

**SCA 68**  
**ISOFLURANE APPLIED DURING ISCHEMIA ENHANCES ISCHEMIC INJURY IN CARDIOMYOCYTES IN PART BY REACTIVE OXYGEN SPECIES**

<sup>1</sup>Dworschak M, <sup>2</sup>Breukelmann D, <sup>3</sup>Hannon J

<sup>1</sup>University Hospital Vienna, Vienna, Austria; <sup>2</sup>University of Muenster, Muenster, Nordrhein, Germany; <sup>3</sup>Mayo Clinic, Rochester, MN, USA

**Background:** The preconditioning effect of isoflurane involves release of reactive oxygen species (ROS), most likely hydroxyl ( $\bullet\text{OH}$ ) and superoxide ( $\bullet\text{O}_2^-$ ) radicals. However,  $\bullet\text{OH}$  is associated with intracellular calcium ( $[\text{Ca}^{2+}]_i$ ) overload during ischemia and reperfusion (1,2). We investigated whether isoflurane applied during ischemia, when ROS scavenging systems are exhausted, modifies ischemic injury of rat cardiomyocytes via generation of ROS.

**Methods:** Experimental procedures were reviewed and approved by the ACUC of the Mayo Foundation and were in accordance with NIH guidelines. Ischemia was simulated for 30 minutes by superfusing ventricular myocytes with an acidic, glucose free Tyrode's solution containing deoxy-glucose to inhibit glycolysis (pH: 6.3). Simultaneously,  $p\text{O}_2$  was reduced to  $< 15$  mmHg. Myocytes were field stimulated throughout the experiment. Isoflurane treated cells (iso) were exposed to 1MAC of isoflurane. In order to test whether ROS are involved with the application of isoflurane, either the hydroxyl scavenger, mercaptopropionylglycine (iso+MPG) or a superoxide dismutase mimetic, Mn(III)tetrakis(4-benzoic acid)porphyrin chloride (iso+MnTBAP), were added to

the superfusion buffer. MPG was also applied to control cells to determine the contribution of  $\bullet\text{OH}$  to  $\text{Ca}^{2+}$  homeostasis during ischemia (control+MPG). Ischemic injury was assessed by measuring  $[\text{Ca}^{2+}]_i$ , systolic cell shortening, and arrhythmic events. The fluorescent dye Fura-2 was employed to determine  $[\text{Ca}^{2+}]_i$ . Analysis of variance was used for statistical analysis and the significance level was set at  $P < 0.05$ .

**Results:** Resting, diastolic  $[\text{Ca}^{2+}]_i$  increased in all groups. However, this was most prominent in isoflurane treated cardiomyocytes and could be mitigated by both scavengers ( $P < 0.001$ ). Isoflurane decreased the rate constant of the  $\text{Ca}^{2+}$  transient decline, i.e. it diminished  $\text{Ca}^{2+}$  clearance. Isoflurane also impaired cell shortening to a greater extent compared to control ( $P < 0.025$ ) while MnTBAP restored it again. Furthermore, the iso group showed more arrhythmic events ( $P < 0.002$ ), which could not be ameliorated by MPG and MnTBAP.

**Conclusion:** Isoflurane when applied during ischemia appears to worsen arrhythmia, cell shortening and  $[\text{Ca}^{2+}]_i$  overload in single ventricular myocytes from the rat. The latter finding may be caused by impeding  $\text{Ca}^{2+}$  efflux (3). As MPG and MnTBAP ameliorated isoflurane's effects on diastolic  $[\text{Ca}^{2+}]_i$ , isoflurane seems to act in part through generation of ROS. MnTBAP improved the decrease of  $\text{Ca}^{2+}$  sensitivity potentially induced by ROS.

**References:**

1. Kaneko M et al. *Am J Physiol* 1989, 256: H368-74,
2. Rowe GT et al. *Circ Res* 1983, 53: 584-91,
3. Hannon JD et al. *Anesthesiology* 2002, 96: 1457-64.