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

AUTHORS JR Shayevitz, M.D., J Varani, Ph.D., PR Knight, M.D., Ph.D.
AFFILIATION Departments of Anesthesiology and Pathology, University of Michigan Medical School, Ann Arbor, MI 48109

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).

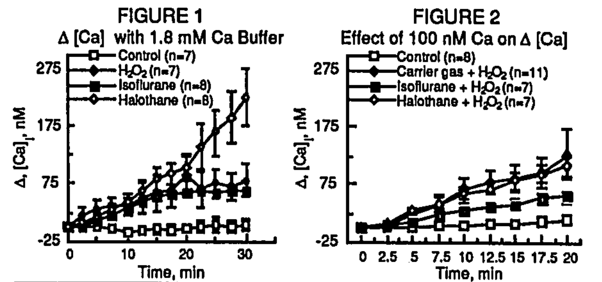
RPAECs isolated as described elsewhere[3] were kindly provided by Dr. Una S. Ryan (University of Miami). The day before the experiment, the cells were seeded onto round coverglasses at a density of $2-3\times10^5$ cells per coverglass and allowed to adhere during an overnight incubation in Minimum Essential 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 μM 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°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 μM 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 μM H_2O_2, with HBSS plus H_2O_2 after equilibration with 1.7% halothane, with HBSS plus 750 μM 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

AUTHORS: T.J. Ebert, M.D., Ph.D., D.D. Kanitz, M.D., R.J. Berens, M.D., J.P. Kampine, M.D., Ph.D.

AFFILIATION: Department of Anesthesiology, The Medical College of Wisconsin and VA Medical Center, Milwaukee, WI 53295

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 μg 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 ± 5*
% Δ SBP, mm Hg	-10 ± 4	5.6 ± 3.1*
% Δ MSNA, freq/100 cardiac cycles	-30 ± 12	-11 ± 7*
% Δ baroreceptor gain		
I MSNA I, bursts/mm Hg	-75 ± 13	-13 ± 14*
R-R interval, msec/mm Hg	-62 ± 12	-16 ± 17*

Data are mean % Δ ± 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.

Downloaded from http://pubs.asahq.org/anesthesiology/article-pdf/73/3A/NA/417602/0000542-199009001-00341.pdf by guest on 27 November 2020