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Plasma membrane structure: Effects of hydrolases on muscle resting potentials

Due to the technical difficulties involved in isolating uncontaminated plasma membrane from cells in sufficient quantity for accurate biochemical analysis, the information regarding its structure is based largely on indirect evidence[1]. One approach is to measure the effects of a spectrum of specific digestive enzymes on functions which are unique to plasma membranes of living cells, for example, bioelectrogenesis[2]. This report describes the effects of various hydrolases on the resting membrane potentials of muscle fibers.

All potentials were recorded using 3 M KCl-filled glass micropipettes, amplified by a high-impedance negative-capacity pre-amplifier, and displayed on conventional Tektronix equipment. Only microelectrodes with tip potentials less than 5 mV were used. The measurements were made on surface fibers of bender (flexor of the propodite) muscles from the walking legs of Maine lobsters *(Homarus americanus)*. The control Ringer solution contained 10 mM KCl, 455 mM NaCl, 24 mM $CaCl_2$, 8 mM $MgCl_2$ and 5.8 mM H_3BO_3 adjusted with NaOH to a pH of 7.4 $\pm$ 0.1. The experimental Ringer solutions were identical to the controls, but also contained a known amount of a specific digestive enzyme. Control and experimental muscles (from contralateral legs of the same animal) were dissected and incubated for 2 h in 25 ml of the appropriate media. These muscles were then washed 5 times with fresh control Ringer and membrane potentials were measured in control Ringer. Incubations and measurements were made at room temperature (21–23°). The ribonuclease (EC 2.7.7.17), lipase 448 (EC 3.1.1.3), phospholipase C (EC 3.1.4.3), deoxyribonuclease (EC 3.1.4.5), alkaline phosphatase (EC 3.1.3.1), chitinase (EC 3.2.1.14), hyaluronidase (EC 3.2.1.35), carboxypeptidase A (EC 3.4.2.1), papain (EC 3.3.3.10), clostridiopeptidase A (EC 3.4.4.19), trypsin (EC 3.4.4.4), and chymotrypsin B (EC 3.4.4.6) were obtained from Nutritional Biochemicals Corporation. The neuraminidase (EC 3.3.1.18) was obtained from General Biochemicals Co., and the pure Nystatin (Mycostatin) from E. R. Squibb Co. Muscle cells were chosen for this study since it had been shown that externally applied hydrolytic enzymes are freely accessible to the plasma membrane[3]. Nerve fibers, on the other hand, are affected by proteases only when internally perfused[4,5], presumably because the Schwann cell surrounding the axon prevents these enzymes from reaching their site of action on the nerve plasma membrane.

Lobster muscle cells behave as Nernst electrode systems for potassium ion, producing a 58-mV drop in resting potential for a 10-fold increase in external potassium concentration[6]. Furthermore, the membrane potentials of these cells are quite insensitive to changes in pH, external calcium ion concentration, or cyanide poisoning (H. GAINER, unpublished data). Therefore, the resting potential is a rather stable measure of the state of the muscle plasma membrane. Any decrease in membrane potential as a result of the enzymatic treatment, must be due either to an altered ion permselectivity of the membrane or to non-specific injury of the membrane causing short-circuiting of the muscle membrane electromotive force. The results of the enzyme experiments are presented in Table I.

