TITLE: ANESTHETICS INCREASE CYTOSOLIC CALCIUM IN MONONUCLEAR CELLS FROM NORMAL AND MH-SUSCEPTIBLE PATIENTS

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INTRODUCTION. Klip *et al.* reported that halothane treatment of peripheral blood mononuclear cells isolated from patients susceptible to malignant hyperthermia causes a rise in the cytosolic concentration of calcium, $[Ca^{2+}]i$ (1-3). These reports indicated that halothane induces minimal changes in $[Ca^{2+}]i$ in cells from normal controls under the same conditions. If confirmed, this result could lead to a test for MH which would be much safer and less invasive than the current muscle biopsy/halothane-caffeine contracture test (4). In order to examine this possibility, we have initiated a study of the effects of halothane (and isoflurane) on the cytosolic calcium concentration in mononuclear cells from normals and MH-susceptible subjects.

METHODS. Informed consent was obtained from all subjects, and the study was approved by the Institutional Review Board. MH susceptibility was confirmed by family or personal history plus a documented positive muscle biopsy test. Blood was drawn into a heparinized syringe, and mononuclear cells (approximately 85% lymphocytes and 15% monocytes) were isolated and incubated with the calcium chelating dyes quin2, indo-1, or fura-2 by established procedures (5). The dye-loaded cells were suspended at a concentration of 10^6 cells/ml in a buffer containing 125 mM NaCl, 5 mM KCl, 5 mM glucose, 10 mM $NaHCO_3$, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 20 mM HEPES, pH 7.4, and maintained at 37° in a thermostatically controlled cuvette. Liquid anesthetic was added via syringe (1-4 µl/ml, see ref 1-3). Calibration of the fluorescence determinations utilized digitonin lysis in the presence of EGTA, and the $[Ca^{2+}]i$ was confirmed by the 340/380 ratio in the case of fura-2. The fluorescence was corrected for external dye, autofluorescence of the cells and added reagents, and quenching of the fluorescence by heavy metals (6).

RESULTS. Addition of halothane caused a significant rise in $[Ca^{2+}]i$, in the suspension of mononuclear cells from both normals and MH-susceptible patients (see Table). A significant rise was seen with all three dyes. The amount of halothane added was 4 µl/ml, although addition of as little as 1 µl/ml gave essentially the same results. Since there was usually no plateau in the halothane-induced rise in $[Ca^{2+}]i$, "+ Halothane" and "Δ $[Ca^{2+}]i$" refer to the calculated $[Ca^{2+}]i$ and the net increase in $[Ca^{2+}]i$, respectively, five minutes after addition of the anesthetic.

CYTOSOLIC CALCIUM (nM)

	fura-2	indo-1	quin2
	NORMAL SUBJECTS		
Resting	94±7 (5)	110±30 (2)	122±5 (2)
+Halothane	199±47	194±7	177±2
Δ $[Ca^{2+}]i$	105	84	55
	MH-SUSCEPTIBLE		
Resting	86±9 (3)		
+Halothane	193±19		
Δ $[Ca^{2+}]i$	107		

$[Ca^{2+}]i$ was determined immediately before and five minutes after the addition of halothane (4 µl/ml). The values represent mean ± SEM of the average of quadruplicate determinations in each subject (number of subjects in parentheses).

Several of these cell suspensions were also treated with isoflurane, with similar results (not shown). There was great variability, both within and between subjects, in the response to halothane or isoflurane. There was no anesthetic-induced change in $[Ca^{2+}]i$ when the medium bathing the cells contained excess EGTA. The addition of 25 µM La^{3+}, a known inhibitor of calcium channels, blocked the response as well.

DISCUSSION. We have shown that halothane and isoflurane induce a rise in $[Ca^{2+}]i$ in mononuclear cells from control and MH susceptible subjects. In contrast to the data of Klip *et al.* (1-3) there appears to be a significant increase in $[Ca^{2+}]i$ in cells from normal subjects. For fura-2 loaded cells, there is no significant difference in the response between MH patients and normal subjects. Although the numbers of patients tested was small, it appears unlikely that any differences, if they do exist, will be of a sufficient magnitude to allow this procedure to serve as a clinically useful diagnostic or screening test for malignant hyperthermia susceptibility, especially given the inter- and intra-subject variability. Of note is the observation that halothane has been reported to increase cytosolic calcium significantly (from 175 to 251 nM) in adipocytes from a non-MH source (7), and also to cause an increase in $[Ca^{2+}]i$ in isolated rat myocytes (8). Pentobarbital (0.1 mM) has been reported to increase cytosolic calcium by 40% in rat thymocytes (9). The mechanism of the increase in $[Ca^{2+}]i$ in mononuclear cells by halothane is unknown, although it appears to involve changes in the plasma membrane permeability to Ca^{2+}, as evidenced by the blocking effect of EGTA and lanthanum, and a report that the increase in $[Ca^{2+}]i$ in MH cells could be prevented by nifedipine, a calcium channel blocker (3).

CONCLUSIONS. 1) Halothane and isoflurane cause a significant increase in the concentration of free cytosolic calcium in mononuclear leukocytes from normal adults.

2) This effect is dependent on external calcium, and may be mediated by plasma membrane Ca^{2+} channels.

3) Halothane induced similar increases in $[Ca^{2+}]i$ in mononuclear leukocytes from 3 patients with documented malignant hyperthermia and in 5 normal adults.

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