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Inhibitory Effects of Thiopental, Ketamine, and Propofol on Voltage-dependent Ca^{2+} Channels in Porcine Tracheal Smooth Muscle Cells

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Background: **Intravenously administered anesthetics directly inhibit airway smooth muscle contraction. Because many anesthetic agents affect membrane ion channel function and sustained contraction of airway smooth muscle requires the influx of Ca^{2+} through voltage-dependent Ca^{2+} channels, it was hypothesized that intravenous anesthetics inhibit airway smooth muscle voltage-dependent Ca^{2+} channels.**

Methods: **Porcine tracheal smooth muscle cells were enzymatically dispersed and studied using whole-cell, patch-clamp techniques. The cells were exposed to thiopental ($10^{-7} - 3 \times 10^{-4}$ M), ketamine ($10^{-6} - 10^{-3}$ M), or propofol ($10^{-7} - 3 \times 10^{-4}$ M) while recording macroscopic voltage-activated Ca^{2+} currents (I_{Ca}).**

Results: **Each intravenous anesthetic tested significantly inhibited I_{Ca} in a dose-dependent manner with 3×10^{-4} M thiopental, 10^{-3} M ketamine, and 3×10^{-4} M propofol each causing ~50% depression of peak I_{Ca}, but with no apparent shift in the voltage dependence of induced I_{Ca}. After pretreatment with the Ca^{2+} channel agonist Bay K 8644, thiopental, but not ketamine or propofol, shifted the maximum I_{Ca} to more positive potentials. All three anesthetics promoted the inactivated state of the channel at more negative potentials, but propofol was less effective than thiopental or ketamine in this regard.**

Conclusions: **Three intravenous anesthetics evaluated in this study decreased the I_{Ca} of porcine tracheal smooth muscle cells but with subtle electrophysiologic differences. Hence, thiopental, ketamine, and propofol each inhibit L-type voltage-dependent Ca^{2+} channels of porcine tracheal smooth muscle cells but the molecular mechanisms involved may be agent specific. This inhibition may contribute to the airway smooth muscle relaxant effects of these agents observed *in vitro* at concentrations greater than those encountered clinically. (Key words: Anesthetics, intravenous: ketamine; propofol; thiopental. Channels, ions; calcium. Muscle, smooth: trachea.)**

INTRAVENOUS anesthetics, especially ketamine,[1–3] are known to cause bronchodilatation and to inhibit bronchoconstriction. This effect may be achieved directly by relaxing smooth muscle cells of the airway and/or indirectly by blocking airway reflexes. For example, ketamine has been shown both to inhibit the excitability of the vagus nerve[2] and to relax airway smooth muscle preparations.[1–3] The effects of thiobarbiturates are more complex.[4–6] Thiobarbiturates inhibit vagus nerve reflexes[7] but may either contract or relax airway smooth muscle, depending on the dose,[8] on the condition of preconstriction,[9,10] and on the species studied.[8] Although it has been shown that the contractile effect of thiopental on airway smooth muscle *in vitro* was mediated by constrictor prostaglandins,[8] the inhibitory mechanism of this anesthetic is still unknown. Recently, propofol (2,6-diisopropylphenol), a new short-acting intravenous anesthetic,[11] was reported to antagonize fentanyl-induced bronchoconstriction during surgery,[12] to inhibit postoperative bronchospasm in patients with hyperreactive airway disease,[13] and to decrease the incidence of wheezing in patients after induction of general anesthesia and tracheal intubation.[14] The mechanism of the direct inhibitory effect of this anesthetic on airway smooth muscle has not yet been determined.[15]

Because the concentration of intracellular free Ca^{2+} ($[Ca^{2+}]_i$) plays a central role in the regulation of airway smooth muscle tone,[16,17] a possible mechanism for relaxation by intravenous anesthetics is a decrease in $[Ca^{2+}]_i$. Furthermore, sustained contraction of airway smooth muscle requires the continued entry of extra-

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cellular Ca^{2+}[18] and blockade of voltage-dependent Ca^{2+} channels (VDCs) suppresses the sustained increase in $[Ca^{2+}]_i$ in agonist-stimulated tracheal smooth muscle.[16] Hence, we hypothesized that intravenous anesthetics attenuate airway smooth muscle contraction by inhibition of VDCs. To test this hypothesis, we used patch-clamp techniques to directly measure the effects of the intravenous anesthetics thiopental, ketamine, and propofol on inward Ca^{2+} currents through VDCs (I_{Ca}) in porcine tracheal smooth muscle cells.

