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A1037

TITLE: ISOFLURANE MODULATES CALCIUM CHANNEL CURRENT IN SPINAL CORD DORSAL HORN NEURONS

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INTRODUCTION: The entry of calcium ions into neurons mediates important cellular events involved in neuronal function and signal processing. Since voltage gated calcium channels are an important site of action of volatile anesthetics in electrically excitable tissues such as myocardium, we investigated whether the effects of isoflurane involve modulation of these channels in central nervous system neurons.

METHODS: The dorsal horn of the spinal cord was dissected from rat embryos 16 days post conception. Neurons were dissociated by trypsin and trituration, plated on an astrocyte monolayer, and maintained in tissue culture for up to 3 weeks. Currents were recorded at room temperature by the whole-cell patch clamp technique[1], using barium as the primary charge carrier, cesium in the electrode solution, and tetrodotoxin in the external solution. To minimize calcium current run-down the electrode solution contained an ATP regenerating system consisting of ATP, creatine phosphate, and creatine phosphokinase. External solution was equilibrated with isoflurane vapor in room air delivered by an Ohmeda vaporizer.

RESULTS: Figure 1 illustrates the effect of 1.5% isoflurane on whole cell calcium channel current evoked by a 350 ms test pulse from a holding potential of -80 mV to +10 mV. Maximal depression of inward current occurred within 2 minutes of isoflurane exposure; the effect was almost completely reversed within 2 minutes of replacing control bath solution. Isoflurane-induced depression of calcium channel current was observed in 14 out of 14 cells tested. Figure 2 shows the effect of isoflurane on a series of increasing step depolarizations from a holding potential of -80mV.

CONCLUSION: Calcium currents play an integral role in a variety of neuronal functions including excitability and neurotransmitter release. These experiments demonstrate that isoflurane reduces calcium channel current in dorsal horn spinal neurons. Since the dorsal horn of the spinal cord is the initial processing and relaying site in the pathway for pain perception, the reduction of calcium current by isoflurane may contribute to the analgesic action of this anesthetic agent.

Reference: (1) Pflugers Arch 391:85-100, 1981.

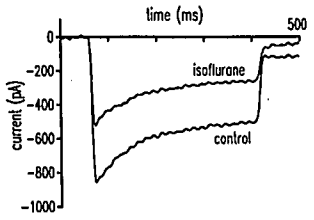

fig 1. Calcium current from dorsal horn neuron before and during the application of 1.5% isoflurane.

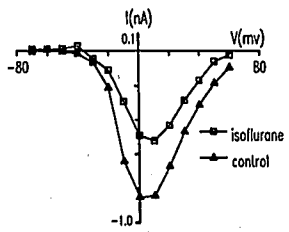

fig 2. I-V curves from same cell as figure 1 showing amplitude of peak calcium current over a range of test potentials. Figure compares I-V curve in presence of 1.5% isoflurane with control.

A1038

TITLE: SUPPRESSION OF A Ca^{2+}-DEPENDENT K^+ CONDUCTANCE IN ADRENAL CHROMAFFIN CELLS BY HALOTHANE

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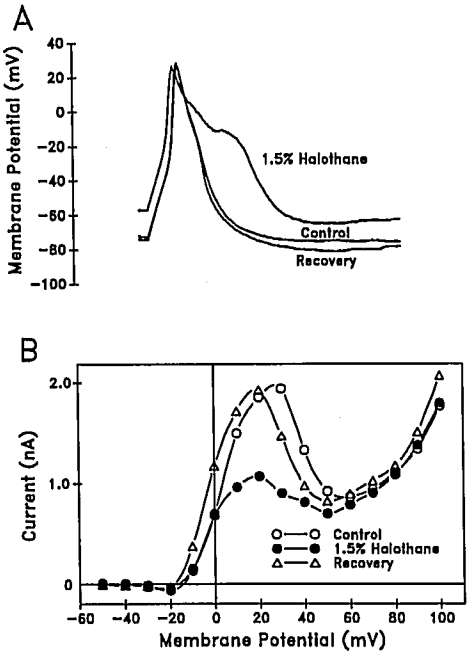
It is well known that anesthetics alter sympathetic function. In order to elucidate any direct effects of anesthetics on a neuronal component of the sympathetic system, we used the whole-cell patch clamp technique to measure the electrical activity of bovine adrenal medullary chromaffin cells in the presence of halothane as well as isoflurane.

Isolated chromaffin cells were affixed to poly-L-lysine-coated coverslips and placed in a chamber over an inverted microscope. The external bathing solution contained (in mM): 140 NaCl, 5 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, pH 7.4. For current-clamp and voltage-clamp measurements of K^+ current, the internal pipette solution contained (in mM): 42 KOH, 100 KCl, 1 $CaCl_2$, 10 EGTA, 2 $MgCl_2$, 5 HEPES, 5 ATP, pH 7.3. In order to measure Ca^{2+} current (I_{Ca}), K^+ was replaced with Cs^+ in the pipette solution and tetrodotoxin was added to the external bath to suppress Na^+ currents. Peak Ca^{2+}-dependent K^+ current ($I_{K(Ca)}$), a graphical method was employed (*J. Physiol.*, 367: 117-141, 1985). Volatile anesthetics were allowed to equilibrate in the external solution for 20-30 min before use. All recordings were performed at room temperature (19-22° C).

Utilizing the current-clamp recording mode to monitor transmembrane potentials, 1.5% halothane induced a reversible depolarization of 17 ± 4 mV (mean ± SEM, n=3) accompanied by an increase in the duration of the evoked action potentials (Fig. A), suggesting that halothane acts to decrease a K^+ conductance. Voltage-clamp measurements revealed that the current largely attributed to the Ca^{2+}-dependent K^+ channel (*J. Physiol.*, 367: 117-141, 1985), i.e. the Ca^{2+}-dependent "hump" in the current-voltage relation, is depressed by halothane by 56 ± 2% (n=5) as shown in Fig. B. In contrast, isoflurane at an equipotent concentration, 2.5%, was largely ineffective in the reduction of $I_{K(Ca)}$; a slight reduction in only 1 of 5 cells was observed. The halothane-induced decrease in $I_{K(Ca)}$ seems to be independent of any alteration in I_{Ca}; 1.5% halothane had no significant effect on peak I_{Ca}.

In summary, halothane appears to depress selectively peak $I_{K(Ca)}$ in bovine adrenal medullary chromaffin cells, which are specialized sympathetic neurons. This depression of $I_{K(Ca)}$ may underlie the observed increase in evoked action potential duration and depolarization in these cells by halothane.



Figures: A, Effect of 1.5% halothane on the evoked and resting transmembrane potentials. B, Current-voltage relation depicting the suppression of the Ca^{2+}-dependent K^+ current "hump" by halothane.