1419 A Recombinant Fab Antibody for Analysis and Isolation of AMPA Receptors.

M.C. Schmidt, L.K. Jespersen, S. Coleman and K.P. Keinanen
Department of Biosciences, PO Box 36, FIN-0014 University of Helsinki, FINLAND

The conformation specific Fab 7 antibody (Jespersen et al., 2000: Eur.J.Biochem. 267:1383-1389) recognizes the N-terminal domain of GluR-B and GluR-D subunits. The Fab fragment was produced in E.coli RV308 in a 7,5 l bioreactor and purified using expanded bead immobilized metal-ion affinity chromatography and Protein G affinity chromatography techniques.

Immunostaining with purified Fab antibody showed expression and localization of the GluR complexes in insect and mammalian cells. Native GluR complexes were isolated from solubilized pig brains by affinity chromatography using the Fab fragment immobilized to a NHS-activated Sepharose matrix. Immunoblot analysis showed all AMPA receptor subunits in the isolated fractions indicating the presence of the complete channel complexes. The purity obtained by our purification scheme was demonstrated by silver staining.

1420 Antibacterial agents berberine, palmatine and benzalkonium are cationic penetrants for model and bacterial membranes

L.L. Severina, M.S. Muntyan and K. Lewis
Moscow State University, Moscow 119899, Russia and Tufts University, Medford, USA

Some hydrophobic cations are known to be electrophoretically imported into bacterial cells and actively extruded from these cells by multidrug resistance (MDR) pumps. We have studied transport of plant antimicrobial agents berberine and palmatine and synthetic agent benzalkonium chloride through black planar phospholipid membrane (BLM) and Staphylococcus aureus membrane. It is found that gradients of these cations across BLM generate an electric potential. Penetrating anion tetraphenyl borate and floretin (a plant substance decreasing membrane dipol potential) stimulate this effect. Under optimal conditions, theoretical value of electric potential is observed i.e. 62 mV/ten-fold cation gradient. The studied cations are accumulated in the S.aureus cells as is shown by direct measurement of the cation concentration with a cation-sensitive electrode. The cation accumulation is abolished by protonophore CCCP and is stimulated by mutation in an MDR pump. It is concluded that studied agents are penetrating cations and substrates of an MDR pump, which should be taken into account when new effective antimicrobial drugs are selected.

1421 Structure of Ryanodine Receptor Isoforms in Different Functional States as Visualized by Three-Dimensional Cryo-electron Microscopy

M.R. Sharma1, I. Joyakumar1, S. Fleischer1,4 and T. Wagenknecht1,2

1 Wadsworth Center, New York State Department of Health, Albany, New York 12201-0509, 2 Department of Biomedical Sciences, Albany, New York 12201-0509, 3 Department of Molecular Biology, and 4 the Department of Pharmacology, at Vanderbilt University, Medical School, Nashville, Tennessee 37232, USA.

The non-selective cation channel, ryanodine receptor (RyR) is ubiquitous in all mammalian tissues. In striated muscle its function is to provide for rapid translocation of calcium ions from the intracellular storage organelle to the cytosol, thereby, facilitating the excitation-contraction (E-C) coupling.

There are three isoforms of RyR: RyR1, RyR2, RyR3, preferably expressed in skeletal muscle, heart muscle, and brain tissues, respectively. These three isoforms are known to have three highly divergent regions of amino acid sequence among them. To pin-point these divergent regions 3D structures of all three isoforms have been studied by 3D cryo-electron microscopy (1,2,3). In addition, 3D maps were also obtained for these isoforms in buffer conditions that specifically favor an open (that conducts calcium ions) or a closed (that do not conduct calcium ions) channel conformations. A comparison of open and closed channel 3D structures will be presented to evaluate/understand the functional difference among the three receptor isoforms (4).


1422 LIV-1 breast cancer protein belongs to a new family of histidine-rich membrane proteins with potential to control intracellular zinc.

Tenovus Cancer Research Centre, Welsh School of Pharmacy, Cardiff University, Cardiff, CF10 3XF, UK

Investigation of the protein product of the oestrogen-regulated gene LIV-1, implicated in metastatic breast cancer (1), has revealed ten protein sequences of unknown function that belong to a new family with potential to control intracellular zinc (2).

Sequence alignment highlights the similarity in transmembrane domains II and V (45-88% and 35-80% respectively) as well as the occurrence of conserved extramembrane charged residues, indicating potential ion transport ability. This family also shares a conserved motif of 66 residues, including a transmembrane domain and consensus sequence of the catalytic zinc-binding site of metalloproteases, HEXPHEXXGD. These proteins have similar predicted secondary structure to zinc transporters and contain more histidine-rich repeats, suggesting an ability to bind or transport zinc across membranes. We propose that these proteins form a new family with potential to control intracellular zinc homeostasis. The only protein of this group with any known function is oestrogen regulated LIV-1, which has been implicated in metastatic breast cancer. Whether the other proteins have a role in cancer and/or disease remains to be determined. However, multiple tissue array analysis suggests that they are predominantly present in hormonally controlled tissues.