AuPS/ASB Meeting - Canberra 2005

Symposium 7: Function and Regulation of Ion Transport Membrane Proteins

Friday 30 September 2005

Chair: Ron Clarke

The transition between gating states in inward rectifier K^+ channels

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Potassium channels are integral membrane proteins that facilitate a controlled flow of charge across cell membranes. Electrical activity depends the capacity of the channel to stably adopt alternate physiological conformers – 'closed' and 'open'. Although previous crystal structures of K^+ channels reveal significant plasticity of the pore, it is unclear whether the conformational differences between individual structures correlate solely with gating state, or if they are representative of familial connections. Two subtly different X-ray structures of a prokaryotic inward rectifier K^+ channel (KirBac3.1) from *Magnetospirillum magnetotacticum* are presented here. The assembly with the more constricted ion conduction pathway is markedly asymmetric in the intracellular domains, whereas the other channel is sufficiently open to allow insertion of a large polyamine into the conduction pathway. The KirBac3.1 structures complement that of a close homologue, KirBac1.1, crystallised in an unequivocally non-conducting 'closed' conformation. By eliminating family-specific differences, the structures define key molecular indicators of gating state. Incremental re-arrangements occurring in the pore and intracellular domains are likely to reflect distinct stages in the closed-to-open transition.

Channelrhodopsin 1,2, a new class of ion channels: functional description and cellular applications

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Microbial-type rhodopsins are found in archaea, prokaryotes and eukaryotes. Some of them represent light activated ion pumps like the well known proton pump bacteriorhodopsin or they act as photosensors for phototactic behaviour in archaea. These proteins have in common the usual rhodopsin-like seven-transmembrane helices motif. By expressing microbial-type rhodopsins from the green alga *Chlamydomonas reinhardtii* in oocytes from Xenopus laevis or in HEK 293 cells we identified two light gated channels. Both channels open rapidly after light excitation and generate a large permeability for protons (ChR1) and for monovalent and divalent cations (ChR2), respectively. The action spectra give strong evidence for the participation of these light gated ion channels on the phototactic behaviour of the alga.

The predicted seven transmembrane α helices structure of ChR1,2 is characteristic for G protein-coupled receptors but reflect a completely new motif for a cation-selective ion channel. Because of its unique properties as a light gated ion channel, which depolarizes cells directly without any delay, ChR2 offers the possibility to use it as a tool for manipulating the electrical properties excitable cells or Ca-dependent processes simply by light in a non-invasive manner.

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Membrane lipid composition and its effect on Na^+,K^+-ATPase molecular activity: insights from mammals, birds and ectotherms

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The basal metabolic rate (BMR) of animals varies dramatically, being several-fold higher in endotherms compared to ectotherms, and much greater, on a mass-specific basis, in smaller vertebrates compared to larger vertebrates. Despite this large variation in metabolic rate between species, a significant and relatively constant proportion of metabolism is associated with membrane-linked energy consuming processes (e.g. Na^+ cycling), regardless of the absolute level of BMR. The majority of these membrane-associated processes are mediated by membrane-bound proteins, and here we have measured the molecular activity (turnover rate) of the Na^+,K^+-ATPase enzyme, a ubiquitous membrane protein that is a significant contributor to BMR, in a range of tissues* from species (five mammals, eight birds and three ectotherms) that vary greatly in their metabolic intensity. Additionally, we have analysed membrane acyl composition in the same tissues to determine the role of the lipid milieu surrounding membrane proteins, in regulating their activity.

Na^+,K^+-ATPase molecular activity varied approximately 20-fold across the different species (1,600 - 29,000 $ATP.min^{-1}$), and was generally greater in animals with a higher BMR (i.e. small vertebrates > large vertebrates and endotherms > ectotherms). These variations in molecular activity were associated with differences in membrane lipid composition, with membranes from more metabolically active species having a higher unsaturation index (i.e. number of double bonds per 100 fatty acid chains). The trends in membrane unsaturation were primarily due to significant and substantial variations in the concentration of the highly polyunsaturated omega-3 fatty acid, docosohexaenoic acid (22:6(n-3)), which ranged between 0.5% and 40% of the total fatty acids across the different species. When linear correlations were calculated between Na^+,K^+-ATPase molecular activity and the relative percentage of individual fatty acids in the membrane, 22:6(n-3) displayed the strongest correlation for any fatty acid in the combined data sets for both the endothermic species ($R=0.69$, $N=39$, $P<0.0001$) and the ectothermic species ($R=0.78$, $N=12$, $P=0.003$). Our results suggest that membrane lipid composition, and particularly 22:6(n-3) content, may play a role in determining the pace (or rate) of metabolism, *via* an effect on the molecular activity of membrane-bound proteins.

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* Tissues were obtained from euthanased animals.