Methods and Materials

Preparation of Dispersed Porcine Tracheal Smooth Muscle Cells

This study was approved by the Johns Hopkins Medical Institutions Animal Care and Use Committee. Pigs (*Sus scrofa,* weighing 30–45 kg) were sedated with 25 mg/kg intramuscular ketamine, anesthetized with 7–8 mg/kg intravenous pentobarbital, and exsanguinated. Tracheas were excised quickly and placed in modified Krebs solution equilibrated with 95% O_2-5% CO_2 at 4°C (composition in mM: NaCl 118, KCl 4.7, $NaHCO_3$ 21, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 10, and $CaCl_2$ 2.5; pH ~ 7.4). Cell dispersion was performed according to previously described methods.[19,20] Briefly, tracheal smooth muscle was minced and incubated for 10 min in Ca^{2+}-free modified Tyrode's solution at room temperature (22–24°C). The modified Tyrode's solution contained (in mM): NaCl 135, KCl 5.4, $MgCl_2$ 1.0, glucose 5.0, N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid] 5.0, and 0.1% (w/v) bovine serum albumin; pH adjusted to 7.4 with 0.5 M tris-[hydroxymethyl]aminomethane. The tissue was then digested for 25 min at 37°C in Ca^{2+}-free modified Tyrode's solution with added 0.08% (w/v) collagenase, 0.05% trypsin inhibitor, and 0.03% protease. Cells were dispersed by trituration, filtered through nylon mesh, and centrifuged. The pellet was resuspended in a modified Kraftbrühe solution[21] and stored at 4°C for up to 5 h before use. The modified Kraftbrühe solution contained (in mM): KCl 85, K_2HPO_4 30, $MgSO_4$ 5.0, Na_2ATP 5.0, pyruvic acid 5.0, creatine 5.0, taurine 20, β-hydroxybutyrate 5.0, and 0.1% (w/v) fatty acid-free bovine serum albumin; pH adjusted to 7.25 with tris-[hydroxymethyl]aminomethane.

Whole-cell Patch Clamp Recording

All experiments were performed at room temperature (22–24°C). Micropipettes were pulled from soda-lime hematocrit tubing (Fisher Scientific, Pittsburgh, PA) and had resistances of 4–6 MΩ when filled with solution. The pipette solution contained (in mM): CsCl 130, $MgCl_2$ 4.0, EGTA 10, Na_2ATP 5.0, and N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid] 10; pH adjusted to 7.2 with tris-[hydroxymethyl]-aminomethane. The bath solution contained (in mM): tetraethylammonium chloride 130, $MgCl_2$ 1.0, $CaCl_2$ 10, glucose 10, and N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid] 10; pH adjusted to 7.4 with tris-[hydroxymethyl]aminomethane.

An aliquot (~0.5 ml) of the cell suspension was placed in a perfusion chamber on the stage of an inverted microscope (Olympus CK2, Leeds Instruments, Minneapolis, MN). A patch pipette was placed against the membrane of a tracheal smooth muscle cell using a three-dimensional oil-driven micromanipulator (MO-102, Narishige, Tokyo, Japan). After obtaining a high-resistance seal (3–20 GΩ) with slight suction, the patch membrane was disrupted by strong negative pressure. Membrane currents were monitored using a List EPC-7 patch clamp amplifier (Medical Systems, Greenvale, NY) and the amplifier output was low-pass filtered at 900 Hz. All data were digitized (2,000 samples/s), stored on a hard disk, and analyzed later.

Whole-cell inward Ca^{2+} currents (I_{Ca}) were elicited at 5-s intervals by 50 ms depolarizing pulses (−50 to +40 mV) from a holding potential of −80 mV. Leak and capacitative currents, estimated by appropriate scaling of currents during 20-mV hyperpolarizing pulses, were subtracted from each of these records. Series resistance was not compensated. Inactivation curves were determined using a double-pulse protocol that consisted of a 1-s duration prepulse to a potential in the range −80 to +20 mV, followed by a 50-ms depolarization to +20 mV. The peak change in current during the test pulse was expressed as a fraction of that obtained with the −80 mV prepulse and this quantity was least-squares fitted to a Boltzmann expression[22,23] to estimate the potential of half-maximal inactivation ($V_{1/2}$) and the slope factor (k).

Voltage-pulse protocols were performed in control solutions for >5 min to obtain a stable baseline. Data from cells that showed unstable I_{Ca} amplitudes, <100 pA of peak I_{Ca}, or a >10% reduction in amplitude during the control recording period were discarded. In some experiments, the Ca^{2+} channel agonist Bay K 8644 was added 5 min before exposure to anesthetic. Cells were then exposed to a single concentration of one of three intravenous anesthetics (thiopental, 10^{-7}

-3×10^{-4} M;, ketamine, $10^{-6} - 10^{-3}$ M; or propofol, $10^{-7} - 3 \times 10^{-4}$ M) by changing the inflow perfusate of the chamber to one of similar composition but with the anesthetic. Replacement of the chamber solution (~3 ml/min) required ~1 min. After a 5-min exposure, the perfusate was reswitched to the control solution. The GΩ-seal was maintained for a period sufficient to evaluate the reversibility of anesthetic effects in 61 of 75 experiments (81%).

