Effects of Isoflurane on Plasma-Membrane Calcium ATPase

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Introduction: Plasma membrane Ca^{2+}-ATPase (PMCA) is a transmembrane enzymatic protein present in human neurons, myocytes, and RBCs which plays a role in controlling cellular Ca^{2+} levels by regulating intracellular ATP.[1] Inhaled anesthetic agents, such as isoflurane, affect transmembrane Ca^{2+} levels by inhibiting PMCA activity.[2] However, the effect of isoflurane on the interaction between ATP and PMCA has not been studied. We hypothesized that the inhibitory effects of isoflurane on PMCA involve modulation of the interaction between ATP and PMCA.

Methods: RBC membranes (containing PMCA) were isolated from human subjects with a previously validated methodology using a calmodulin affinity chromatographic technique.[3] The membranes were treated with isoflurane using human serum albumin as an intermediary with transfer occurring via a dialysis membrane. The treated membranes were then assayed for PMCA activity using the Malachite Green Colorimetric Assay. Activity of PMCA was determined by measuring the release of inorganic phosphate. Varying concentrations of ATP were used to determine the inhibitory effect on PMCA by isoflurane.

Results: Isoflurane inhibited basal and CaM-stimulated PMCA activity (nmol of inorganic phosphate per mg PMCA per minute) by 28.6% ($P < 0.04$) and 66.9% (saturating concentration of 300nM) ($P < 0.02$), respectively. Significant inhibition of PMCA activity (77.7%) also occurred at CaM concentration as low as 2nM. Isoflurane also inhibited PMCA activity at ATP concentrations between 10μM and 1000μM.

Conclusion: Isoflurane inhibited basal and calmodulin-stimulated PMCA activity. Significant inhibition of PMCA activity occurred at all concentrations of CaM and as low as 2nM. Isoflurane suppressed the Michaelis-Menton kinetics of PMCA activity as a function of ATP at concentrations from 10μM to 1000μM.

Discussion: We showed for the first time that isoflurane decreases PMCA activity as a function of ATP concentration. We also illustrated that isoflurane decreases the ability of CaM to stimulate PMCA activity. In addition, we introduced a novel physiologic delivery system for isoflurane using albumin as a surrogate transfer molecule. The concentration of isoflurane used for these experiments was not determined. Due to difficulties in studying neuronal PMCA, the RBC isoform was used as a substitute. Further investigations could include exploring the direct effects of isoflurane on CaM and ATP using neuronal isoforms of PMCA, and ensuring clinically relevent concentrations of isoflurane. These data would suggest that a primary biochemical target for isoflurane neural inhibition is intracellular ATP via a direct effect on PMCA activity.

References:

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