TABLE I

EFFECTS OF ENZYMATIC DIGESTION ON THE RESTING MEMBRANE POTENTIAL OF MUSCLE

Experiment No.	*Enzyme*	*Concentration (mg/ml)*	*Number of cells*	*Resting potential (mV)**	*Potential difference from paired control (mV)***	*P****
1–11	Control	(untreated)	150	81 ± 0.32	—	—
A.	Proteases					
1	Trypsin	0.01	20	76 ± 1.12	5	<0.01
3	Trypsin	0.1	10	61 ± 1.99	18	<0.01
7	Carboxypeptidase	1.0	20	66 ± 1.71	13	<0.01
8	Papain	1.0	30	71 ± 0.77	8	<0.01
7	Clostridiopeptidase A	1.0	20	66 ± 1.82	13	<0.01
7	Chymotrypsin B	1.0	10	55 ± 3.02	24	<0.01
B.	Lipases					
3	Lipase	0.05	10	60 ± 5.20	19	<0.01
1	Lipase	0.1	10	57 ± 5.02	24	<0.01
1	Phospholipase C	0.02	20	64 ± 2.23	17	<0.01
3	Phospholipase C	0.05	10	51 ± 2.41	28	<0.01
11	Acetone†	50	10	56 ± 2.23	22	<0.01
10	Saponin†	0.01	10	67 ± 2.55	14	<0.01
12	Nystatin†	1000§	20	69 ± 1.43	12	<0.01
C.	Other enzymes					
2	Hyaluronidase	1	20	81 ± 1.39	−3	>0.1
9	Neuraminidase	1	20	84 ± 1.55	0	—
7	Deoxyribonuclease	0.5	10	79 ± 1.01	0	—
7	Chitinase	1	10	78 ± 0.74	1	>0.5
7	Ribonuclease	1	10	80 ± 0.96	−1	>0.5
8	Alkaline phosphatase	1	20	79 ± 0.84	0	—

* Mean ± S.E.

** The positive and negative values represent depolarization and hyperpolarization, respectively, from the paired controls.

*** The probability (*P*) was determined using Student's "*t*" test.

† Non-enzymatic lipolytic agents.

§ Concentration in units/ml.

Only proteolytic and lipolytic agents (Table I, A, B) reduced the membrane potential. The enzymes acting on polysaccharides, nucleic acids and orthophosphoric monoesters were without effect (Table I, C). Heat denaturation of the proteases and lipases (at 100° for 60 min) eliminated their effectiveness as depolarizing agents, which suggests that their enzymatic activity was necessary for the depolarization. The conditions of the experiments described in Table I (*i.e.*, 2-h incubation at the given enzyme concentrations) were chosen so that membrane potentials could be measured in cells with no obvious injury. Longer incubation times or higher protease and lipase concentrations invariably produced damaged muscle cells usually with retraction clots. The depolarizations produced by the action of the enzymes were always accompanied by substantial decreases in effective membrane resistances (H. GAINER, unpublished results). Lobster muscle-fiber resting potentials are very sensitive to the external potassium ion concentration, but are relatively insensitive to cesium ions added to the external medium[6]. Tests of the relative sensitivities of enzymatically depolarized cells to these two ions showed no increase of permselec-

tivity to cesium (H. GAINER, unpublished results) which suggests that the mechanism for depolarization was by the production of micro-injuries to the plasma membrane which were sufficient to short-circuit the potential and lower effective resistance, but not to produce coarse structural damage.

It is difficult from these data to come to definite conclusions about the specific composition or structure of the muscle plasma membrane. However, it is clear that the membrane must be composed of a lipid–protein matrix and that both components are involved in maintaining the permeability barrier to ions. The greater effectiveness of the lipases in causing depolarization at lower concentrations than that of the pure proteases, indicates that the lipolytic activities of these lipases (as opposed to their protease contaminants) were involved. It is also of interest that acetone which dissolves the neutral lipids[7] of the membrane, and saponin and Nystatin which act specifically on cholesterol in membranes[8], all produced considerable depolarization. Hence, it is likely that cholesterol is present in the structure of muscle plasma membrane. While polysaccharides, nucleic acids, and sialic acid have been associated with cell surfaces[9–11] the data in Table I (C) indicates that these components may not be fundamental in maintaining the permeability barrier function of plasma membranes.

The results of this study are consonant with either the unit membrane[12] or the lipid micellar model[13] of membrane structure. However, the apparent easy access of such large enzymes as phospholipase C to the phospholipid substrate in the membrane, is suggestive that large exposed lipid patches may exist on the surface of the muscle plasma membrane. An alternative possibility is that the slight proteolytic activity of the phospholipase C may remove enough of the protein coat so that the large enzyme can freely approach its substrate. The latter view has been proposed for the lytic action of phospholipase A on human erythrocytes[14].

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