Materials

The following drugs and chemicals were used: type I-S trypsin inhibitor (from soybean), bovine serum albumin, Na_2ATP, pyruvic acid, creatine, taurine, β-hydroxybutyrate, ketamine hydrochloride (Sigma Chemical, St. Louis, MO), type-I collagenase (Gibco Laboratories, Grand Island, NY), protease, Bay K 8644 (Calbiochem, La Jolla, CA), sodium thiopental (Pentothal; Abbott Laboratories, North Chicago, IL), propofol (Diprivan; Stuart Pharmaceuticals, Wilmington, DE), and Intralipid 10% (Kabi Vitrum, Alameda, CA). Bay K 8644 was dissolved in ethanol (0.01% final concentration). Sodium thiopental (from 9.5×10^{-2} M solution in 15 mM Na_2CO_3) had no effect on pH of the bath solution within the range 10^{-7} to 3×10^{-4} M. Propofol was diluted from an aqueous emulsion (5.6×10^{-2} M) in 10% (v/v) soybean oil, 2.25% glycerol, and 1.2% purified egg lecithin.

Statistical Analysis

Data are expressed as mean ± SEM. Changes in peak I_{Ca} or in the inactivation parameters $V_{1/2}$ and k with exposure to each drug were compared at each applied potential by the paired, two-tailed t test. The percent of control peak I_{Ca} and the values of $V_{1/2}$ and k after treatment were compared between anesthetics at equieffective concentrations using one-factor analysis of variance and Fisher's *a posteriori* test. In all comparisons, $P < 0.05$ was considered significant.

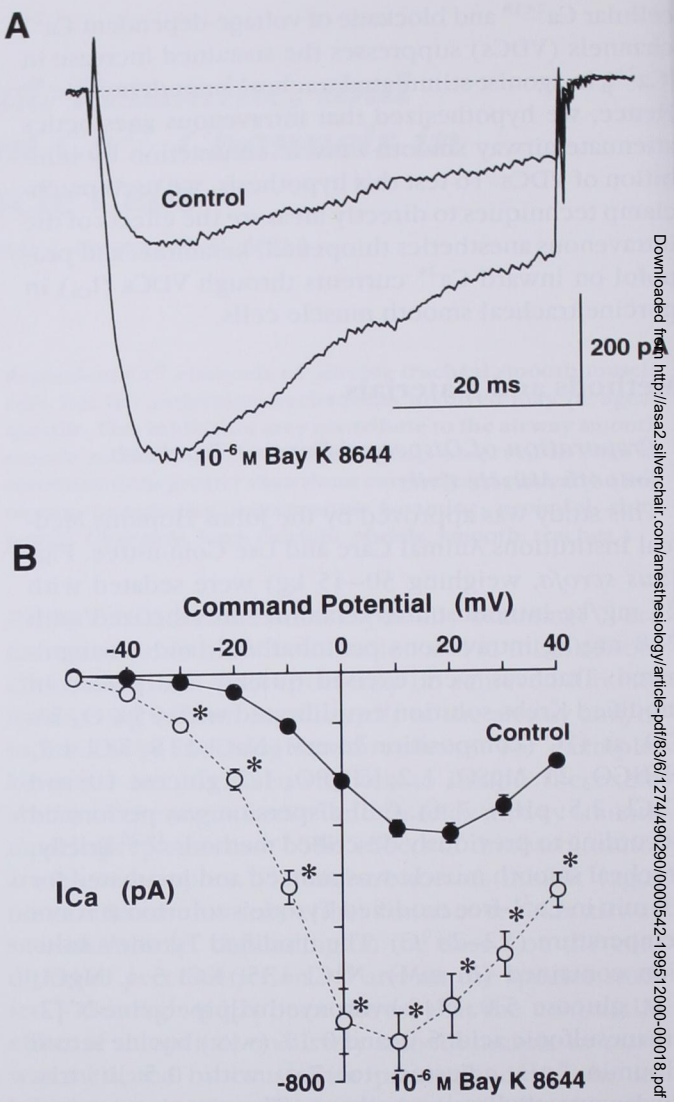

Fig. 1. Effects of Bay K 8644 on depolarization-induced inward Ca^{2+} currents (I_{Ca}). (*A*) Typical recordings of I_{Ca} induced by depolarizing pulses to +10 mV in the absence and presence of 10^{-6} M Bay K 8644. Dashed line denotes zero current. (*B*) Relationship between peak I_{Ca} and applied potential before (●, solid line) and after (○, dashed line) exposure to 10^{-6} M Bay K 8644. Symbols represent mean ± SEM (n = 5, *$P < 0.05$).

