Tu-Pos33

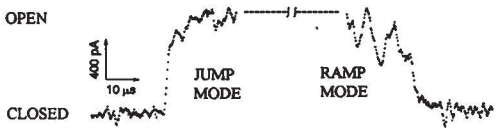
POTASSIUM CHANNEL OPENER, RP 66471, INDUCES MEMBRANE DEPOLARIZATION OF RAT LIVER MITOCHONDRIA. ((A.Szewczyk, G.Wójcik and M.J. Nałęcz)) Nencki Inst. of Exp. Biol., Warsaw, Poland. (Spon. by A. Azzi)

The potassium channel sensitive to ATP (K_{ATP}) has been described in the inner membrane of rat liver mitochondria. Activation of this channel should lead to the depolarization of the inner mitochondrial membrane. Hence, effect of potassium channel openers on membrane potential of rat liver mitochondria were studied. It was shown that potassium channel opener RP 66471 induces depolarization of the mitochondrial membrane. The depolarization of mitochondrial membrane was induced exclusively by RP 66471 and not by other related compounds (nicorandil, aprykalim, minoxidil sulfate, Ro 31-6930 and KRN 2391). Amplitude of depolarization was significantly larger in the presence of K^+ and Rb^+ ions than in the presence of Li^+ and Na^+ ions. The effect of RP 66471 appears specific since neither the inhibition of mitochondrial respiration nor the uncoupling of mitochondria was observed concomitantly. It was shown that effect of RP 66471 on membrane potential was caused by increasing permeability of inner mitochondrial membrane to potassium ions. Summarizing, our results suggest that mitochondrial K_{ATP} channel is involved in regulation of membrane potential of liver mitochondria and, as consequence, in regulation of substrate transport into mitochondria.

Tu-Pos34

OPENING AND CLOSING TRANSITIONS OF A LARGE MITOCHONDRIAL CHANNEL WITH MICROSECOND TIME RESOLUTION. ((J.M. Tang, R.A. Levis, K.G. Lynn, and Bob Eisenberg)) Department of Physiology, Rush Medical College, Chicago, IL 60612 and Department of Physics, Brookhaven National Laboratory, Upton, NY 11973.

We have investigated the single channel current of a large conductance channel from the outer mitochondrial membrane. The gating characteristics of the channel were studied with the tip-dip technique and high bandwidth recording. This channel has a maximum conductance of 7 to 8 nS when recorded in symmetrical 3M KCl solution at room temperature. The channel has several distinct sets of conductance states. It shows different modes of opening and closing behaviors. The time course of opening and closing of the channel has been studied, with resolution of 1.2 μsec at a bandwidth of about 300 kHz. Initial data showed the channel able to open and close in less than 1.2 μsec. Occasionally, this channel appears to gate in a ramp mode (see figure). The time course is then much slower, often many μsec.



Tu-Pos35

INDICATIONS OF A COMMON FOLDING PATTERN FOR VDAC CHANNELS FROM ALL SOURCES. ((J.M. Song, M. Colombini)) Dept. Zoology, Univ. of Maryland, College Park, MD 20742. (Spon. by S. Gupte)

VDAC (mitochondrial porin) is a large channel found in the outer membrane of mitochondria. The structure of the open channel isolated from yeast (S. cerevisiae), as mapped by site-directed mutations, consists of 1 alpha helix and 12 beta strands. The functional properties of VDAC isolated from mitochondria of very diverse organisms is highly conserved. This includes single-channel conductance, ion selectivity and voltage dependence. Analysis of the primary sequences now available for VDAC from higher plants, fungi, invertebrates, and mammals indicate that all form channels using homologous regions of the primary sequence. The transmembrane regions are constrained by the necessity to form sided structures, one surface facing the hydrophobic regions of the membrane interior and the other the aqueous pore. The constraints on the beta strands preclude the presence of adjacent charged residues or proline residues. The regions with the appropriate constraints are also those that have homology with the corresponding regions in the sequences from other species. Competing proposals utilizing 16 transmembrane beta strands are in conflict with experimental observations. (Supported by ONR grant # N00014-90-J-1024)

Tu-Pos36

TOWARDS CRYSTALLIZATION OF THE MITOCHONDRIAL CHANNEL PROTEIN, VDAC ((D. Koppel[1], P. Masters[1], L. Shao[2], C.A. Mannella[1,2])) The Wadsworth Center, Empire State Plaza, Albany, NY 12201-0509; Departments of [1]Biomedical Sciences and [2]Physics, The University at Albany.

