Difference in Saponin Sensitivity between Myotubes and Mononucleated Cells from Chick Breast Muscle

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ABSTRACT. Cultured muscle cells treated successively with saponin at varied concentrations released protein initially at 2×10^{-5} g/ml saponin and then at 6×10^{-5} g/ml saponin. The first release was accompanied with creatine kinase activity, whereas the second release was not. Morphological observations indicated that the first protein leakage was derived mainly from myotubes and the second release mainly from mononucleated cells.

Saponin, a plant glycoside, was recently shown (5) to make the plasma membrane in several kinds of cells permeable to large protein molecules. Cells thus treated have been utilized for examining the cellular distribution of contractile proteins using macromolecular tracers, such as specific antibody or heavy meromyosin (3, 5, 7). The concentrations of saponin required have been in the range from 10^{-5} to 10^{-4} g/ml, depending on the cell species (4).

In the present study, we investigated the effective range of saponin concentration for protein molecules to pass through the surface membrane of cultured muscle cells. Myotubes were found to be affected by lower saponin concentration than mononucleated cells.

Single cells were dissociated from breast muscle of 11 or 12 day chick embryos, and 10^6 cells were inoculated to 100mm Falcon plastic petri dishes (1). The cells were cultured for 3 days in medium composed of 84.5% Eagle's MEM (Nissui Co., Tokyo), 15% horse serum (Flow Laboratories, Rockville, Maryland) and 0.5% adult chick serum. Then, the culture medium was replaced by salt solution containing 150 mM NaCl, 5 mM $MgCl_2$, 0.8 mM $CaCl_2$, 10 mM Tris-HCl (pH 7.6) and varied concentrations of saponin (NBC) at 20°C. The solution was renewed every 5 minutes. The protein released into the solutions was determined by the biuret reaction and the creatine kinase activity was measured by the method of Noda, Kuby and Lardy (2).

The protein leakage from cells was observed in saponin concentrations higher than 2×10^{-5} g/ml. Fig. 1 shows that protein was released biphasically from cells during treatment with three different concentrations of saponin. The first protein release occurred at 2×10^{-5} g/ml saponin, and the second release at 6×10^{-5} g/ml. The first leakage was accompanied with the increase in creatine kinase activity, whereas the second leakage was not. A further increase in saponin concentration to 1×10^{-4} g/ml

Fig. 1. Time course of protein and creatine kinase release from cultured muscle cells during saponin treatment. Ordinates indicate protein (upper) and creatine kinase (lower) released from cells of one dish. The cells were treated with salt solution containing 0, 2×10^{-5} , 6×10^{-5} or 1×10^{-4} g/ml saponin. The solution was changed every 5 minutes. It was spun down at 3,000 rpm for 5 minutes. The resulting supernatant was used for protein and kinase determinations. Concentrations of saponin in solutions are shown in the figure.

did not induce a protein release.

As creatine kinase is known to increase after muscle cell fusion (6), the myotube is probably responsible for leakage at 2×10^{-5} g/ml saponin. Observation by phase contrast microscopy actually revealed that myotubes appeared flattened in the presence of 2×10^{-5} g/ml saponin, whereas mononucleated cells remained unchanged. Electron microscopic investigation also demonstrated that ferritin particles penetrated into most large myotubes but not into mononucleated cells treated with 2×10^{-5} g/ml saponin (Fig. 2).

When the cells were treated with saponin at 2×10^{-5} g/ml for 15 minutes and further cultured in the medium, myotubes degenerated and detached from the culture dish within several hours, but most mononucleated cells proliferated and myotubes reappeared after a few days.

In the presence of 6×10^{-5} g/ml saponin, mononucleated cells also degenerated, retracting their processes and becoming rather round. Ferritin particles were observed to enter into all cultured cells.

These findings indicate that saponin perforates the cytoplasmic membranes of myotubes at 2×10^{-5} g/ml and those of mononucleated cells at about 6×10^{-5} g/ml. This change in saponin sensitivity to myogenic cells during myogenesis *in vitro* is probably related to the differentiation of cytoplasmic membrane.

Fig. 2. Entry of ferritin particles into cultured muscle cells treated with saponin. The cells were treated with 2×10^{-5} g/ml saponin for 15 minutes, incubated in solution containing ferritin for 15 minutes, then fixed with glutaraldehyde (2%) and OsO_4 (1%), dehydrated by ethanol series and embedded in Epon. Thin sections were cut on a Porter Blum MT II B microtome using a diamond knife. The specimens were slightly stained with lead and observed by a JEOL 100B electron microscope. Fig. 2a. Part of myotube. Ferritin particles (encircled) are found in the cytoplasm (cyt). Fig. 2b. Part of mononucleated cell. Ferritin particles are not observed in cytoplasm (cyt) and nucleus (N). Magnification $\times 100,000$

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