Glutathione transferase mu-2 modulation of the activity of muscle sarcoplasmic reticulum

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Glutathione transferases (GST) are versatile enzymes with a wide range of activities ranging from conjugation of glutathione to toxic xenobiotics to maintaining protein disulfide bonds (Sheehan *et al*., 2001).

Members of the GST family have been shown by our group to affect the activity of both skeletal and cardiac ryanodine receptors (RyR) (Dulhunty *et al*., 2001). The present work focuses on glutathione transferase mu-2 (GST mu-2), one of the major GST isoforms in humans (Van Bladeren *et al.*, 2000).

The effects of GST mu-2 on skeletal and cardiac RyRs (isolated from New Zealand rabbit back and hind leg and pig heart respectively) were observed at the single channel level using planar lipid bilayers using symmetrical caesium methansulphonate as current carrier (250/250 mmol/l; *cis* (cytoplasmic*)/trans* (luminal)). $CaCl_2$ (100 μmol/l) was used to activate the channel on the cytoplasmic side at ± 40 millivolt of holding potentials. When GST mu-2 was added to the cytoplasmic side of the skeletal RyR, different effects were observed depending on the holding potential. At +40 millivolt there was a decline in the activity of the channel in a dose dependent manner. Up to approx 80% decrease in the relative mean current (mean current in presence of GST mu-2 compared to control) was observed with 16 μmol/l GST mu-2 (*n*=8). Further investigation revealed that the effect of GST was voltage dependent (14 out of 16 channels) as at -40 millivolt GST mu-2 significantly activated the channel showing about 3 fold increase in the relative mean current (*n*=8). In the presence of GST mu-2, cardiac ryanodine receptor showed a decrease in activity at -40 millivolt, the inhibition being more prominent at +40 millivolt. 2 μmol/l GST mu-2 led to approx 40% decrease in the relative mean current at -40 millivolt compared to approx 60% decrease at +40 millivolt (*n*=6).

The effect of GST mu-2 on calcium induced calcium release and calcium uptake was investigated in skeletal muscle sarcoplasmic reticulum using stopped-flow technique. 8 μmol/l GST mu-2 reduced the rate constant of calcium release by approx 40% (average of 50 traces in 6 experiments) while 10 μmol/l GST mu-2 increased calcium uptake rate constant by approx 13% (average of 40 traces in 6 experiments).

In conclusion (1) GST mu-2 inhibited skeletal RyR at + 40 millivolt while it activated it at 40 millivolt; indicating that GST mu-2 modulation of the skeletal RyR was voltage dependent; (2) GST mu-2 inhibited cardiac RyR, the inhibition being more prominent at positive voltage; (3) GST mu-2 inhibited calcium induced calcium release in skeletal sarcoplasmic reticulum; (4) GST mu-2 potentiated calcium uptake by the skeletal sarcoplasmic reticulum; (5) GST mu-2 effect on calcium release and uptake is potentially a product of the interaction with both the ryanodine receptor and the calcium pump.

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