Effect of volatile anaesthetics on the calcium release channel in the heart

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Although volatile anaesthetics serve a crucial role in preventing pain they continue to have a number of serious side effects. One of these is their ability to excite Ca^{2+} release from the intracellular Ca^{2+} stores (sarcoplasmic reticulum, SR) *via* calcium release channels named ryanodine receptors (RyR). This can result in fatal episodes of malignant hyperthermia in otherwise normal patients harbouring mutations in the skeletal muscle RyR isoform (RyR1, MacLennan & Chen, 1993). The cardiac isoform of the RyR (RyR2) plays a key role in cardiac muscle contraction, pacemaking and rythmicity (Vinogradova *et al.,* 2005). During periods of ischemia, changes in the intracellular milieu cause a decrease in RyR activity and consequently an increase in SR Ca^{2+} load. Upon reperfusion of the heart tissue, recovery of RyR2 activity in the presence of abnormally high store loads leads to cardiac arrhythmias. Evidence now indicates that activation of RyR2 by volatile anaesthetics protects against myocardial injury and arrhythmias following ischemia and reperfusion (Yang *et al.*, 2005). Here we report the first detailed investigation on the effects of volatile anaesthetics on the function of cardiac RyRs.

RyRs were isolated from sheep hearts and incorporated into artificial lipid bilayers that separated baths corresponding to the cytoplasm and SR lumen. The activity of RyRs was measured using single channel recording. Volatile anaesthetics were added to the baths by injection of solutions from sealed reservoirs. These solutions contained the desired concentration of anaesthetic (either halothane or isoflurane) and were carefully titrated for various levels of free Ca^{2+} and Mg^{2+}. We found that clinical doses of these anaesthetics increased RyR2 open probability (P_o) *via* increases in the channel mean open time and opening frequency. The K_as for halothane and isoflurane were 1 mmol/l and 3 mmol/l, respectively. However, the maximal effect of halothane (5-fold increase in P_o) was ~3-fold larger than that for isoflurane. These agents were shown to activate RyRs by interacting with their cytoplasmic domains. Furthermore, anaesthetic site of action was found to be distinct from the adenine nucleotide activating sites, contrary to previous suggestions (Yang *et al.*, 2005).

The effects of halothane on RyR2 regulation by cytoplasmic and luminal Ca^{2+} and Mg^{2+} were accurately fitted by a model based on a tetrameric RyR structure with four Ca^{2+} sensing mechanisms on each subunit (Laver, 2007; Laver & Honen, 2008); two activation sites (the luminal *L*-site with 40 μmol/l affinity and the cytoplasmic *A*-site with 1 μmol/l affinity) and two cytoplasmic inactivation sites (I_1-site with 10 mmol/l affinity and the I_2-site with 1 μmol/l affinity). Halothane did not appear to alter the ion binding affinities for these sites. Rather, it increased channel opening rate and decreased the channel closing rate associated with Ca^{2+} binding to the two activation sites.

Laver DR.(2007) *Biophysical Journal*, **92:** 3541-55.

Laver DR & Honen BN. (2008) *Journal of General Physiology*, **in press**.

MacLennan DH & Chen SR. (1993) *Annals of the New York Academy of Science,* **707:** 294-304.

Vinogradova TM, Maltsev VA, Bogdanov KY, Lyashkov AE & Lakatta EG. (2005) *Annals of the New York Academy of Science,* **1047:** 138-56.

Yang Z, Harrison SM & Steele DS. (2005) *Cardiovascular Research,* **65:** 167-76.