Attempts at crystallizing the mitochondrial porin, VDAC, have been hampered by limiting amounts of the purified protein and by lack of knowledge of its structure in detergents under varying conditions. We have been able to express yeast and fungal VDAC genes (gift of M. Forte, Oregon Health Sciences Univ.) in E. coli as fusion proteins with maltose binding protein, using the pMAL expression system. Conditions that optimize cleavage by factor Xa are found to differ for fusion proteins containing the yeast and fungal proteins. Yields of pure yeast VDAC of 20-40 mg/L of bacterial culture are obtained, compared to 0.1-0.2 mg/L of pure VDAC from fungal cultures. Circular dichroism (CD) of VDAC isolated from fungal mitochondria indicates that a high β-sheet structure, characteristic of VDAC's open state in liposomes, is preserved in detergents like octylglucoside and LDAO over a wide range of temperature (4-25°), pH (6-11) and ionic strength. Low pH induces a reversibly closed state with lower β-sheet content, having a CD spectrum like that observed in SDS at pH 7. (Supported by NSF grant MCB9219353.)

Tu-Pos37

EXPERIMENTAL AND COMPUTATIONAL APPROACHES TO DETERMINING HOW VDAC FOLDS. ((C.A. Mannella[1], E. Dolginova[1], S. Stanley[1], D. D'Arcangelis[1], C.E. Lawrence[1,2], A.F. Neuwald[2])) [1]The Wadsworth Center, Empire State Plaza, Albany, New York, 12201-0509; [2]National Center for Biotechnology Information, NLM, NIH, Bethesda, MD 20894.

While there is no obvious homology between the primary structures of bacterial and mitochondrial porins, circular dichroism (CD) indicates the two classes of membrane proteins have similar secondary structure (Shao et al. 1994, Biophys. J. 66:A21). Proposals for the number of β-strands in VDAC's putative β-barrel range from 12 to 19. Constraints on modeling VDAC are being provided by low-resolution molecular envelopes from electron crystallography and by immuno-topological studies, along with CD studies of short peptides representing subsequences in VDAC. Also, we have detected regions in VDAC sequences that are similar to an 11-residue model (identified using a new local multiple alignment algorithm, the Gibbs sampler, Lawrence et al., 1993, Science 262:208) corresponding to amphipathic β-strands tilted 30° in bacterial porins of known structure. (Supported in part by NSF grant MCB9219353.)

Tu-Pos38

PHYSIOLOGICAL AND PHARMACOLOGICAL ACTIVATORS OF THE MITOCHONDRIAL K_{ATP} CHANNEL. ((P. Paucek, V. Yarov-Yarovoy, X. Sun, and K. D. Garlid)). Dept. of Chemistry, Biochemistry, and Molecular Biology, Oregon Graduate Institute of Science & Technology, Portland, OR 97291-1000.

The mitochondrial K_{ATP} channel is inhibited by ATP ($K_i \approx 50$ μM) in the presence of Mg^{2+}, which raises the question of how the channel is opened in vivo [Paucek et al. (1992) J. Biol. Chem. *267*, 26062]. We now report physiological and pharmacological activation of this K_{ATP} channel. For these experiments, the partially purified, reconstituted K_{ATP} channel was inhibited by 500 μM ATP in 2 mM Mg^{2+}. (i) K^+ flux was completely reactivated by GTP ($K_a \approx 7$ μM) and GDP ($K_a \approx 140$ μM). Guanine nucleotides were without effect on the active state in the absence of ATP. GTP also activated the ATP-inhibited K_{ATP} channel in intact mitochondria respiring in K+ succinate media ($K_a \approx 3$ μM in 60 μM ATP). GTP-γS was as effective as GTP. (ii) K^+ flux was completely reactivated by smooth muscle relaxants known to be K^+ channel openers. Potent pharmacological activators of the mitochondrial K_{ATP} channel include diazoxide ($K_a \approx 3$ μM), cromakalim ($K_a \approx 1$ μM), and two developmental derivatives of cromakalim obtained from E. Merck ($K_a \approx 7$ nM). Hill slopes are consistent with tetrameric structure. (iii) The mitochondrial K_{ATP} channel is regulated by nucleotides only on the cytosolic side. (Supported by NIH Grant GM 31086)