Anesthesiology
V 75, No 3A, Sep 1991

POSTER SESSION XII

A364

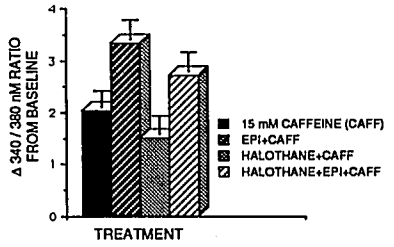
TITLE INTERACTION OF EPINEPHRINE AND HALOTHANE ON CAFFEINE INDUCED CALCIUM TRANSIENTS FROM SINGLE VENTRICULAR MYOCYTES

AUTHORS P.R. Knight, M.D., Ph.D., D. Wilde, Ph.D., M. Haney, M.D.

AFFILIATION Department of Anesthesiology
University of Michigan Medical School
Ann Arbor, Michigan 48109-0048

The inhibition of caffeine-induced Ca^{2+} release following exposure of cardiac myocytes to halothane probably results from a decrease in the amount of available Ca^{2+} in the sarcoplasmic reticulum (SR). This may be due to either SR leakage of Ca^{2+}, inhibition of SR re-uptake or both. Epinephrine (EPI) increases the $[Ca^{2+}]$ available for release from the SR and enhances Ca^{2+} re-uptake after contraction. We report the effects of EPI on the Ca^{2+} transient induced by caffeine in isolated myocytes exposed to halothane.

Single, quiescent rat ventricular myocytes were enzymatically isolated by a modified Langendorff collagenase perfusion of isolated hearts. After isolation, the cells were loaded with 4 μM Fura-2 AM for 10 min at 22°C and placed in a controlled temperature-atmosphere chamber at 37°C. The cells were superfused with an oxygenated Tyrode's solution containing 1.8 mM Ca^{2+}. $[Ca^{2+}]_i$ induced changes in fluorescence were measured using a MPV photometer mounted on a Leitz Diavert microscope equipped with quartz optics and alternating 340 nm and 380 nm excitation filters. $[Ca^{2+}]_i$ was determined by standard methods. Myocytes were treated with 15 mM caffeine with or without 1μM EPI, and in the presence or absence of 1.5% halothane. A ten minute recovery period was allowed between all drug challenges. Statistical inference was determined using Students unpaired "t" test corrected for multiple comparisons.



Resting $[Ca^{2+}]_i$ in the quiescent rat ventricular myocytes is 79±5 nM. EPI 1μM induces spontaneous Ca^{2+} transients with contractions in 30% of the cells. Exposure of the cardiac myocytes to 15 mM caffeine increased $[Ca^{2+}]_i$ on average, by 68±14 nM ($p<0.05$, n=6). Halothane decreased the caffeine induced increase in $[Ca^{2+}]_i$ by 35±14% ($p<0.05$, n=6). EPI, 1μM, increased the magnitude of the caffeine induced rise in $[Ca^{2+}]_i$ by 64±18% ($p<0.05$, n=8) in cells not exposed to halothane and reversed the reduction of the 1.5% halothane effect on caffeine induced Ca^{2+} transients to above preanesthetic levels ($p<0.05$, n=8).

Caffeine, 15 mM, induces calcium transients in myocytes by promoting release of Ca^{2+} from the SR. This action is inhibited by halothane which suggests that halothane either directly inhibits the release of Ca^{2+} from the SR or depletes the SR of available Ca^{2+}, or both. EPI increases Ca^{2+} current at the sarcolemma and increases Ca^{2+} stores by stimulating uptake. EPI reverses the depression by halothane of caffeine-induced release of Ca^{2+} from the SR, supporting the hypothesis that halothane effects can be countered by stimulation of SR Ca^{2+} uptake.

