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# Activation of Ca++ Transporting Systems in Plasma Membranes

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presented by

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# 6. SUMMARY

The plasma membrane of virtually all cells contains 3 pathways for the movement of Ca++ into and out of the cell. Two of these, the Ca++ pumping ATPase and the Na+ - Ca++ exchanger have been examined and found to respond to physiologically related stimuli. The work presented here focussed on mammalian cardiac sarcolemma, and erythrocyte ghosts. The following major points were found.

1. A protein activator exists in sarcolemmal membranes which can stimulate the Ca++ATPase to levels of activity above those previously found. This activation could be observed for the isolated sarcolemmal or erythrocyte Ca++ATPase and in hemolysed erythrocyte ghosts. The activating factor was enriched by a scheme in which a 2.5% triton x 100 extract of sarcolemmal vesicles was precipitated with 60% ammonium sulfate and fractionated by DEAE column chromatography. The final enriched fraction contained major proteins of 56 and 60 kDa. The activating protein(s) were found to be distinct from calmodulin due to the sensitivity to boiling and to the fact that activation occured after controlled trypsinization of the Ca++ATPase, a condition which nullifies calmodulin stimulation.

2. The Na+ - Ca++ exchanger of heart sarcolemma is electrogenic, as suggested by the formation of a

transmembrane potential during its function. This was first suggested by the increased rate of Ca++ uptake into heart sarcolemmal vesicles in the presence of K+ and valinomycin. The potential was monitored by the use of a lipophilic cation, tetraphenylphosphonium, and an electrode selective to it. Examination of the exchange system over a wide range of pH showed that under some conditions, Ca++ and TPP+ were co - transported in a 1:1 ratio. The implication is that Na and Ca++ are exchanged in a ratio of 3:1.

3. The Na+ - Ca++ exchanger was further studied for activation by ATP. The data suggest that the nucleotide may stimulate the exchange in a non - energizing manner.

4. The Ca++ pumping ATPase of the plasma membrane from heart cells and erythrocytes was isolated and phosphorylated by the cAMP - dependent protein kinase. The phosphorylatable site is completely dependent on the presence of the kinase and is not Ca++ dependent. Preliminary results indicate that an increase in Ca++ pumping takes place after phosphorylation of the enzyme.

## ZUSSAMENFASSUNG

Die Ca++ATPase von Sarkolemmen und Erythrocytenmembranen wurde untersucht; folgende Hauptpunkte sind gefunden worden:

1. In Sarkolemmen existiert ein Aktivatorprotein das die Ca++ATPase in Gegenwart von Calmodulin hoch stimulieren kann. Eine Reinigungsanleitung fuer die Aktivierungsproteine ist unternommen worden und 2 Proteine, der Einer von 56 kDa und der Zweiter von 60 kDa, wurden gefunden.

2. Der Na+ - Ca++ Austauscher von Herzsarkolemmen hat elektrogenische Charakteristiken. Ein elektrogenische Potential wurde waehrend des Na+ - Ca++ Austausches mit dem lipophilen Kation TPP+ untersucht.

3. Der Na+ - Ca++ Austauscher wird durch µM Konzentrationen von ATP activiert.

4. Die isolierte Ca++ATPase von Plasmamembranen wird durch die cAMP - abhaenigige Protein Kinase phosphoryliert.