca^{2+}-free elasmobranch physiological solution (in mM: NaCl 270, urea 250, TMAO 70, KCl 5, HEPES 10, $MgCl_2$ 3, dextrose 1; pH = 7.2 with NaOH & equilibrated with 100% O_2 containing 30 U/ml of heparin). The rectal gland with the cannulation tube intact was then cut off the animal and was immersed in the same heparinized and Ca^{2+}-free solution. The cannulated tube of the rectal gland was connected to a Langendorff apparatus and was subsequently perfused at 5 ml/min with different solutions, which were maintained at 32°C and equilibrated with oxygen. The gland was first perfused with Ca^{2+}-free elasmobranch physiological solution for 5 min, and then, the perfusion was switched to an oxygenated Ca^{2+}-free solution containing 0.4 mg/ml collagenase (Type A, Boehringer Mannheim Co., IN) and 0.1 mg/ml protease (Type XIV, Sigma) for about 10 min. Finally, an elasmobranch solution containing 200 μM Ca^{2+} was used to wash away the enzymatic solution for 5 to 7 min. The rectal gland was then cut open along its long axis and single cells were released into the 200 μM Ca^{2+}- containing elasmobranch solution by gentle agitation.

Table 1 lists the cell types that were identified based on electrophysiological and morphological properties of the cells. The A type population was primarily granular in appearance and strongly expressed the inwardly rectifying K^+ channels (I_{K1}). Cell type B also had a granular appearance but expressed primarily I_{Cl}. C type cells expressing primarily the I_{Cl} were much smaller but contained little or no granular materials and because of their appearance could be easily distinguished from somewhat larger B type cells. Other distinguishing differences between the 3 cell types listed in Table 1, include the membrane resting potential (Vm), the reversal potential (E_{Rev}), and the membrane capacitance. Since A and B cell types looked morphologically similar, the population in each category was determined based on the number of the cells expressing I_{K1} or I_{Cl} as a fraction of total cells patched randomly during the experimental period.

Figure 1 shows tracings of membrane current recorded from an A type cell showing a predominant inwardly rectifying K^+ current. The reversal potential of the current was about -72 mV which approximated the measured resting membrane potential (-78 mV). Rapid (<50 ms) application of 100 μM Ba^{2+} reversibly blocked I_{K1}, unmasking a small linear current with a reversal potential around zero mV (Fig. 1, filled circles) the equilibrium potential of Cl^- (242 mM in patch pipette and 291 mM in the bath). Thus cell type A appears to express both K^+ and Cl^- channels.

Figure 2 illustrates the membrane current characteristics of type B and C cells. The reversal potential of B and C cell types was around zero mV,

approximating the Cl^- equilibrium potential. Thus, even though these cells were freshly isolated from the same dogfish rectal gland, they expressed only the Cl^- channel. Comparison of Cl^- currents in A with those of B and C cell types suggests that although the kinetic and the voltage-dependence of Cl^- channel in both cell types were similar, the density of Cl^- current was about 5 times higher in B and C cell types compared to the A cell type. It is likely that the larger resting potentials (-70mV to -85mV) of the A cell type result from the presence of the inwardly rectifying K^+ channel in these cells. It should be noted that the classification of cell types presented in this communication is primarily based on electrophysiological measurement with only partial supporting data from morphological observations. Although the presence of more than one cell type in the rectal gland would not be particularly surprising, it is also possible that the isolated cells, losing their electrically polar conditions, either over express the Cl^- current (cell type B and C) or down regulate (cell type A) it. Alternatively, the K^+ current may run down or be over expressed resulting in the dominance of K^+ conductance. It is of interest in this regard that type A cells appear to have both K^+ and Cl^- channels. The freshly isolated single rectal gland cells represent a new cell source for electrophysiological studies and as such may become particularly useful for comparison with the data previously described in cultured cells.

