Tu-AM-Sym I-1

MOLECULAR INSIGHTS INTO THE STRUCTURE AND MODE OF ACTION OF THE MITOCHONDRIAL CHANNEL, VDAC.

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The outer membrane of mitochondria from cells representing all eukaryotic kingdoms contains large aqueous pores formed by VDAC channels. The results of the research performed by many investigators yield a rapidly evolving picture of a large (≈3nm in diam.) aqueous pore formed by a relatively small amount of protein (282-283 amino acids, depending on the source). The wall of the pore is composed primarily of an antiparallel beta sheet although one alpha helix is also present. The weak anion selectivity of the channel is primarily the result of the large number of charged side chains extending into the aqueous pore. Channel closure results in a narrower pore (≈1.8nm in diam.) that shows preference for cations. The "closed" states show only reduced permeability to simple salts but are much less permeable or impermeable to the physiologically relevant organic anions. Channel closure results in a large volume change within the pore and the translocation of protein domains from the walls of the pore probably to the membrane surface. The charge on the mass that is translocated, in association with channel gating, is probably responsible for the voltage dependence of the gating process. Evidence indicates VDAC's voltage sensor is positively charged. Since each channel can close at both positive and negative potentials, regions of the protein wall forming the aqueous pore and carrying a net positive charge, may translocate in one direction or another depending on the sign of the applied potential. There is some evidence for a small aqueous domain that could act as a binding site for a soluble modulating protein or hexokinase. Thus, VDAC seems to be a very efficient protein, performing many tasks with a small amount of protein mass.

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Channels in Mitochondrial Membranes. M.C.Sorgato and°O.Moran Dip. di Chimica Biologica, Univ. di Padova, Italy and°Settore di Biofisica, S.I.S.S.A., Trieste, Italy

Since the discovery of the voltage dependent 107 pS conductance inner mitochondrial membrane channel, several other conductances,with different electrical properties, have been found in the inner membrane of the organelle. The work carried out in our laboratories has recently aimed at assigning the different channels to biochemically and morphologically distinct regions of the inner membrane. This has been achieved by patch clamping either the inner membrane in situ or large liposomes containing the isolated inner membrane or a mitochondrial fraction enriched in the so called contact sites, particular regions where the inner and outer membranes are tightly juxtaposed. This complementary study has therefore offered the possibility to distinguish between the physical location of conductances ranging from approx. 5 to 100 pS (more rarely upto 200 pS), consistently present in the "naked" inner membrane, and of larger conductances (with a more frequent peak of approx. 500 pS), which seem to be localised preferentially in the contact site regions. The function of these channels is yet unknown. Nonetheless, if the interpretation of our experiments is correct, mitochondrial contact site channels, in particular, display an electrical behaviour which render them suitable for the transport of macromolecules, a function which has been ascribed to these regions.

The study of the outer mitochondrial membrane with the patch clamp technique, in situ or reconstituted in liposomes, has also been carried out and has shown the presence of currents corresponding to low conductances (>50 pS), found also in the other membrane fractions, as well as of four distinct and voltage dependent conductances of 99,152,220 and 307 pS, respectively (in 150 mM KCl). The lack of detection, under our experimental conditions, of a channel with characteristics similar to those described for the voltage-dependent-anion-channel, will be discussed. The work in progress on the isolation of the channel(s)-forming protein of the inner mitochondrial membrane will also be presented.

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MODULATION OF INNER MITOCHONDRIAL CHANNEL ACTIVITY BY Ca^{2+}, pH AND PHARMACOLOGICAL AGENTS. K.W. Kinnally, Dept. of Biology, Siena College, Loudonville, N.Y. and Dept. Biol. Scie., S.U.N.Y. Albany

We have investigated the effect of several conditions and pharmacological agents on the behavior of channels of the inner mitochondrial membrane. Using patch-clamp techniques on excised inside-out patches derived from mitoplasts, we have evidence for the following behavior. (1) Ca^{2+} concentration and the history of the previous exposure to Ca^{2+} are significant. Isolation of the mitoplasts in solutions containing a background of Ca^{2+} (10^{-7} to 10^{-6} M) favors multiconductance activity up to 1nS (or higher). Isolation in the presence of EGTA or exposure to free Ca^{+2} at 10^{-9} M or below after isolation, often permits the observation of the ~110 pS channel. (2) Perfusion with an alkaline medium (pH 8.3, matrix side) increases the conductance of the membrane by 80±25 pS, an effect reversed by return to the original pH of 6.8 or addition of Mg^{2+}. (3) Amphiphilic drugs (amiodarone, propranalol and quinine) which reverse the conductance increase produced by alkaline pH also block multiconductance channel activity and sharply increase the resistance of the membrane (frequently from 5-10 GΩ to 50-100 GΩ). Amiodarone decreases the open time of the ~110 pS channel and the channel conductance observed often increases to 140 to 160 pS. Antimycin A also decreases channel activity, an effect which is probably unrelated to metabolism since rotenone has no effect. The possible physiological significance of the channels will be discussed. Aided grants from NSF DCB-8818432 and USIA 1A-1AEMP-G8193395 and 193692.

