Title : CALCIUM CURRENTS ARE DECREASED BY HALOTHANE, ENFLURANE AND ISOFLURANE IN ISOLATED CANINE VENTRICULAR AND PURKINJE CELLS

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INTRODUCTION. The slow inward or calcium current (I_{Ca}) is an important determinant of the slow diastolic depolarization, plateau and repolarization phases of the cardiac action potential. In addition, I_{Ca} is essential in the initiation and maintenance of myocardial contraction. It has been shown that halothane (H), enflurane (E) and isoflurane (I) exert their negative inotropic effects in part by reducing the intracellular calcium concentration during the contraction phase.[1] Although the level of intracellular calcium is influenced by a number of factors, I_{Ca} is one of the major determinants. In order to assess directly the effects of H, E and I on I_{Ca}, we conducted whole-cell voltage clamp experiments in freshly isolated canine ventricular and Purkinje cells obtained by enzymatic dissociation.

METHODS. Dogs were anesthetized with H and the hearts rapidly excised and rinsed with cold Krebs solution. Thin strips of ventricular tissue were obtained using a biopsy needle, while Purkinje strands were taken from the left ventricle by isolating the free running fibers. The tissues were incubated at 37°C for 2 hours in Ca^{++}-free collagenase solution.[2] Dispersed myocytes were washed and stored in recovery solution (MEM, Gibco). The cells were placed in a perfusion chamber containing Tyrodes solution (22°C), and voltage-clamped with patch pipettes filled with Cs glutamate solution. The cells were then superfused with solution containing 5 mM Ca^{++} or Ba^{++} and 135 mM TEA as a substitute for Na^+. These recording solutions eliminated K^+ and Na^+ currents, and allowed for isolation of the inward Ca^{++} current. During whole-cell recording, I_{Ca} was measured during 200 ms depolarizations from a holding potential of -80 mV to consecutively more positive command potentials (10 mV increments). I_{Ca} was measured before, during and after introduction of low and high anesthetic concentrations: H: 0.40 and 0.88 mM, E: 0.83 and 1.41 mM and I: 0.68 & 1.12 mM. Whenever possible the effects of all 3 anesthetic agents were tested in the same cardiac cell.

RESULTS. Current-voltage curves for I_{Ca} activation are plotted from data recorded before and 5 min after exposure to H, E or I in ventricular cells in Figure 1. At the lower concentrations (Fig. 1a), the three anesthetic agents reduced the magnitude of I_{Ca}, with a maximal I_{Ca} attained at a membrane potential of about 10 mV. The I-V curves for I_{Ca} were depressed further with higher concentrations of these agents (Fig. 1b). Representative tracings of peak I_{Ca} in a ventricular cell recorded during depolarizing pulses from -80 mV to + 10 mV are shown in Figure 2. The lower levels of H, E and I reduced peak I_{Ca}, but did not shift the voltage-dependency of the I-V curve. Similar effects of H, E and I on I_{Ca} in Purkinje cell are plotted in Figure 3. As in ventricular cells, I_{Ca} in Purkinje myocytes was depressed during exposure to the three anesthetic agents.

DISCUSSION. It has been shown that H, E and I decrease Ca^{++} dependent action potentials in cardiac muscle.[3,4] Depression of I_{Ca} by H in single, voltage-clamped cells has been documented recently using rat myocytes.[5] The results of our experiments indicate that: (1) H, E and I produce a dose-dependent depression of I_{Ca} in canine ventricular muscle cells, and (2) all three agents also depress I_{Ca} in Purkinje fibers. This depression of I_{Ca} is evident in the presence of H, E and I at clinically relevant concentrations. Their negative inotropic and chronotropic effects on the myocardium are in part related to this depression of inward calcium current.

REFERENCES.
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Figure 1a

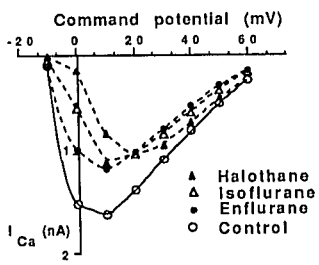


Figure 1b

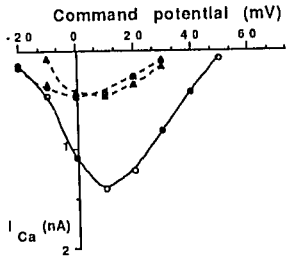


Figure 2

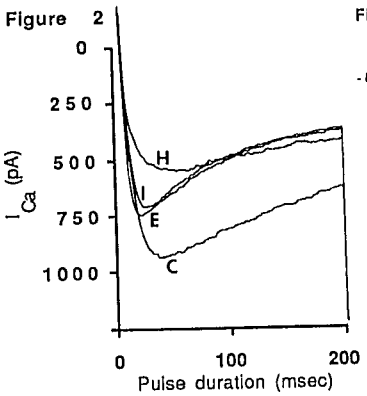
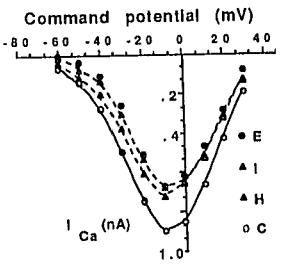


Figure 3



Effects of anesthetic agents on Purkinje fiber calcium current