

## INTRODUCTION

- The plasma membrane Ca<sup>2+</sup> - ATPase (PMCA) is an integral membrane protein present in all cells including human neurons, myocytes, and RBCs. The PMCA uses the metabolic energy of ATP hydrolysis to pump Ca<sup>2+</sup> across the plasma membrane and plays an important role in regulating cellular Ca<sup>2+</sup> levels.
- Inhaled anesthetic agents, such as isoflurane, affect transmembrane Ca<sup>2+</sup> levels by inhibiting PMCA activity.
- However, the effect of isoflurane on the interaction between ATP and PMCA has not been studied.

## HYPOTHESIS

- 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 and treated with isoflurane using human serum albumin as an intermediary.
- Treatment of RBC membranes was as follows:
  - Isoflurane was loaded onto albumin (5mg/mL for 30min) via direct application.
  - Albumin was then placed in a dialysis bag and incubated (2hrs) with RBC membranes, which were then assayed for PMCA activity.
- PMCA activity was carried out using a 25mM Tris buffer (pH 7.4) containing 50mM KCl, 0.2mM EGTA, 1mM MgCl<sub>2</sub>, 10μM CaCl<sub>2</sub>(free), 1mM ATP and 340nM calmodulin (CaM) with ouabain, thapsigargin and oligomycin to inhibit other ATPases.
- In some experiments ATP and CaM concentrations were varied.

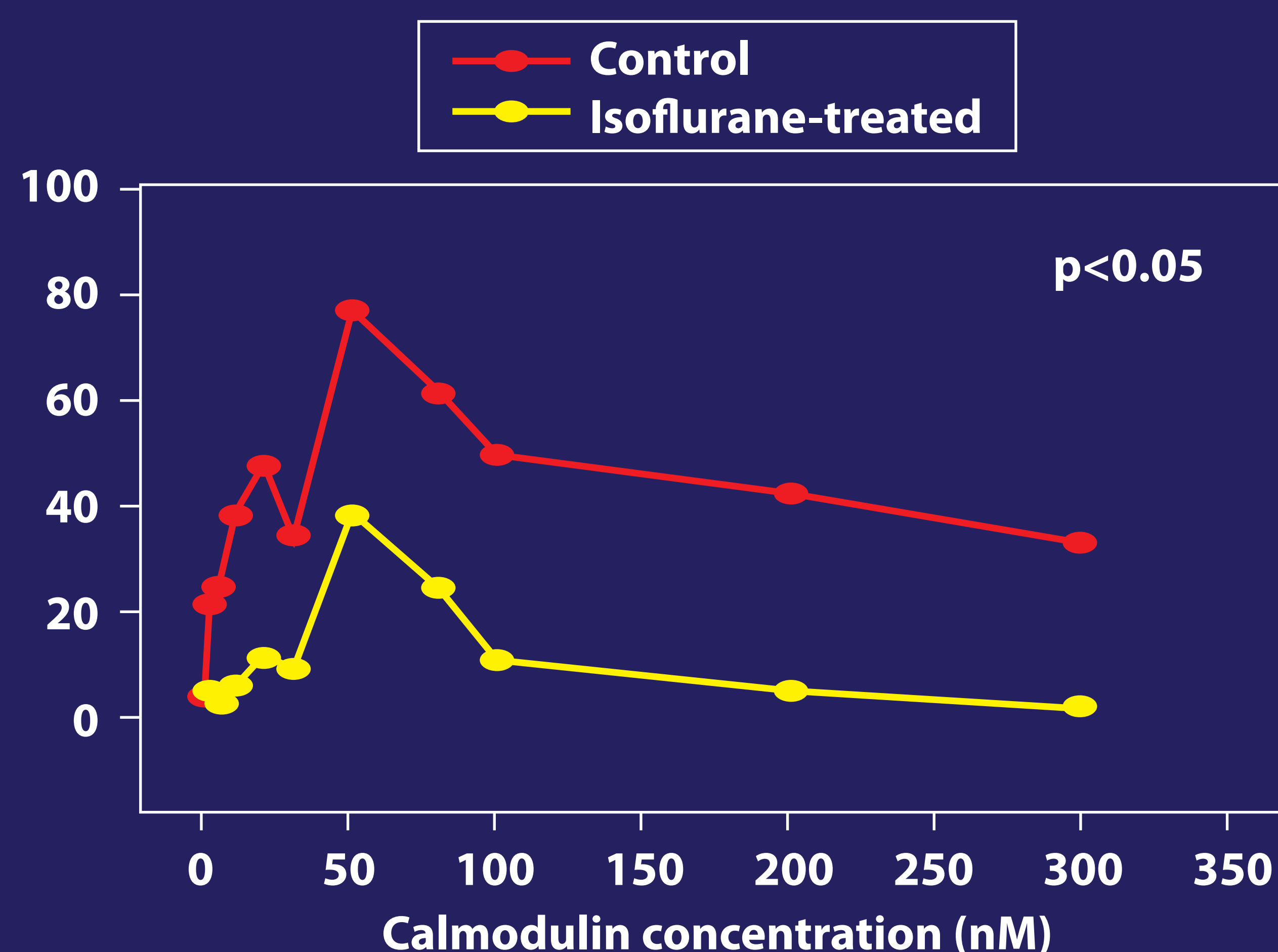
## METHODS

- The treated membranes were then assayed for PMCA activity using the Malachite Green Colorimetric Assay.
- Activity of PMCA was determined by measuring the resultant release of inorganic phosphate.
- Data was analyzed using t-tests and significance was noted at p<0.05.

