Title: HALOTHANE TRANSIENTLY INCREASES FREE INTRACELLULAR CALCIUM CONCENTRATION IN ISOLATED ADULT RAT HEART CELLS

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INTRODUCTION. The negative inotropic effect of halothane (H) in cardiac muscle has been shown to be related to a decrease in the amount of calcium available to the myofilaments during a contraction.[1] Since H alters Ca transport functions at both the cell membrane and the sarcoplasmic reticulum (SR),[2-4] the mechanism by which it brings about this reduction in free intracellular Ca concentration (Ca_f) with each beat is unclear. To better understand this action of H, we have studied Ca_f in quiescent heart cells so that effects of the agent independent of the cardiac action potential could be observed.

METHODS. The hearts of 2 month old Wistar rats were rapidly excised and perfused via the aortic root with Earle's salt solution (ESS) containing 50μM Ca and 1mg/ml collagenase. After 25 min of collagenase perfusion, the left ventricle was minced and strained, and the cells obtained were suspended in ESS with 250μM Ca. After settling, sequential resuspensions were done in ESS containing 1mM Ca and then in ESS plus 1mM Ca, 10mM HEPES, 20mM glucose, and 2.5mg/ml bovine serum albumin and adjusted to pH 7.4. All subsequent manipulations of the suspension were done in this latter solution. All solutions contained 1mM Mg and were equilibrated with 95%O_2/5%CO_2. The cells consist primarily of rod-shaped forms (structurally and functionally intact) and do not contract unless electrically stimulated. The Ca-sensitive, fluorescent dye quin2 was loaded into the cells by a 30 min exposure to a 50μM concentration of the acetoxymethylester of quin2 (quin2 AM). After loading, the suspension was centrifuged and resuspended without quin2 AM, then placed in a continuously stirred cuvette at 37°C for fluorimetry. Excitation wavelength was 333nM and fluorescence at wavelengths >480nM was monitored. H was predissolved in aqueous solution, at a concentration of 11mM, prior to its addition to the suspension.

RESULTS. When acutely introduced to a heart cell suspension, H (0.55mM) produced a transient increase in Ca_f as monitored by quin2 fluorescence, followed by a return to baseline within 1.5 min (see Figure 1). Application of a concentration of caffeine (10mM) sufficient to essentially empty the SR of Ca caused a qualitatively similar, but typically more marked, transient increase in Ca_f. If H was applied 1 to 3 min after 10mM caffeine, no change in Ca_f occurred with H. When the order of additions was reversed, caffeine did produce a transient increase in Ca_f when added after H, but the transient was of less magnitude than that in response to caffeine without prior addition of H (see Figure 2; $P<0.01$ by paired t-test comparing changes in Ca_f as measured from baseline to peak of transient; n=13). When EGTA was added to the suspension to reduce the free extracellular Ca concentration to approx. 100nM one min prior to H, the response of Ca_f to H was not distinguishable from that in 1mM Ca. H had no effect on the autofluorescence of these suspensions, nor did it alter the quin2-Ca fluorescence in the absence of cells.

DISCUSSION. The immediate and transient rise in Ca_f in rat heart cells upon exposure to H is likely due to a release of Ca from the SR. Reduction of free extracellular Ca to 100nM did not attenuate the response, an indication that the source of the increased Ca was not extracellular. The lack of response to halothane after caffeine points to the SR (emptied by caffeine in this case) as the likely source of the Ca. The attenuation of the caffeine-induced Ca_f increase by prior exposure to H suggests that the SR stores of Ca remain depleted in H at least for several minutes. This reduction in the Ca stores of the SR could result in diminished Ca release with each beat and explain the negative inotropic effect of the agent.

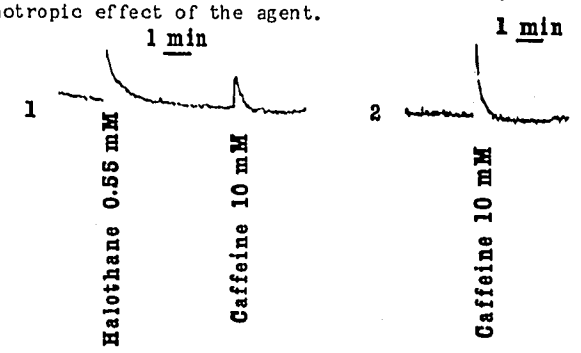

FIGURE 1. Quin2 fluorescence of rat heart cells (74% rod-shaped forms) in response to H and caffeine. Increased fluorescence is upward.

FIGURE 2. Quin2 fluorescence of an aliquot of the same suspension from which figure 1 originated showing the response to caffeine without prior administration of H. The fluorescence minima and maxima (0 Ca and excess Ca responses; not shown), the extracellular fluorescence, and fluorescence prior to drugs were similar for these two aliquots.

REFERENCES.

1. Bosnjak ZJ and Kampine JP: Effects of H on transmembrane potentials, Ca transients, and papillary muscle tension in the cat. Am J Physiol 251 (Heart Circ Physiol 20): H374-H381, 1986.
2. Su JY and Kerrick WG: Effects of H on caffeine-induced tension tansients in functionally-skinned myocardial fibers. Pflugers Arch 380: 29-34, 1979.
3. Komai H and Rusy BF: Effect of H on rested-state and potentiated-state contractions in rabbit papillary muscle: relationship to negative inotropic action. Anesth Analg 61:403-409, 1982.
4. Lynch C, III: Differential depression of myocardial contractility by H and isoflurane in vitro. Anesthesiology 64:620-631, 1986.

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