Results

Characteristics of Calcium Currents

As previously reported,[19,20] I_{Ca} seen in enzymatically dispersed porcine tracheal smooth muscle cells during step depolarizations from −80 mV peaked at ~10 ms and slowly inactivated (fig. 1A). Under baseline conditions, threshold activation of I_{Ca} occurred at ~ −20 mV and maximum peak current amplitude was obtained at ~ +20 mV. In 45 cells, the maximum peak I_{Ca} was −301 ± 3 pA (range −178 to −509 pA). As shown in a representative trace for depolarization from −80 to +10 mV (fig. 1A), Bay K 8644 (10^{-6} M) enhanced the magnitude of I_{Ca} but did not appear to alter the time course of the currents. Bay K 8644 significantly enhanced I_{Ca} at step potentials in the range −30 to +40 mV and increased the maximum peak I_{Ca} from −319 ± 18 pA at +20 mV to −656 ± 60 pA at +10 mV

(~ 2.1 fold, n = 5). There was a ~ 10 mV shift of the peak I_{Ca} *versus* applied potential curve toward more negative potentials (fig. 1B). Inward currents with a similar time course were observed in the inactivation experiments. The inactivation parameters obtained in 15 cells under control conditions were $V_{1/2} = -19.9 \pm 0.3$ mV and $k = 7.2 \pm 0.2$ mV.

Effects of Intravenous Anesthetics on the Activation of Macroscopic Voltage-activated Ca^{2+} Currents

As shown in a representative trace for depolarization from −80 to +20 mV (fig. 2A), thiopental (3×10^{-4} M) inhibited the magnitude of I_{Ca} but did not obviously alter the time course of the currents. Peak I_{Ca} obtained with repeated steps to +20 mV increased over a few minutes after obtaining the whole cell configuration at *time 0* to a stable plateau, decreased rapidly ~ 50% during exposure to 3×10^{-4} M thiopental, and recovered completely with washout (fig. 2B). Similar results were obtained with ketamine and propofol. Figure 3 shows the relationship between peak I_{Ca} *versus* applied potential before and after exposure to 3×10^{-4} M thiopental, 10^{-3} M ketamine, or 3×10^{-4} M propofol. Each of these intravenous anesthetics significantly inhibited I_{Ca} at step potentials in the range −20 or −10 to +40 mV and decreased the peak I_{Ca} at +20 mV by approximately 50% (n = 5). The actual percent inhibitions of peak I_{Ca} achieved by these agents at these concentrations (51.5 ± 6.9, 49.2 ± 6.1, and 47.9 ± 3.9, respectively) were not significantly different. There was no apparent shift in the voltage-dependence of induced I_{Ca} with any anesthetic.

Dose-dependence of the Inhibition of Macroscopic Voltage-activated Ca^{2+} Currents by the Intravenous Anesthetics

We determined the dose-dependence of the inhibition of peak I_{Ca} by each of these intravenous anesthetics. Figure 4 shows the relationship between the percent of control peak I_{Ca} at +20 mV and the concentration of the anesthetic in the bath solution (M). Each of the three intravenous anesthetics significantly inhibited peak I_{Ca} in a dose-dependent manner. Based on total concentration in the solution, thiopental and propofol had similar potency whereas ketamine required somewhat greater concentrations to achieve the same inhibitory effect.

