

TITLE MODULATION OF INTRACELLULAR CALCIUM FLUXES IN OXIDANT-INJURED ENDOTHELIAL CELLS BY HALOTHANE AND ISOFLURANE

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Volatile anesthetics increase the sensitivity of rat pulmonary artery endothelial cells (RPAECs) to oxidant-mediated injury.^{1,2} This effect is due to an anesthetic-induced alteration in the function of the pulmonary vascular endothelium. We have begun exploring the mechanisms of these alterations by following the time course of changes in intracellular free calcium ($[Ca^{2+}]_i$) in single cells superfused with hydrogen peroxide (H_2O_2).

RPAECs isolated as described elsewhere³ were kindly provided by Dr. Una S. Ryan (University of Miami). The day before the experiment, the cells were seeded onto round coverglasses at a density of $2-3 \times 10^5$ cells per coverglass and allowed to adhere during an overnight incubation in Minimum Essential Medium supplemented with 10% fetal bovine serum. This density resulted in a monolayer less than 50% confluent.

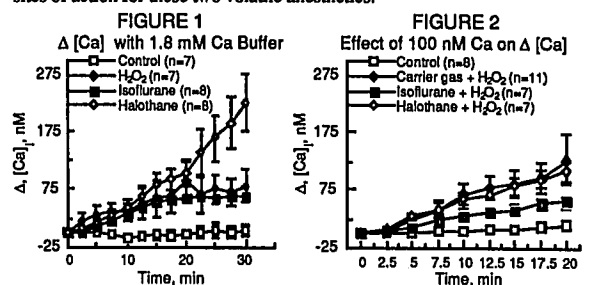
On the day of the experiment, cells were loaded for 20 min at room temperature with $12.5 \mu M$ Fura-2 as the acetoxymethyl ester. After the loading buffer was removed, the coverglasses were mounted in a temperature- and atmosphere-controlled chamber on the stage of a Leitz Diavert microscope. The cells were superfused with a Hank's Balanced Salt Solution (HBSS) based buffer at a rate of 30 mL/hr, maintained at a temperature of $35^\circ C$. The emission intensity signals from single cells at 515 nm were analyzed after alternating excitation at 340 nm and 380 nm. At the conclusion of each experiment, an *in vitro* calibration of each cell was attempted by first superfusing the cell with $10 \mu M$ ionomycin in $1.8 mM$ Ca^{2+} HBSS, followed by $7 mM$ EGTA in Ca^{2+} -free HBSS. $[Ca^{2+}]_i$ was calculated by the method of Grynkiewicz *et al.*, using a dissociation constant of 224.⁴

Each cell was superfused with either $1.8 mM$ or $100 nM$ Ca^{2+} HBSS and treated in one of four different ways: with HBSS plus $750 \mu M$ H_2O_2 , with HBSS plus H_2O_2 after equilibration with 1.7% halothane, with HBSS plus $750 \mu M$ H_2O_2 after

equilibration with 2.8% isoflurane, or with HBSS alone. Data in each Ca^{2+} group were analyzed separately using 2-way ANOVA. If the F-ratio for the treatment-time interaction term was significant ($P < 0.05$), multiple comparisons were performed using the Games-Howell test.

In $1.8 mM$ Ca^{2+} (fig. 1) equilibration with halothane ($n=7$) was associated with a significant rise in $[Ca^{2+}]_i$ over the 30 min treatment with H_2O_2 , compared with either isoflurane pretreatment ($n=8$), or with cells treated with H_2O_2 alone ($n=8$). In $100 nM$ Ca^{2+} (fig. 2), $[Ca^{2+}]_i$ increased in all three groups of injured cells, although isoflurane equilibration was associated with a smaller rise.

Halothane exacerbates the increase in $[Ca^{2+}]_i$ in RPAECs injured with H_2O_2 in normal Ca^{2+} buffer. This effect is not seen with isoflurane pretreatment at an equipotent concentration. In $100 nM$ Ca^{2+} buffer, a significant increase in $[Ca^{2+}]_i$ is seen in injured cells, which is attenuated in the presence of isoflurane. Early in the course of injury (before 20 min) Ca^{2+} is mobilized from sequestered internal stores, which process may be inhibited in the presence of isoflurane; however, after 20 min halothane may increase the influx of Ca^{2+} from the extracellular environment. These effects support the concept of different cellular sites of action for these two volatile anesthetics.



¹ Anesthesiology 71:A212, 1989.

² Anesthesiology 71:A213, 1989.

³ J Tissue Cult Methods 10:9-13, 1986.

⁴ J Biological Chem 260:3440-3450, 1985.

A342

TITLE: ETOMIDATE INDUCTION MAINTAINS SYMPATHETIC OUTFLOW IN HUMANS: DIRECT OBSERVATIONS FROM SYMPATHETIC RECORDINGS

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Etomidate induction in both healthy and compromised patients has minimal effects on cardiovascular function. However, *in vitro* studies suggest that etomidate has direct negative inotropic effects. One mechanism which might contribute to the observed minimal *in vivo* effects of etomidate may be mediated by compensatory sympathetic reflexes which might over-ride direct inotropic actions. In the present research, recordings of sympathetic outflow directed to skeletal muscle blood vessels (MSNA) were obtained during induction of anesthesia with etomidate ($0.3 mg/kg$) and compared to responses recorded during sodium thiopental induction ($4-5 mg/kg$).

ASA class I patients scheduled for elective surgery signed consent forms approved by the Human Studies Committee and were instrumented with ECG, radial artery catheter and were given $10 ml/kg$ of IV saline. MSNA was recorded from a 5μ-tipped needle positioned in a sympathetic nerve within a muscle fascicle of the peroneal nerve. Measured parameters (HR,

MAP, MSNA) prior to induction were similar between etomidate and thiopental groups. Moreover, reflex increases in HR and MSNA elicited by a brief hypotensive stimulus ($100 \mu g$ bolus of nipride) did not differ between groups while awake. Average % changes in parameters which occurred during the 4 minute period after induction and prior to intubation are shown below.

	% Δ during induction	sodium thiopental n=7	etomidate n=6
% Δ HR, b/min		24 ± 5.3	$13 \pm 5^*$
% Δ SBP, mm Hg		-10 ± 4	$5.6 \pm 3.1^*$
% Δ MSNA, freq/100 cardiac cycles		-30 ± 12	$-11 \pm 7^*$
% Δ baroreceptor gain			
MSNA , bursts/mm Hg		-75 ± 13	$-13 \pm 14^*$
R-R interval, msec/mm Hg		-62 ± 12	$-16 \pm 17^*$

Data are mean % $\Delta \pm$ SEM, * = $p < 0.05$ compared to thiopental

Thus, the maintained blood pressure during etomidate induction is in part due to a minimal reduction in sympathetic outflow and a maintained ability of the baroreceptor reflex to augment MSNA and heart rate.