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Patch clamp techniques to study effects of anesthetics on airway smooth muscle cells

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Inhalation anesthetics have a direct relaxant effect on airway smooth muscle. Recent research has done much to clarify the cellular mechanisms for contraction and relaxation in airway smooth muscles and has suggested a possible mechanism of action for inhalation anesthetics in these muscles [1,2]. As in other muscle cells, the free intracellular concentration of Ca^{2+}($[Ca^{2+}]_i$) plays a central role in regulation of airway smooth muscle tone [3]. Yamakage [1] demonstrated that relaxation of precontracted tracheal smooth muscle by halothane was associated with a decrease in $[Ca^{2+}]_i$. Because sustained contraction of this tissue requires the continued entry of extracellular Ca^{2+}, a possible mechanism for the airway relaxant effect of inhalation anesthetics is inhibition of voltage-dependent Ca^{2+} channels (VDC) [4].

Patch clamp techniques provide a means for directly studying properties of membrane ion channels [5]. Applications of these methods to airway smooth muscle cells have demonstrated a variety of K^+ and Ca^{2+} channels that are likely of functional importance [6,7]. One patch clamp method, whole-cell recording, is particularly useful for quantifying Ca^{2+} current (I_{Ca}) through VDC. In this report, we describe the results of a preliminary study that demonstrates inhibition by enflurane of I_{Ca} in isolated airway smooth muscle cells.

Porcine tracheal smooth muscle, dissected free of epithelium and connective tissue, was minced. The tissue fragments were digested in Ca^{2+}-free Tyrode's solution containing 0.1% collagenase, 0.05% trypsin inhibitor, and 0.03% protease for 30 min at 37°C. Single cells were dispersed by repeated gentle aspiration into a plastic pipette, filtered through nylon mesh, and centrifuged at 200 *g* for 3 min. The cell pellet was resuspended in modified "K-B" solution [8] and stored at 4°C for up to 6 h before use.

Micropipettes, manufactured using a Brown-Flaming horizontal puller, were coated with silicone rubber and heat-polished. These had resistance of 3–5 MΩ when filled with the recording solution. Recording solutions were chosen to inhibit K^+ currents and enhance Ca^{2+} currents. The pipette solution contained (in mM): CsCl 130, $MgCl_2$ 4, EGTA 10, Na_2ATP 5, and HEPES/Tris 10 (pH 7.2). The bath solution contained (in mM): TEACl 130, $MgCl_2$ 1, $Cacl_2$ 10, glucose 10, and HEPES/Tris 10 (pH 7.4).

A small volume of the cell suspension was placed in a perfusion chamber on the stage of the inverted microscope (Olympus CK2, Leeds Instruments, Minneapolis, MN, USA). At 400 × magnification, a three-dimensional oil-driven micromanipulator (MO-102, Narishige, Tokyo, Japan) was used to position the patch pipettes against the membranes of the airway smooth muscle cells. Slight suction was applied to the pipette interior to obtain a high-resistance seal (3–10 GΩ), and the patch membrane was disrupted by strong negative pressure. This process provided low-resistance electrical access to the cell interior and allowed the diffusional replacement of cytoplasm with the pipette solution. Pulses (150 ms) were applied from a holding potential of −80 mV to command potentials of −50 to +40 mV to elicit whole-cell I_{Ca}. Membrane currents were amplified by a List EPC-7 patch clamp amplifier (List-Electronics, Great Neck, NY, USA) and the amplifier output was low-pass-filtered at 900 Hz. Threshold activation of I_{Ca} occurred at −20 mV, and the peak current amplitude (−252 ± 18 pA) was reached at +10 mV.