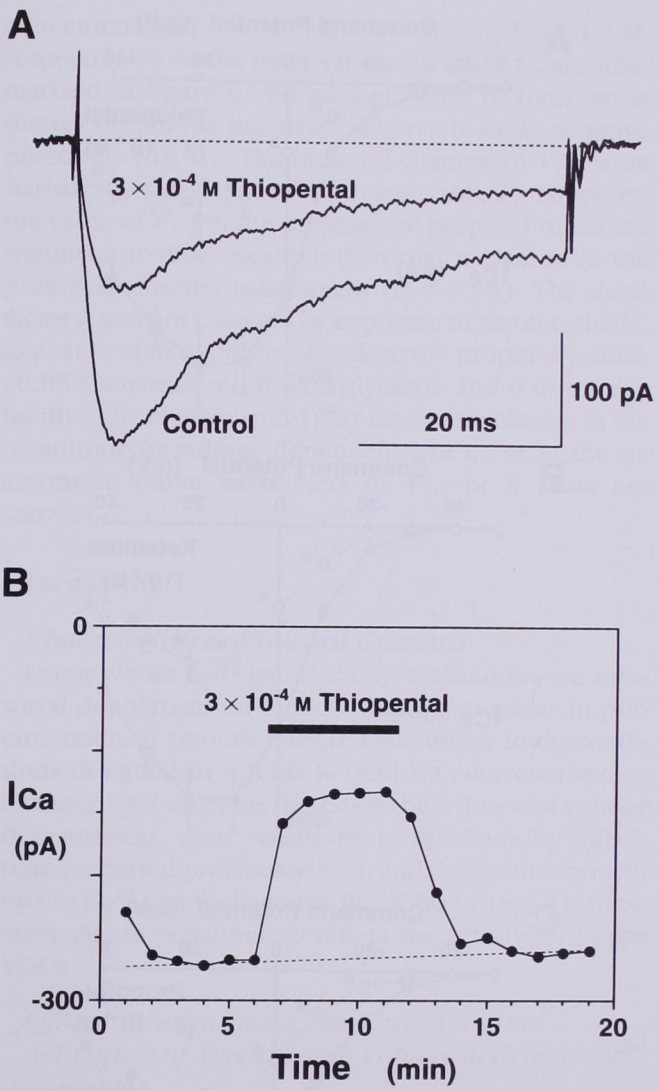

Fig. 2. Effects of thiopental on depolarization-induced I_{Ca}. (*A*) Typical recordings of I_{Ca} induced by pulses to +20 mV in the absence and presence of 3×10^{-4} M thiopental. Dashed line denotes zero current. (*B*) Representative time course of peak I_{Ca} at +20 mV before and after exposure to 3×10^{-4} M thiopental.

Effects of Intravenous Anesthetics on Macroscopic Voltage-activated Ca^{2+} Currents after Activation with Bay K 8644

Pretreatment with the Ca^{2+} channel agonist Bay K 8644 did not prevent the anesthetic-induced inhibition of I_{Ca}. Figure 5 shows the time course of the peak I_{Ca} obtained in a representative cell with repeated steps to +10 mV during exposure to 10^{-6} M Bay K 8644 and 10^{-3} M ketamine. Despite a substantial enhancement of the magnitude of peak I_{Ca} by Bay K 8644, 10^{-3} M

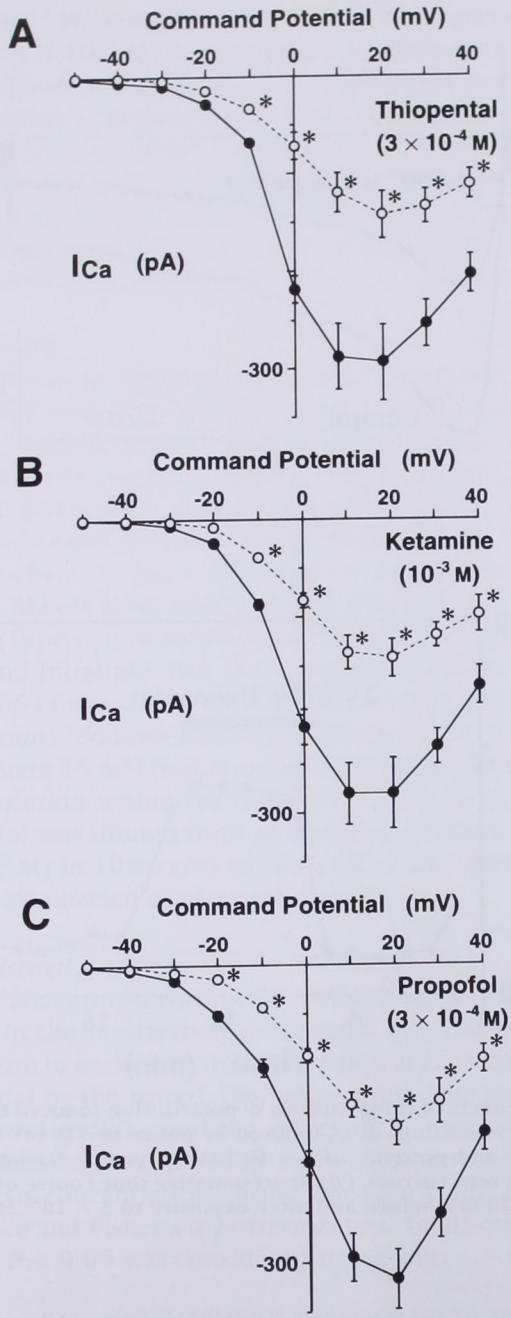

Fig. 3. Relationship between peak I_{Ca} and applied potential before (●, solid line) and after (○, dashed line) exposure to the intravenous anesthetics 3×10^{-4} M thiopental (*A*), 10^{-3} M ketamine (*B*), and 3×10^{-4} M propofol (*C*). Symbols represent mean ± SEM (n = 5, *$P < 0.05$).

ketamine still induced a ~ 50% inhibition. Similar results were obtained with 3×10^{-4} M thiopental and 3×10^{-4} M propofol in the presence of 10^{-6} M Bay K 8644. However, thiopental caused a shift in the relationship between peak I_{Ca} and applied voltage to more positive potentials. The maximum value occurred at +10 mV in the presence of Bay K 8644 and +30 mV in the presence of both Bay K 8644 and thiopental.