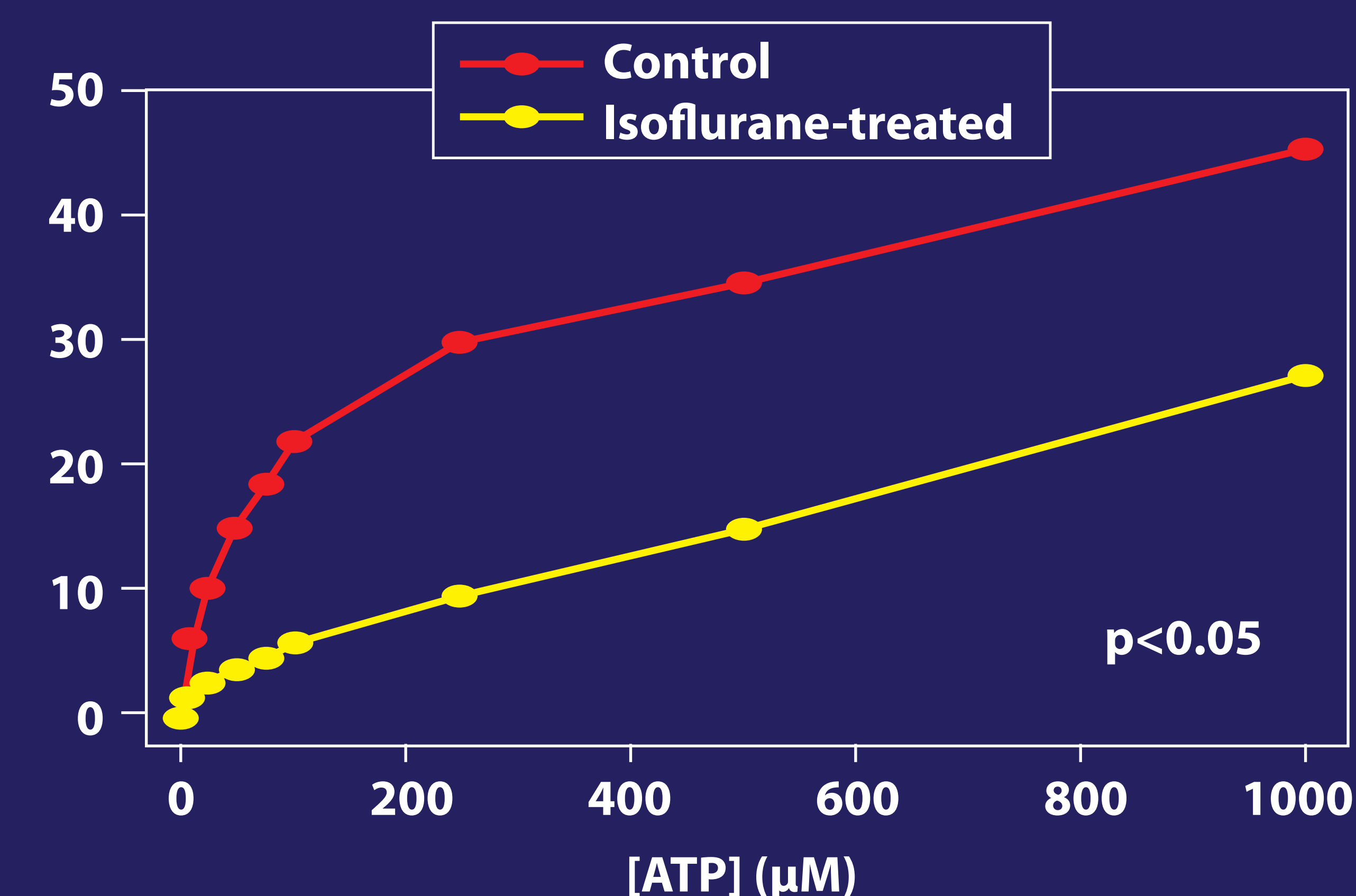
## RESULTS

- Isoflurane inhibited basal PMCA activity (nmol of inorganic phosphate per mg PMCA per minute) by 28.6% (P < 0.04).
- Isoflurane inhibited PMCA activity in the presence of saturating concentrations of CaM (300nM) by 66.9% (P < 0.02).
- Isoflurane also inhibited PMCA activity at ATP concentrations between 10μM and 1000μM.

### EFFECTS OF ISOFLURANE ON CAM-STIMULATED PMCA ACTIVITY



### EFFECTS OF ISOFLURANE ON MICHAELIS-MENTON KINETICS OF PMCA ACTIVITY



## CONCLUSION

- Isoflurane inhibited basal PMCA activity.
- Isoflurane inhibited PMCA activity in the presence of CaM at concentrations of CaM between 2nM and 300nM.
- Isoflurane suppressed the Michaelis-Menton kinetics of PMCA activity as a function of ATP at concentrations from 10μM-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.
- Due to difficulties in studying neuronal PMCA, the RBC isoform was used as a substitute.
- These data would suggest that a primary biochemical target for isoflurane neural inhibition is intracellular ATP via a direct effect on PMCA activity