Block of the divalent anion channel in the SR of rabbit skeletal muscle by disulfonic stilbene derivatives

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Previous studies have identified two types of anion channels in the sarcoplasmic reticulum (SR) of rabbit skeletal muscle (Kourie *et al.*, 1996). One of these anion channels has an appreciable anion conductance (saturating at 20pS for phosphate (P_i) and 60pS for SO_4^{2-}). It was proposed that these channels could be responsible for movement of phosphate across the SR membrane (Laver *et al.*, 2001). To further investigate this hypothesis we have searched for specific inhibitors of the divalent anion channel with the rationale that these inhibitors would prevent P_i transport across the SR.

SR vesicles containing the anion channels were isolated from rabbit skeletal muscle that was removed from dead rabbits. Transport of P_i across the SR vesicle membrane was inferred from the P_i assisted component of Ca^{2+} uptake by the SERCa pump. Ca^{2+} uptake was measured from the optical absorbance of antipyrylazo III (Dulhunty *et al.*, 1999). Ca^{2+} uptake experiments were carried out using solutions containing 100 mM KCl or KP_i, 4mM $MgCl_2$, 1mM ATP, 5μM ruthenium red, 0.5 mM antipyrylazo III, 5 mM TES (pH 7). Anion channels were incorporated into lipid bilayers using standard techniques (Laver *et al.*, 2001). Cytoplasmic solutions contained 260 mM Mg^{2+} (250 mM $MgSO_4$ and 10 mM $MgCl_2$), 1 mM $CaCl_2$ 10 mM TES at pH 7.4. Luminal solutions contained 60 mM Mg^{2+} (50 mM $MgSO_4$ and 10 mM $MgCl_2$), 10 mM TES, pH 7.4.

Lipid bilayer studies showed that the divalent anion channels were inhibited by the disulfonated stilbene derivatives, Diisothiocyanostilbene-2',2'-di-sulfonic acid (DIDS), 4,4'-dinitrostilbene-2,2'-disulfonic acid (DNDS), 4,4'-dibenzamidostilbene-2,2'-disulfonic acid (DBDS) and by suramin. Reversible block by these compounds inhibited these channels with high affinity from the cytoplasmic side (~0.1-1μM) and low affinity from the luminal side (0.1 - 1 mM). The voltage-dependent kinetics of drug binding and dissociation indicated that these compounds can dissociate from the channel to either side of the membrane (*i.e.* they are permeant blockers). DIDS also produces non-reversible inhibition of the channel.

Measurements of P_i facilitated calcium uptake by rabbit SR vesicles were used to assay the degree of P_i transport across the SR membrane. The presence of DBDS at concentrations sufficient to block the divalent anion channel in lipid bilayers (~1-10μM) had no effect on P_i transport. Thus it appears that while this channel conducts P_i, it is not the major pathway for P_i in the SR membrane.

Dulhunty, A.F., Laver, D.R., Gallant, E.M., Casarotto, M.G., Pace, S.M. &Curtis, S. (1999) *Biophysical Journal*, **77:**189-203

Kourie, J.I., Laver, D.R., Junankar, P.R., Gage. P.W. & Dulhunty, A.F. (1996) *Biophysical Journal*, **70:**202-221.

Laver, D.R., Lenz, G.K.E., Dulhunty, A.F. (2001) *Journal of Physiology*, **535:**715-728.