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Effect of phospholipase C on surviving muscle preparations

In addition to the surface membrane, two distinct membrane systems have been implicated in excitation–contraction coupling in skeletal muscles[1–4]. The transverse system provides the channel for the inward conduction of the excitation wave[2,3], which ultimately reaches the sarcoplasmic reticulum and acts as a regulatory signal for the operation of the Ca^{2+} pump[4,5]. The transverse tubules are represented as invaginations of the surface membrane of muscle cell[6], their lumen being continuous with and permeated by the external medium[7–10].

In view of these characteristic structural relationships, the selective modification of muscle cell surface membranes was attempted using phospholipase C (EC 3.1.4.3) which is able to hydrolyse membrane-bound lecithin into diglycerides and phosphorylcholine[11,12].

Frog sartorius muscles incubated at room temperature in frog Ringer solution with 0.5–1.0 mg/ml phospholipase C, lost their contractility to supramaximal tetanic stimuli within 10–30 min. The loss of contractile response was accompanied by a great increase in the rate of potassium release from phospholipase C-treated muscles (Table I). No detectable Ca^{2+} release was observed. Prolonged phospholipase C treatment produced only slight changes in the release of inorganic phosphate or the uptake of glucose by rat diaphragm muscles.

TABLE I

EFFECT OF PHOSPHOLIPASE C ON THE RELEASE OF POTASSIUM FROM MUSCLE

Muscles were quickly dissected from the animals, rinsed with ice cold frog Ringer (frogs) or Krebs bicarbonate buffer (rat diaphragm) and incubated in 5 ml of the same solutions at room temperature (frog) or at 36° (rat) for 1 h. The incubation medium of phospholipase C-treated muscles contained 0.5–1.0 mg phospholipase C per ml (obtained from Sigma). The potassium content of the incubation medium was assayed by flame spectrophotometry (Zeiss PMQ II spectrophotometer), and the potassium release was calculated on the basis of the wet weight of the muscle at the beginning of the incubation. The numbers in parentheses represent the number of independent experiments. The contractility of the muscles was monitored with a Sanborn recorder using an FTA 1-10 force transducer. Muscles were stimulated with supramaximal tetanic stimuli of 1-sec duration applied at intervals of 60 sec.

	Release of K^+ (μmoles K^+/g fresh muscle)	
	Control	*Phospholipase C-treated*
Frog gastrocnemius	3.8 ± 0.32 (2)	13.1 ± 2.2 (2)
Frog sartorius	17.2 ± 2.3 (9)	82.0 ± 9.1 (9)
Rat diaphragm	21.3 ± 4.1 (8)	39.2 ± 3.5 (8)

Muscles which became insensitive to electric stimulation and lost most of their potassium content as a result of phospholipase C treatment, were washed free of excess enzyme and used for preparation of microsomes as described earlier[11,13]. The yield of microsomes from phospholipase C-treated muscles was similar to that of the control (3–4 mg microsomal protein per g wet muscle weight). The ATPase activity and Ca^{2+} transport of microsomes isolated from control and phospholipase C-treated

TABLE II

Ca^{2+} UPTAKE AND ATPase ACTIVITY OF MICROSOMES ISOLATED FROM PHOSPHOLIPASE C-TREATED FROG MUSCLES

Phospholipase C treatment of muscles was carried out as described in the legend to Table I. Tetanic response to electric stimulation, while fully maintained in the controls, was completely lost after 1-h incubation in muscles treated with phospholipase C. Microsomes were prepared, ATPase and Ca^{2+} transport activities were measured as described earlier[11,13,14]. Microsomal protein concentration in both assays was 0.01–0.07 mg/ml. ATPase activities were measured at 37°, Ca^{2+} transport at room temperature. The numbers in parentheses indicate the number of microsome preparations; the number of independent assays on each preparation ranged from 6 to 11. The standard error of each series was less than 10% of the mean. C, control; PC, phospholipase C-treated.

Muscle	*Ca^{2+} uptake (μmoles Ca/mg protein)*				*ATPase activity (μmoles P_i/mg protein per min)*					
	No oxalate		*5 mM oxalate*		*No addition*		*5 mM EGTA*		*5 mM oxalate*	
	C	*PC*	*C*	*PC*	*C*	*PC*	*C*	*PC*	*C*	*PC*
Gastrocnemius	0.42 (2)	0.42 (2)	2.86 (3)	2.64 (3)	2.60 (5)	2.79 (5)	1.72 (5)	1.94 (5)	1.84 (1)	1.58 (1)
Sartorius	—	—	2.28 (1)	2.35 (1)	0.76 (1)	0.97 (1)	0.63 (1)	0.65 (1)	—	—

muscles were, within error, identical under a variety of experimental conditions (Table II). Electron micrographs of microsome preparations obtained from phospholipase C-treated muscles did not show the diglyceride droplets which are regularly observed in microsome suspensions treated with phospholipase C *in vitro*[12] and their lecithin content was similar to that of the control.

The mechanism of action of phospholipase C on the surface membrane of muscle cell is not known. Phospholipase C preparations did not contain measurable proteolytic, alkaline phosphatase or sialidase activities in the concentrations presently employed. The phospholipase C preparations used in these experiments were shown to hydrolyse readily the lecithin component of different biological membranes with the demonstrated liberation of diglycerides and phosphorylcholine[12,15].

On these grounds, the effect of phospholipase C on the potassium release of skeletal muscle is tentatively attributed to the hydrolysis of lecithin in the surface membrane of the muscle cell. Definitive proof for this suggestion would require not only the demonstration of diglyceride and phosphorylcholine release, but also evidence that they originate from the surface membrane of the cell, which is technically not feasible.

The ATPase activity and Ca^{2+} uptake of isolated sarcoplasmic reticulum fragments is highly sensitive to phospholipase C[11,12,15]. Since these functions remained unaffected during phospholipase C treatment of whole muscle, it appears that the effect of phospholipase C under the conditions presently described is confined to the surface region of the muscle cell. It remains to be determined whether these chemically modified muscles offer sufficient practical advantage over the NATORI-fibers[16] for biochemical studies on excitation–contraction coupling.

The effect of phospholipase C on the potassium permeability provides an explanation for its influence on the electrical properties of skeletal muscle surface membrane[17,18].

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The entry of sugars into bone cells. Independence from parathyroid extract and thyrocalcitonin effects

The importance of glucose as a substrate for bone made it of interest to examine whether it is actively accumulated in bone cells, as are amino acids, and whether parathyroid hormone and thyrocalcitonin affect its metabolism by modifying its transfer across the bone cell membrane. Although ADAMSON, LANGELUTTIG AND ANAST[1] could find no evidence of active transport of 3-*O*-methylglucose in embryonic bone, a transport system important in adult life might not appear until later in development. The kinetics of entry of 3-*O*-methylglucose, a non-metabolizable analog of glucose, into isolated bone cells from adult animals was, therefore, determined and the effect of parathyroid extract and thyrocalcitonin on it explored.

Intact bone cells were isolated from the femoral and tibial metaphyses of 38–41-day-old male rats of the Charles River strain following the procedure of WOODS AND NICHOLS[2]. The technique for studying transport phenomena in these cells was based on methods used by CHRISTENSEN AND RIGGS[3].

The cell pellet obtained from 12 rats was suspended in 3.5 ml of Krebs–Ringer bicarbonate buffer (pH 7.4). Aliquots of this suspension (0.5 ml) were equilibrated