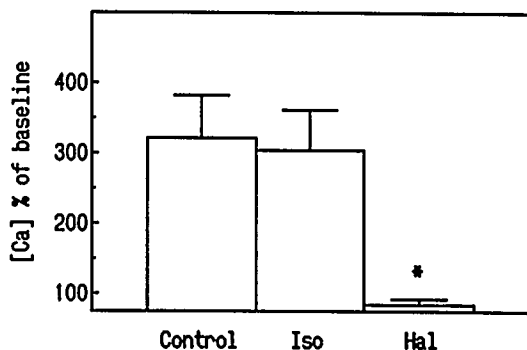


A533

**TITLE:** ENDOTHELIAL CELL CALCIUM MOBILIZATION IS ALTERED BY VOLATILE ANESTHETICS  
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The volatile anesthetics isoflurane and halothane have differing effects on regional blood flow and hemodynamics. Although the mechanisms by which these anesthetics act to modify circulatory control are not completely defined, one mechanism that may contribute to these differences is an anesthetic-induced modulation of endothelial cell signal transduction. Indeed, several studies have suggested that anesthetics can modify calcium mobilization in several cell types. Release of endothelium-derived vasoactive mediators can be directly correlated with changes in cytosolic calcium. Therefore, anesthetic-induced alteration of calcium mobilization in endothelial cells could subsequently alter vascular responsiveness. To explore this possible mechanism of anesthetic action, bovine aortic endothelial cells grown on glass coverslips were loaded with the fluorescent indicator fura-2/AM (5  $\mu$ M) for 30 min. The cells were studied using a microscope modified to measure fluorescence from single cells. Fluorescence emission following excitation at 340 and 380 nm was determined, and cytosolic calcium concentrations were calculated using the ratio method. Baseline calcium averaged  $155 \pm 24$  nM. Responses of the endothelial cells to challenge with the endothelium-dependent vasodilators, bradykinin and ATP were studied before and after the addition of halothane or isoflurane. The absolute magnitude of the responses to agonists varied; therefore, all data were normalized to reflect changes in cytosolic calcium relative to baseline (100%). When the agonist bradykinin (10 nM) was applied to single cells using a pressurized micropipette, a transient increase in cytosolic calcium was observed ( $321 \pm 61\%$  of control). ATP (10  $\mu$ M) increased cytosolic calcium ( $293 \pm 22\%$  of control). Solutions containing either halothane or isoflurane (0.3 mM final concentration) were then added to the cell preparations and a transient increase in cytosolic calcium was observed ( $192 \pm 52\%$  and  $235 \pm 64\%$ , respectively), indicating that both agents affect resting calcium. Halothane, but not isoflurane, significantly attenuated the calcium transient evoked by subsequent addition of bradykinin, as shown below (n=3, ANOVA p<0.05). Responses to ATP were not altered by the anesthetics. These findings indicate that these volatile anesthetics alter specific pathways involved in calcium mobilization in endothelial cells and thereby alter endothelial cell responses to vasoactive agonists.



A534

**TITLE:** ISOFLURANE DOES NOT VASODILATE RAT THORACIC AORTIC RINGS BY EDRF OR OTHER CYCLIC GMP MEDIATED MECHANISMS  
**AUTHORS:** JK Brendel, M.D., G Proctor, B.S., RA Johns, M.D.  
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**INTRODUCTION:** Isoflurane has been reported to cause endothelium-derived relaxing factor (EDRF)-induced dilation of dog coronary arteries<sup>1</sup>, while others have demonstrated that isoflurane does not cause EDRF-induced dilation of cat<sup>2</sup> or rabbit<sup>3</sup> cerebral arteries. The purpose of this work was to directly examine the role of EDRF in isoflurane mediated vasodilation.

**METHODS:** Isolated rat thoracic aorta rings were suspended on Grass force transducers at 2 gm resting tension in Krebs' solution (37°C) aerated with 95% O<sub>2</sub>/5% CO<sub>2</sub> for the measurement of isometric tension. Three groups were studied: endothelium intact (EI), endothelium denuded (ED) and nitro-L-arginine (NLA; a specific inhibitor of EDRF synthase) treated rings. Rings were washed and constricted with either a 50% maximal dose of phenylephrine (PE) or KCL ( $4 \times 10^{-2}$ M). Isoflurane 1, 2 or 3% was added in a cumulative manner allowing 10 minutes for each concentration to equilibrate. Indomethacin was present in all experiments at  $2.8 \times 10^{-5}$ M. Methacholine challenge was used to confirm endothelial status at the beginning and end of all experiments. As EDRF causes vascular relaxation by stimulating vascular smooth muscle guanylate cyclase, the effect of isoflurane on vascular ring cyclic GMP content was determined as a specific indicator of EDRF mediated dilation. Rings were isolated as described above, constricted with PE ( $1 \times 10^{-7}$ M), and challenged with MCH ( $1 \times 10^{-6}$ M) as the positive control or by 1, 2 or 3% isoflurane. After eight minutes of isoflurane exposure, the rings were flash frozen in dry ice-cooled acetone, homogenized in 1N HCl, and the tissue supernate analyzed for cyclic GMP by radioimmunoassay.

Data are expressed as mean  $\pm$  SEM and each point represents 18 rings from 6 animals, (N=6) in isometric tension studies and 4 rings in each group (N=4) in cyclic GMP studies. Data were analyzed by ANOVA with multiple range testing where needed.

**RESULTS:** Isoflurane caused dose-dependent vasodilation of both KCL and PE constricted rings. In the PE group, at 2% and 3% isoflurane, ED and NLA treated rings relaxed to a greater extent than EI rings (p<0.01; Fig 1). There were no significant differences in isoflurane-induced relaxation of any of the KCL constricted groups. MCH, an endothelium-dependent vasodilator, increased cyclic GMP concentration of EI vascular rings significantly (p<0.001) above control. Isoflurane, 1, 2, or 3% had no effect on cyclic GMP content of EI or ED vessels (Fig 2).

**CONCLUSIONS:** Vasodilation by isoflurane is due to a direct effect and is independent of EDRF or other cyclic GMP or endothelial mediated mechanisms. Phenylephrine causes EDRF production which modulates its vasoconstricting effect on VSM. The decreased relaxation to isoflurane seen in the endothelial-intact PE constricted rings may represent EDRF inhibition by isoflurane, consistent with our previous findings<sup>4</sup>.

FIGURE 1:

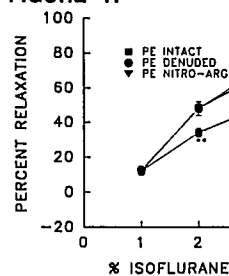
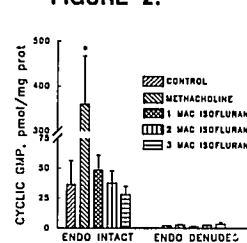


FIGURE 2:



**REFERENCES:** 1. Anesthesiology 67:513-17, 1987; 2. Anesthesiology 71:A577, 1990; 3. Anesthesiology 71:A648, 1990; 4. Anesthesiology 71:A604, 1990