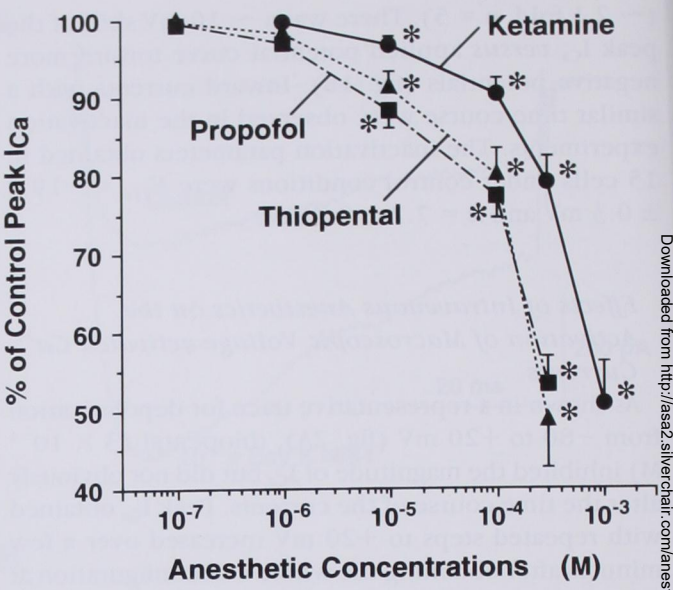

Fig. 4. Relationship between peak I_{Ca} at +20 mV, expressed as a percent of control, and the bath concentrations of the intravenous anesthetics thiopental (▲), ketamine (●), and propofol (■). Symbols represent mean ± SEM (n = 5). *$P < 0.05$ comparison to percent of control peak I_{Ca} at the lowest concentration.

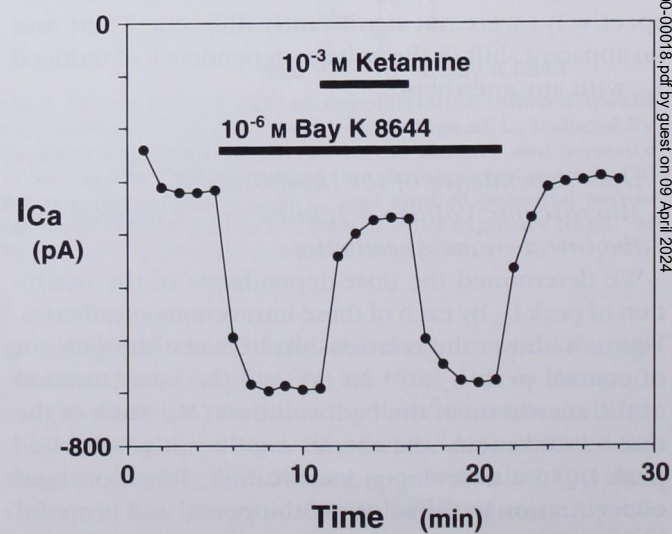

Fig. 5. Representative time course of peak I_{Ca} obtained with repeated steps to +10 mV during sequential additions of 10^{-6} M Bay K 8644 and 10^{-3} M ketamine.

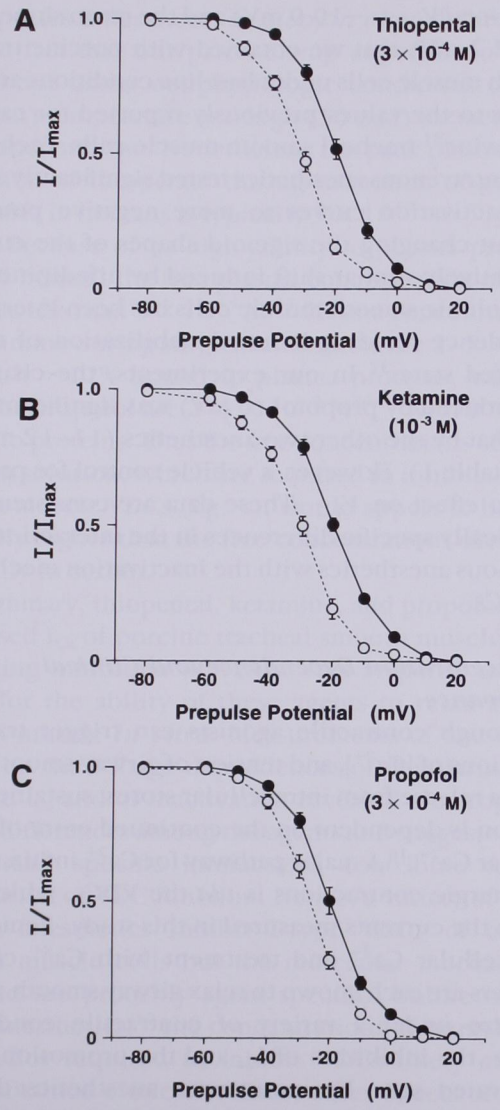

Fig. 6. Effects of the intravenous anesthetics thiopental (*A*), ketamine (*B*), and propofol (*C*) on voltage-dependent steady-state inactivation of I_{Ca}. Inactivation curves were generated under control conditions (●, solid line) and then repeated in presence of one intravenous anesthetic (○, dashed line). Symbols represent mean ± SEM (n = 5).