Testing the membrane pacemaker model of metabolism

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Much of the metabolic chemistry of life occurs in the lipid rich environment of membranes. Although membrane lipid composition is often seen as relatively constant this belies both processes that continuously remodel these structures as well as the differences between species. In a series of comparisons that differ greatly in metabolic rate (namely ectotherms *vs* endotherms, newborn *vs* adult rats, large mammals *vs* small mammals, large birds *vs* small birds) we have observed a correlation with the fatty acid composition of cellular membranes and metabolic rate. Low metabolic rates are associated with monounsaturated (i.e. lipids with fatty acids with only one C=C) and high metabolic rates are associated with polyunsaturated (i.e. lipids with fatty acids with two or more C=C) membranes. In essence there is a link between membrane lipid composition and metabolism; this link forms the basis for the membrane pacemaker theory of metabolism.

Much of our work on the relationship between membrane lipid composition and metabolism has been derived from examining the sodium pump (Na^+K^+-ATPase). Constituting up to 20% of the resting metabolism, the sodium pump in different species has vastly different rates of molecular activity (i.e. rate of substrate turn-over) with higher rates of molecular activity associated with polyunsaturated and lower rates associated with monounsaturated membranes. In order to test these correlations, species membrane lipid cross-over experiments were performed. Basically, sodium pumps from tissues (kidney or brain) of species with high and low sodium pump molecular activities were crossed-over with membrane lipid from the same tissue of each species (namely; rat against toad, cow against crocodile and adult rat against neonate rat). In all cases, the results showed molecular activities shifted in the direction of the added membrane lipid source. Namely, original membrane lipid restored original molecular activity, sodium pumps with high molecular activity when added with lipid from membrane with low sodium pump molecular activities resulted in decreased activity and conversely sodium pumps with low molecular activity when added with lipid from membrane with high sodium pump molecular activities resulted in increased activity. The order of change in some cases was as much as a 2-3 fold increase or decrease in molecular activity. These results clearly suggest that membrane lipid composition may play a significant role in determining the molecular activity of membrane bound proteins such as the sodium pump, and in so doing set the pace of metabolism. One consequence of this possibility is that membrane lipid composition can be influenced by dietary fat intake and that may have significant implications for metabolic based processes.

Regulation of the Na,K-ATPase

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Na^+,K^+-ATPase, the enzymatic equivalent of the membrane Na-K pump, maintains transmembrane electrochemical gradients for Na^+ and K^+. These gradients serve in secondary active transport processes that regulate cellular ions as well as organic compounds. The Na^+-K^+ pump therefore has a central role in cell function, and its activity is tightly regulated by a variety of hormones, acting on the pump *via* cell surface receptors coupled to intracellular messenger pathways.

Regulation of the Na^+-K^+ pump is of particular interest in the heart because of the role intracellular Na^+ plays in excitation-contraction coupling and in the "electro-mechanical phenotype" of heart failure. Effects of hormones are controversial. Most controversies arise from inappropriate experimental methods, not taking into account the pump's dependence on both transported ligands at intracellular and extracellular sites and on membrane voltage.

We use the whole-cell patch clamp technique to measure electrogenic Na^+-K^+ pump current (I_p, arising from the 3:2 Na^+:K^+ exchange ratio) in ventricular myocytes. Provided wide-tipped patch pipettes are used, the technique allows accurate control of pump ligands on both sides of the cell membrane and control of membrane voltage. We have examined effects of hormones coupled to protein kinases A, C and G (PKA, PKC, PKG). Direct phosphorylation of the Na^+-K^+ pump by protein kinases have been implicated in its regulation for many years. However, such phosphorylation is difficult to demonstrate *in vitro* unless the pump molecule is denatured.

We examined the effect of the catecholamine noradrenaline (NA), typically believed coupled to PKA activation *via* β_1 and β_2 adrenergic receptors. NA induced an increase in I_p. However, the increase persisted after blockade of β_1/β_2 or inhibition of PKA. In contrast, NA-induced pump stimulation was abolished by ODQ-induced inhibition of nitric oxide-activated guanylyl cyclase, an enzyme coupled to the β_3 receptor. Stimulation was reproduced by the selective β_3 agonist BRL 37344.

Angiotensin II induced a decrease in I_p that was abolished by inhibition of PKC, a kinase often implicated in pump phosphorylation/regulation. However, PKC-mediated phosphorylation of the pump molecule itself seemed unlikely because additional experiments indicated that the effect of PKC was was dependent on NAD(P)H oxidase activation; the Na^+-K^+ pump may be regulated by a direct effect of reactive oxygen species on the pump molecule itself.

Atrial natriuretic peptide (ANP) induced an increase in I_p. Most effects of ANP are mediated by the NPRA receptor, a 'membrane guanylyl cyclase' that is insensitive to ODQ. However, ODQ abolished ANP-induced pump stimulation implicating nitric oxide-activated guanylyl cyclase. In agreement with this inhibition of cGMP-activated protein kinase (PKG) also abolished stimulation.

It is concluded that hormone, receptor and protein kinase-mediated Na^+-K^+ pump regulation is intricately related to nitric oxide and reactive oxygen species metabolism and that direct phosphorylation of the pump molecule is probably not involved.