After several minutes of recording, cells were exposed to enflurane for 7 min by changing the inflow

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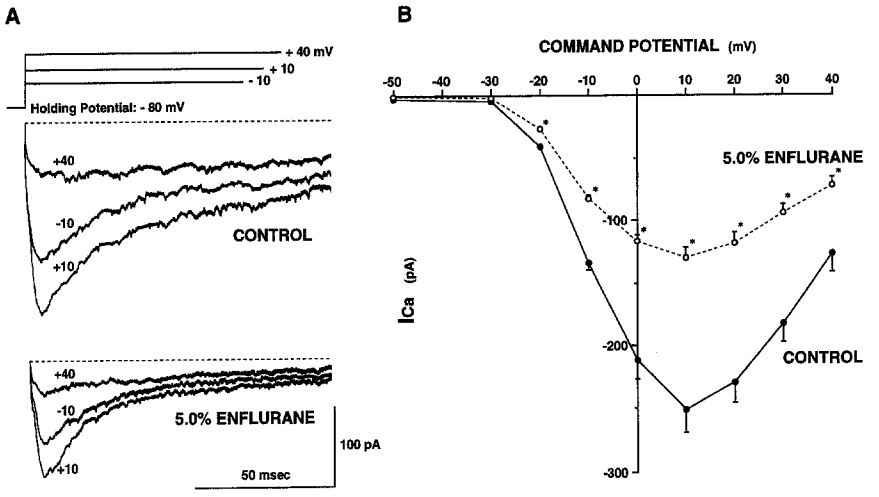

Fig. 1A, B. Effect of enflurane on inward Ca^{2+} currents through voltage-dependent calcium channels. **A** Recordings of Ca^{2+} current generated by progressive depolarizing pulses (only −10, +10, and +40 mV are shown). **B** The relationship of peak Ca^{2+} current and applied voltage. Each symbol represents mean ± SEM of data from 5 cells. $^*P < 0.05$ *vs.* control

perfusate to one vigorously bubbled with the anesthetic (5.0% in air). The anesthetic significantly inhibited I_{Ca} throughout the voltage range studied (Fig. 1A). Enflurane produced a 48 ± 2% reduction (mean ± SEM, $n = 5$) from the control peak current at +10 mV. There was no apparent shift in the voltage-dependence of induced Ca^{2+} currents (Fig. 1B).

We conclude that enflurane has an inhibitory effect on VDC of procine airway smooth muscle cells. These results are similar to those reported with halothane and/or isoflurane in canine coronary artery and guinea-pig portal vein smooth muscle cells [9,10]. Our studies suggest that one mechanism by which inhalation anesthetics decrease the intracellular Ca^{2+} concentration and cause smooth muscle relaxation is by direct inhibition of VDC.

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References

1. Yamakage M (1992) Direct inhibitory mechanisms of halothane on canine tracheal smooth muscle contraction. Anesthesiology 77:546–553
2. Jones KA, Housmans PR, Warner DO, Lorens RR, Rehder K (1993) Halothane alters cytosolic transient in tracheal smooth muscle. Am J Physiol 265:L80–L86
3. Adelstein RS, Pato MD, Conti MA (1980) Regulation of smooth muslce myosin kinase activity by phosphorylation and dephosphorylation. In: Ebashi S, Murayama K, Endo M (ed) Muscle Contraction: Its regulatory mechanisms. Jpn Sci Soc, Tokyo, pp 303–313
4. Yamakage M, Kawamata T, Kohro S, Namiki A (1994) Inhibitory effect of halothane on high-K^+-induced canine tracheal smooth muscle contraction and intracellular Ca^{2+} increment. Acta Anaesthesiol Scand 38 (in press)
5. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch 391:85–100
6. McCann JD, Welsh MJ (1986) Voltage-gated Ca-activated K channels in isolated canine airway smooth muscle cells. J Physiol (Lond) 372:113–127
7. Kotlikoff MI (1988) Calcium currents in isolated canine airway smooth muscle cells. Am J Physiol 254:C793–C801
8. Isenberg G, Klockner U (1982) Calcium tolerant ventricular myocytes prepared by preincubation in a "KB medium". Pflügers Arch 395:6–18
9. Buljubasic N, Rusch NJ, Marijic J, Kampine JP, Bosnjak ZJ (1992) Effects of halothane and isoflurane on calcium and potassium channel currents in canine coronary arterial cells. Anesthesiology 76:990–998
10. Wilde DW (1992) Effects of isoflurane on $I_{K,Ca}$ and L-channel Ba^{2+} current in single, vascular smooth muscle cells from guinea-pig portal vein. Anesthesiology 77:A685