This change in voltage dependence was not seen in the absence of Bay K 8644 (fig. 3) or with either of the other two anesthetics tested.

Effects of Intravenous Anesthetics on Steady-state Inactivation of Macroscopic Voltage-activated Ca^{2+} Currents

The effects of the intravenous anesthetics thiopental, ketamine, and propofol at equi-effective inhibitory concentrations (3×10^{-4} M, 10^{-3} M, and 3×10^{-4} M, respectively) on the inactivation curves of I_{Ca} are summarized in figure 6 and table 1. Each of these anesthetics shifted the inactivation curve to more negative potentials (fig. 6). The induced changes in $V_{1/2}$ were statistically significant in each case (table 1). However, the value of $V_{1/2}$ in the presence of propofol remained significantly more positive than that measured in the presence of either other agent ($P < 0.05$). The slope factor k was not changed by exposure to any anesthetic. Exposure of five additional cells to the propofol vehicle (0.05% soybean oil, 0.01% glycerol, and 0.006% egg lecithin; from Intralipid 10%) caused no change in the magnitude or voltage dependence of I_{Ca} or in the inactivation curve parameters of $V_{1/2}$ or k (data not shown).

Discussion

Characteristics of Inward Currents

Using whole-cell, patch-clamp techniques we measured depolarization-induced inward currents in porcine tracheal smooth muscle cells under ionic conditions designed to inhibit K^+ and Na^+ currents and to enhance Ca^{2+} currents. Based on their time and voltage dependences, their sensitivity to blockade by nifedipine (reported previously[19,20]), and their enhancement by the Ca^{2+} channel agonist Bay K 8644 (fig. 1), these currents are presumed to reflect the activity of L-type VDCs.[24,25]

Effects of Intravenous Anesthetics on the Activation of Macroscopic Voltage-activated Ca^{2+} Currents

Each of the intravenous anesthetics tested inhibited I_{Ca} through VDCs of porcine tracheal smooth muscle

Table 1. Effects of Intravenous Anesthetics on the Inactivation Parameters of I_{Ca}

	Thiopental (3×10^{-4} M)	Ketamine (10^{-3} M)	Propofol (3×10^{-4} M)
Percent inhibition of peak I_{Ca} at +20 mV	53.1 ± 5.4	55.7 ± 7.2	51.1 ± 4.5
Potential of half-inactivation ($V_{1/2}$, mV)			
Control	−19.6 ± 0.7	−19.9 ± 0.5	−20.0 ± 0.6
Anesthetic	−31.2 ± 0.8*	−29.6 ± 0.7*	−26.4 ± 0.4*†
Slope factor (k, mV)			
Control	7.2 ± 0.4	7.4 ± 0.3	7.0 ± 0.5
Anesthetic	7.1 ± 0.5	7.5 ± 0.4	6.9 ± 0.2

* $P < 0.05$, t test comparison with control.

† $P < 0.05$, Fisher's test comparison with each other agent.

cells without an apparent change in the time course of the currents (fig. 2A). The onset of inhibition was rapid and the effect was reversible (fig. 2B) and dose-related (fig. 4). None of the intravenous anesthetics altered the voltage dependence of I_{Ca} (fig. 3). These data demonstrate a cellular effect of intravenous anesthetics that can account for the airway smooth muscle relaxant effects of these agents.[1–3,9,15] The relatively high concentrations of thiopental and ketamine required to inhibit VDCs are similar to those required to directly relax preconstricted airway smooth muscle preparations. Our results are also consistent with data obtained with vascular smooth muscles in which indirect evidence has suggested inhibition of VDCs by barbiturates[26] and propofol[27,28] and in which ketamine has been shown to inhibit whole-cell patch clamp I_{Ca}.[29]

Effects of Intravenous Anesthetics on Macroscopic Voltage-activated Ca^{2+} Currents in the Presence of Bay K 8644

To investigate the molecular basis of intravenous anesthetic effects on VDCs, we performed additional experiments with the Ca^{2+} channel agonist Bay K 8644. Bay K 8644 (10^{-6} M) enhanced I_{Ca} ~ 2 fold and caused a slight shift of the peak I_{Ca} *versus* applied potential curve to more negative potentials (fig. 1). However, Bay K 8644 did not prevent inhibition of I_{Ca} by thiopental, ketamine, or propofol. Interestingly, thiopental but not ketamine or propofol caused a substantial shift in the voltage dependence of induced I_{Ca} to more positive potentials. This distinctive action of thiopental indicates that, in addition to physicochemical or nonspecific interactions with VDCs, intravenous anesthetics have chemically specific effects on VDC properties. One possible explanation for the additional effect of thiopental is that it interacts with the portion of the channel protein that functions as the voltage sensor for activation.