A365

TITLE: VOLATILE ANESTHETICS AFFECT TIME COURSE AND AMPLITUDE OF SR CA^{2+} RELEASE BY CAFFEINE IN CARDIAC MYOCYTES

AUTHORS: D.W. Wilde, Ph.D., B.A. Davidson, B.S., P.R. Knight, M.D., Ph.D.

AFFILIATION: Dept. Anes., University of Michigan Medical Center, Ann Arbor, MI 48109

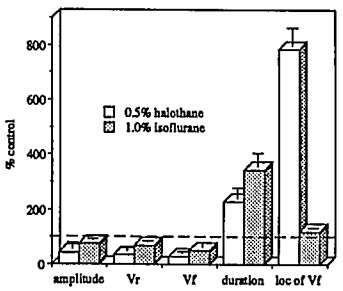
Halothane (HAL) and isoflurane (ISO) reduce the amount of Ca^{2+} released from cardiac SR following exposure of the cell to caffeine.[1,2] The depletion of SR stores of Ca^{2+} by these anesthetics contributes to their negative inotropic effect on the heart. Using the rat cardiac myocyte, a cell principally dependent on SR Ca^{2+} release for contraction, we have analyzed the time course of the caffeine induced Ca^{2+} release in the presence or absence of HAL and ISO. High resolution computer analysis was used to examine the rates of SR Ca^{2+} release and reuptake-extrusion.

Single, quiescent, rat ventricular myocytes were isolated by collagenase dispersion. Cell suspensions were loaded in 4 μM FURA-2 AM for 10 min. at 22°C. Cells were placed in a controlled atmosphere-temperature bath on the stage of a Leitz inverted fluorescence microscope equipped with 340 nm and 380 nm excitation filters. Fluorescence output at either excitation wavelength was captured by photomultiplier and digitized at 10 kHz on a Macintosh IIfx using acquisition software written in LabVIEW 2®. $[Ca^{2+}]_i$ was determined by standard methods[3]. Cells in the 1.5 ml bath were superfused at 3.5 ml/min with oxygenated HEPES-Tyrodes at 35°C. Cells were exposed to 15 mM caffeine in the superfusate for 20 sec and the resultant Ca^{2+} transient recorded. The superfusate and bath atmosphere were then equilibrated for 10 minutes with HAL or ISO in 100% O_2, delivered by vaporizer, and the cells reexposed to caffeine. Caffeine stimulated Ca^{2+} transients were analyzed for peak amplitude, maximum upstroke rate (Vr), maximum rate of fall (Vf), the location of these parameters, and duration.

At roughly equal MAC, HAL (0.5%) reduced transient amplitude and slowed Vr and Vf to a greater degree than ISO (1%) (fig.). Both agents increased transient duration. In 0.5% HAL, amplitude fell from a control value of 254±34 nM to 111±52 nM. 1% ISO reduced amplitude from 340±23 nM to 263±6 nM. HAL (0.5%) reduced Vr from a control value of 18530±1586 nM/sec to 6655±2113 nM/sec. Vf was reduced from 145±16 nM/sec in the control to 42±8 nM/sec. All differences from control were significant at $p<0.05$ determined by the unpaired Student's t-test.

The data show that HAL and ISO reduce the amount of Ca^{2+} released from the cardiac myocyte SR during caffeine exposure and alter the time course of the Ca^{2+} transient. The delayed attainment of Vf and increased duration caused by HAL and ISO indicate possible interference with Ca^{2+} reuptake-extrusion mechanisms. This effect would increase the likelihood of net Ca^{2+} loss from the myocyte during anesthetic exposure and exacerbate the negative inotropic actions of these anesthetics.

References
1. *Anesthesiology* 72:911-920, 1990.
2. *Mechanisms of Anesthetic Action in Muscle* , in press.
3. *J Biological Chem* 260:3440-3450, 1985.



Comparison of the effects of equipotent doses of HAL and ISO on Ca^{2+} transient amplitude, max. rate of rise (Vr), max. rate of fall (Vf), duration and the location of Vf in caffiene-stimulated cardiac myocytes.