Magnesium inhibition of skeletal muscle ryanodine receptors modified by DIDS, ryanodine and ATP

E.R. O'Neill[1], G.D. Lamb[2] and D.R. Laver [1], [1]School of Biomedical Sciences, University of Newcastle, Australia and [2]Department of Zoology, LaTrobe University, Australia.

In skeletal muscle the activity of ryanodine receptor (RyR) calcium release channels in the sarcoplasmic reticulum is regulated by the dihydropyridine receptor (DHPR) voltage sensors in the t-tubule membrane. Ca^{2+}, Mg^{2+} and ATP are potent intracellular regulators of RyRs. The effects of these substances on isolated RyRs are well characterised yet it is not clear how they regulate RyR opening under voltage-sensor control. RyRs are activated by μM cytoplasmic Ca^{2+} and mM ATP while physiological [Mg^{2+}] (~1 mM) in the cytoplasm fully inhibits them. It is proposed that during muscle contraction, DHPRs transiently relieve Mg^{2+} inhibition which then permits activation of RyRs by ATP (Lamb *et al.*, 1991).

Mg^{2+} is thought to inhibit RyRs by binding both to low affinity sites that show little specificity between divalent ions (I-sites) and to high affinity sites for Ca^{2+} (A-sites) thus preventing Ca^{2+} from activating the channel (Laver *et al.*, 1997). However, ATP is known to activate RyRs in the absence of cytoplasmic Ca^{2+} so it is not clear how Mg^{2+} at the A-sites affects channel opening under physiological conditions. Here we investigate the mechanism of Mg^{2+} inhibition in the presence of ATP and two drugs, 4,4'-diisothiocyano-stilbene-2,2'-disulfonic acid (DIDS) and ryanodine, which also activate RyRs in the absence of Ca^{2+}.

RyRs were isolated from rabbit skeletal muscle and incorporated into lipid bilayers using standard techniques (O'Neill *et al.*, 2003). Skeletal muscle was removed from dead rabbits. Cytoplasmic solutions contained 250 mM Cs^+ (230 mM $CsCH_3O_3S$ and 20 mM CsCl) 10 mM TES at pH 7.4. Luminal solutions contained 50 mM Cs (30 mM $CsCH_3O_3S$ and 20 mM CsCl), 10 mM TES, pH 7.4.

DIDS decreased I-site affinity for Mg^{2+} and Ca^{2+} by 10 fold and ryanodine abolished binding completely. Cytoplasmic Mg^{2+} inhibited RyRs via the Ca^{2+} activation site even in the absence of Ca^{2+} indicating that Mg^{2+} inhibition is not merely due to the prevention of Ca^{2+} binding. In the case of ryanodine modified RyRs, monovalent ions (Cs^+) could also activate the channel. RyR activity in the virtual absence of Ca^{2+} (~1 nM) was not due to sensitisation of the channel to Ca^{2+} as previously thought (Du *et al.*, 2001; Masumiya *et al.*, 2001) but was due to Ca^{2+}-independent channel opening by ryanodine. The apparent Mg^{2+} affinity at the A-site was decreased by cytoplasmic Cs^+ and Ca^{2+} as well as by luminal Ca^{2+} in a way which suggests that cytoplasmic Mg^{2+}, Cs^+ and Ca^{2+} compete for a site near the cytoplasmic entrance. Ions at this site may progress to the A-site further into the pore. Binding of these ions at the A-site is in competition with luminal Ca^{2+} and leads to either activation (2 × Cs^+ or Ca^{2+}) or inhibition (Mg^{2+}) of RyRs.

Du, G.G., Guo, X, Khanna, V.K. & MacLennan, D.H. (2001) Ryanodine sensitizes the cardiac Ca^{2+} release channel (ryanodine receptor isoform 2) to Ca^{2+} activation and dissociates as the channel is closed by Ca^{2+} depletion. *Proceedings of the National Academy of Sciences of the United States of America,* 98: 13625-30.

Lamb, G.D. & D.G. Stephenson (1991). Effect of Mg^{2+} on the control of Ca^{2+} release in skeletal muscle fibres of the toad. *Journal of Physiology,* 434: 507-528.

Laver, D.R., T.M. Baynes & A.F. Dulhunty (1997). Magnesium inhibition of ryanodine-receptor calcium channels: evidence for two independent mechanisms. *Journal of Membrane Biology,* 156: 213-229.

Masumiya, H., Li, P., Zhang, L., & Chen, S.R. (2001) Ryanodine sensitizes the Ca^{2+} release channel (ryanodine receptor) to Ca^{2+} activation. *Journal of Biological Chemistry,* 276: 39727-35.

O'Neill E.R., Sakowska, M.M., & Laver D.R. (2003) Regulation of the calcium release channel from skeletal muscle by suramin and the disulphonate stilbene derivatives DIDS, DBDS, and DNDS. *Biophysical Journal,* 84: 1-16,