Effects of Intravenous Anesthetics on the Inactivation of Macroscopic Voltage-activated Ca^{2+} Currents

To further examine the inhibitory actions of these intravenous anesthetics on VDCs of tracheal smooth muscle cells, we studied the effects of these anesthetics on steady-state, voltage-dependent inactivation of I_{Ca}. During prolonged depolarization a fraction of the VDCs enters an unavailable or "inactivated" state. The degree of steady-state inactivation depends on the prepulse potential (fig. 6). The mean potential of half inactivation ($V_{1/2} = -19.9$ mV) and the mean slope factor ($k = 7.2$ mV) that we obtained with porcine tracheal smooth muscle cells under baseline conditions are each similar to the values previously reported for canine[30] and bovine[31] tracheal smooth muscle cells. Each of the three intravenous anesthetics tested significantly shifted the inactivation curves to more negative potentials without changing the sigmoid shapes of the curve. A qualitatively similar shift induced by nifedipine in canine colonic smooth muscle cells has been interpreted as evidence for drug-induced stabilization of the inactivated state.[32] In our experiments, the change in $V_{1/2}$ induced by propofol (6 mV) was significantly less than that by the other two anesthetics (11–12 mV; fig. 6 and table 1). However, a vehicle control for propofol had no effect on $V_{1/2}$. These data are consistent with chemically specific differences in the interaction of intravenous anesthetics with the inactivation mechanism of VDCs.

Concentration Dependence and Clinical Relevance

Although contractile agonists can trigger transient elevations of $[Ca^{2+}]_i$ and tension of airway smooth muscle *via* release from intracellular stores, sustained contraction is dependent on the continued entry of extracellular Ca^{2+}.[16] A major pathway for Ca^{2+} influx during cholinergic contractions is *via* the VDCs, which give rise to the currents measured in this study. Removal of extracellular Ca^{2+} and treatment with Ca^{2+} channel blockers are each known to relax airway smooth muscle *in vitro* under a variety of contractile conditions. Hence, the inhibition of I_{Ca} and the promotion of the inactivated state by intravenous anesthetics demonstrated in this study are both effects that would be expected to antagonize bronchoconstriction. However, these effects are of clinical interest only if they occur at concentrations that are usually attained during anesthesia.

Thiopental, ketamine, and propofol each showed concentration-dependent inhibition of I_{Ca} (fig. 4). Based on the total solution concentration, ketamine was less potent than the other agents by a factor of ~3. Because propofol was added as an emulsion it is likely that its free concentration was substantially less than the total and that this drug is, in fact, more potent than thiopental for inhibition of I_{Ca}. We suggest that the potency order of these agents is propofol > thiopental > ketamine. Extrapolation of our data to the clinical situation must be viewed with caution because of pos-

sible species differences, *in vivo/in vitro* differences, and the fact that our patch clamp experiments were carried out under nonphysiologic conditions of low (ambient) temperature and high (10 mM) extracellular Ca^{2+} concentration. Nonetheless, the peak plasma concentration of thiopental during induction of general anesthesia in humans is typically $5 \times 10^{10-}$ to 3×10^{-4} M.[33,34] Because of its high affinity to plasma proteins (~90% bound) the free plasma concentration of thiopental is less than 5×10^{-5} M.[34] Plasma concentrations for ketamine during surgical anesthesia are $2–5 \times 10^{-6}$ M[35] with ~12% bound to plasma proteins, whereas those for propofol are $2–5 \times 10^{-5}$ M[36,37] with 97–99% bound to proteins. Thus the free concentrations of these drugs in solution, which are required to inhibit airway smooth muscle VDCs (fig. 4), would appear to be substantially higher than the free concentrations observed clinically in serum.

In summary, thiopental, ketamine, and propofol each decreased I_{Ca} of porcine tracheal smooth muscle cells indicating inhibition of VDCs. This response can account for the ability of these agents to relax airway smooth muscle *in vitro*. Measurements of anesthetic effects on the voltage dependences of depolarization-induced I_{Ca} and of inactivation revealed electrophysiologic differences among the agents and suggested that structurally specific interactions contribute to the modulation of VDC function. Hence, a variety of intravenous anesthetics inhibit L-type VDCs of airway smooth muscle cells but the molecular mechanisms involved are probably agent specific. Unlike inhalational anesthetics,[19] the concentrations of intravenous anesthetics required to inhibit VDCs likely exceed those obtained clinically. Thus, other effects of these agents such as inhibition of neural reflexes[3,8,11] are more likely to be responsible for their bronchodilatory effects *in vivo*.

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