Figure 1

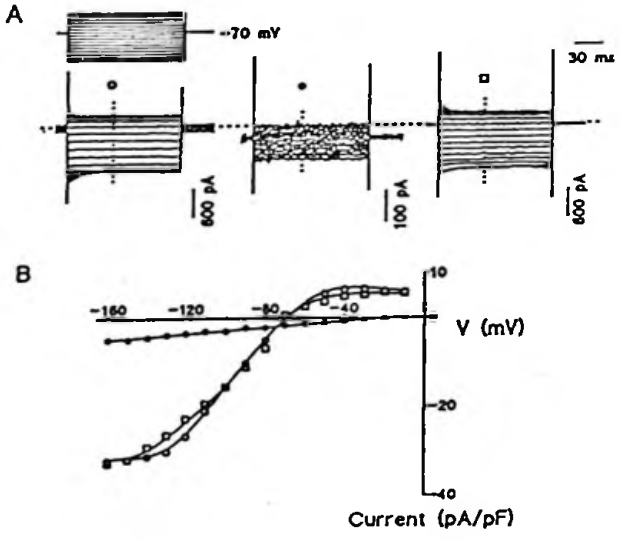

Figure 1. Inwardly rectifying K^+ currents recorded by whole-cell voltage clamp method. A. Superimposed original currents were evoked by 200 ms pulses from a holding potential of -70 mV in 10 mV increments to the identical potential (-10 to -160 mV) every 5 s (see inset). The membrane resting potential (Vm) was -78 mV and the cell capacitance was 28 pF. The dashed horizontal line represents the 0 mV level. (O), control; (●), 100 μM Ba^{2+}; (□), washout. Note different scale was used for the original current tracings following Ba^{2+} exposure. B. Current-voltage relations for control, and 100 μM Ba^{2+} containing solution and following washout of Ba^{2+}. Currents were measured at the point of dotted vertical lines and current densities were calculated by dividing the measured currents by the cell capacities. 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 bath solution contained (in mM) 270 NaCl, 250 urea, 5 $CaCl_2$, 5 KCl, 20 HEPES, 3 $MgCl_2$, 1 Glucose and pH was adjusted to 7.2 with NaOH. Experiments were carried out at room temperature (19-22°C).

Figure 2

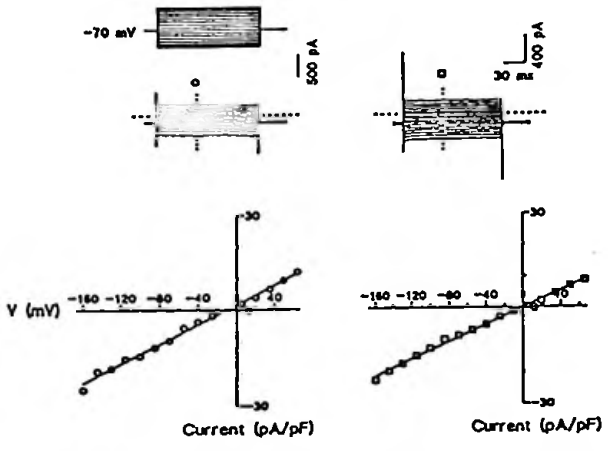

Figure 2. Voltage-dependence of membrane current found in cell type B and C. Upper panel, original current traces recorded from cell type B (O) and C (□). Currents were activated by 200 ms pulses from a holding potential of -70 mV to potentials ranging from -160 to 65 mV applied in 15 mV increments and at 5 s intervals. The dashed horizontal lines represent the 0 mV level. Lower panel, current-voltage relations were constructed by measuring the currents at the time indicated by dotted vertical lines for cell type B (O) and C (□). I-V relation reversed near zero mV, consistent with the assumption that the current was carried primary by Cl^- (symmetrical Cl^- concentration). The left panels were obtained from the cell type B and had a resting potential of -5 mV and cell capacitance of 22 pF; the right panels represent C type cells with -3 mV resting potential and cell capacitance of 17 pF. The internal and external solutions were the same as described in Fig. 1.

Table 1. Different cell types from freshly isolated dogfish rectal gland

Cell type	Channel expressed	Current (pA/pF)	E_{Rev} (mV)	V_m (mV)	C_m (pF)	Diameter (μM)	Granular material	Population %
A	I_{K1}	-18±3	-73±3	-74±3	45±7	8-15	+	65 (n=19)
	I_{Cl}	- 5±2	-4±3		35±7	8-15	+	(n=2)
B	I_{Cl}	-24±5	-14±5	-11±6	57±14	8-15	+	30 (n=9)
C	I_{Cl}	-15±3	2±4	3±6	16±1	< 8	-	5 (n=3)

The diameter of cells was measured using X400 magnification. Current density (pA/pF) was measured at -120 mV and is normalized relative to the cell capacitance. I_{k1} and I_{Cl} were activated by 200 ms pulses from a holding potential of -70 mV to -120 mV applied at 5 s interval. E_{Rev} = reversal potential, V_m = membrane resting potential, C_m = membrane capacitance. (+), cells had granular particles in cytoplasm; (-) cells did not have particles. Values are expressed as mean ± SEM.

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