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IONIC CHANNELS OF THE SARCOPLASMIC RETICULUM OF LOBSTER REMOTOR MUSCLE.

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The membranes inside a muscle fiber are embedded in the contractile proteins they control. Those proteins impede the study of channels in the sarcoplasmic reticulum (*SR*) membrane. The lobster remotor muscle has much more *SR* than most muscle fibers and gigaseals are easily made once its inner membranes are exposed by splitting the fiber. Gigaseals presumably form after membrane lipid flows into the pipette, driven by its large free energy of binding to glass, favored by the plasticity of the *SR* membrane. Recordings can be routinely made of single K^+ channels similar to (but distinct from) those reconstituted into artificial bilayers from vertebrate muscle. Ca^{++} channels sensitive to ryanodine and ruthenium red can also be studied, repeatedly but not routinely. The channels studied are in a reasonably physiological environment but are unlikely to remain attached to structures in the T membrane. The split remotor fiber gives repeated caffeine contractions: its *SR* is able to release and reaccumulate Ca^{++}. It is as viable as skinned preparations of most other muscles.

"Whole-*SR*" recordings are badly needed, because direct measurements of *SR* membrane potential are not available, and only a little is known of *SR* membrane conductance and capacitance. It would be comforting to acquire data supporting the common assumption that the membrane potential of the *SR* is identically and unchangably zero, having no role in controlling the functional movement of ions (e.g. Ca^{++}), so unlike the role of the potential across most membranes. We have made some whole-*SR* recordings of membrane potential, finding a value close to zero, but the common occurrence of rescaling, which may reflect the plasticity of the *SR*, prevents systematic investigation. Our study of macroscopic electrical properties of the *SR* has been limited because the compartment accessed in whole-*SR* recordings has little capacitance, presumably because it is tiny in area. Step currents produce voltage transients too fast to record reliably but the steady voltage displacement suggests the *SR* is not very leaky even in the skinned preparation.

Tu-AM-Sym I-5

ION CONDUCTIVITY OF THE NUCLEAR ENVELOPE.

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The nuclear envelope is a structure of particular interest because it is involved in diverse steps of protein synthesis, is a support for hormone receptors and is an active participant in mitosis. Nucleo-cytoplasmic communication is endowed with large protein complexes in which circular association of eight globular subunits forming a central canal where, it is assumed, bidirectional transport of RNA and proteins occurs. Well maintained isolated nuclei present a structure in the canal called the central granule. Measurements of passive diffusion define open pathways with a functional diameter of 9 nm. However, using voltage sensitive microelectrodes it was shown that certain nuclei are able to maintain a potential difference with respect to the cytoplasm. Although the value of 10-15 mV could be explained by a Donnan equilibrium due to large non diffusible proteins in the nucleus, different nucleo-cytoplasmic ionic concentrations were reported and a dependency of nuclear membrane potential on cytoplasmic ionic strength was demonstrated. Both these facts are against the concept of free diffusion as far as ions are concerned. Using on-nucleus patch-clamp technique on pronuclei obtained manipulating the mouse zygote, it was shown that nuclear envelope contains potassium selective ionic channels. That the nuclear external membrane contains ion channels is not exceptional. Other organelles such as mitochondria and endoplasmic reticulum contain ionic channels. In particular since the outer nuclear membrane is continuous with the endoplasmic reticulum, it is not surprising that a similar channel is present on both the surfaces. What was unexpected was the possibility, using single channel recording technique, to visualize the current flow through nuclear pores, since they are considered open pathways. Electron microscopy of pronuclei envelopes reveals a pore density of more than 3 per um^2. In other preparations this value is even higher. Patch-clamp electrodes delimited 3-4 um^2 of membrane. The membrane area of interest in the experiment should contain several pores but certainly not less than one. Assuming a water hole of 9 nm diameter, 80 nm long, filled with 100 ohm·cm resistant solution, it should be possible to visualize a 1000 pS ionic channel. The ionic pathway recorded on the pronucleus envelope is 200 pS. thus, either nuclear pores are closed and the channel is a separate entity, or the pore itself forms the ionic pathway, but is constricted, showing a lower conductance than expected.