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Inhibitory Effects of Etomidate and Ketamine on Endothelium-dependent Relaxation in Canine Pulmonary Artery

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Background: **The authors recently demonstrated that acetylcholine-induced pulmonary vasorelaxation had two primary components, nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF). The goal was to investigate the effects of etomidate and ketamine on the NO- and EDHF-mediated components of pulmonary vasorelaxation in response to acetylcholine, bradykinin, and the calcium ionophore, A23187.**

Methods: **Canine pulmonary arterial rings with an intact endothelium were suspended in organ chambers for isometric tension recording. The effects of etomidate and ketamine (10^{-5} M and 10^{-4} M) on vasorelaxation responses to acetylcholine, bradykinin, and A23187 were assessed in phenylephrine-contracted rings. The NO- and EDHF-mediated components of relaxation were assessed using a NO synthase inhibitor (*N*-nitro-L-arginine methylester [L-NAME]: 10^{-4} M) and a Ca^{2+}-activated potassium channel inhibitor (tetrabutylammonium hydrogen sulfate [TBA]: 10^{-3} M) in rings pretreated with a cyclooxygenase inhibitor (ibuprofen: 10^{-5} M). Intracellular calcium concentration ($[Ca^{2+}]_i$) was measured in cultured bovine pulmonary artery endothelial cells loaded with acetoxylmethyl ester of fura-2.**

Results: **Etomidate and ketamine attenuated pulmonary vasorelaxation in response to acetylcholine and bradykinin, whereas they had no effect on the response to A23187. The relaxant responses to acetylcholine and bradykinin were attenuated by L-NAME or TBA alone and were abolished by combined inhibition in rings pretreated with ibuprofen. Etomidate and ketamine further attenuated both L-NAME—resistant and TBA-resistant relaxation. These anesthetics also inhibited increases in endothelial $[Ca^{2+}]_i$ in response to bradykinin, but not A23187.**

Conclusion: **These results indicate that etomidate and ketamine attenuated vasorelaxant responses to acetylcholine and bradykinin by inhibiting both NO- and EDHF-mediated components. Moreover, our results suggest that these anesthetics do not directly suppress NO or EDHF activity, but rather inhibit the endothelial $[Ca^{2+}]_i$ transient in response to receptor activation.**

THE endothelium plays a crucial role in regulating the tone of vascular smooth muscle *via* the release of vasoactive mediators in response to various neurohumoral agonists, including acetylcholine and bradykinin. Nitric oxide (NO), synthesized by the L-arginine–NO synthase pathway, and prostacyclin, produced by the arachidonic acid–cyclooxygenase pathway, have been identified as endothelium-derived mediators.[1,2] In addition, the endothelium can release endothelium-derived hyperpolarizing factors (EDHFs),[3–5] although their identity has not been firmly established. The relative contribution of NO and EDHFs to total endothelium-dependent relaxation depends on the endothelial stimulus and the vascular bed investigated.[6,7]

We recently demonstrated in isolated canine pulmonary artery that acetylcholine-induced relaxation has two primary components, NO and EDHF, and that the intravenous anesthetic, propofol, selectively attenuates acetylcholine-induced relaxation by inhibiting both of these endothelium-derived mediators.[8] Etomidate and ketamine are intravenous anesthetics that are recognized to have minimal inhibitory effects on clinically measured end points of cardiovascular function. As a result, they are often used as induction agents in patients with hemodynamic instability. Although both etomidate and ketamine are thought to inhibit endothelium-dependent vasodilation in the systemic circulation, little is known about their effects or mechanism of action on endothelium-dependent relaxation in the pulmonary circulation.

The first goal of this *in vitro* study was to investigate the effects of etomidate and ketamine on the NO- and EDHF-mediated components of pulmonary vasorelaxation in response to receptor-dependent and -independent endothelial activators. A second goal was to test the hypothesis that these intravenous anesthetics attenuate endothelium-dependent vasorelaxation by reducing increases in intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ in endothelial cells in response to receptor activation.

Materials and Methods

All experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee at The Cleveland Clinic Foundation (Cleveland, OH).

Preparation of Pulmonary Arterial Rings

Twenty healthy male mongrel dogs weighing 24–32 kg were anesthetized with intravenous pentobarbital sodium (30 mg/kg) and fentanyl citrate (15 μg/kg). After tracheal intubation, the lungs were mechanically ventilated. A catheter was placed in the right femoral artery, and the dogs were exsanguinated by controlled hemorrhage. A left lateral thoracotomy was performed through the fifth intercostal space, and the heart was arrested

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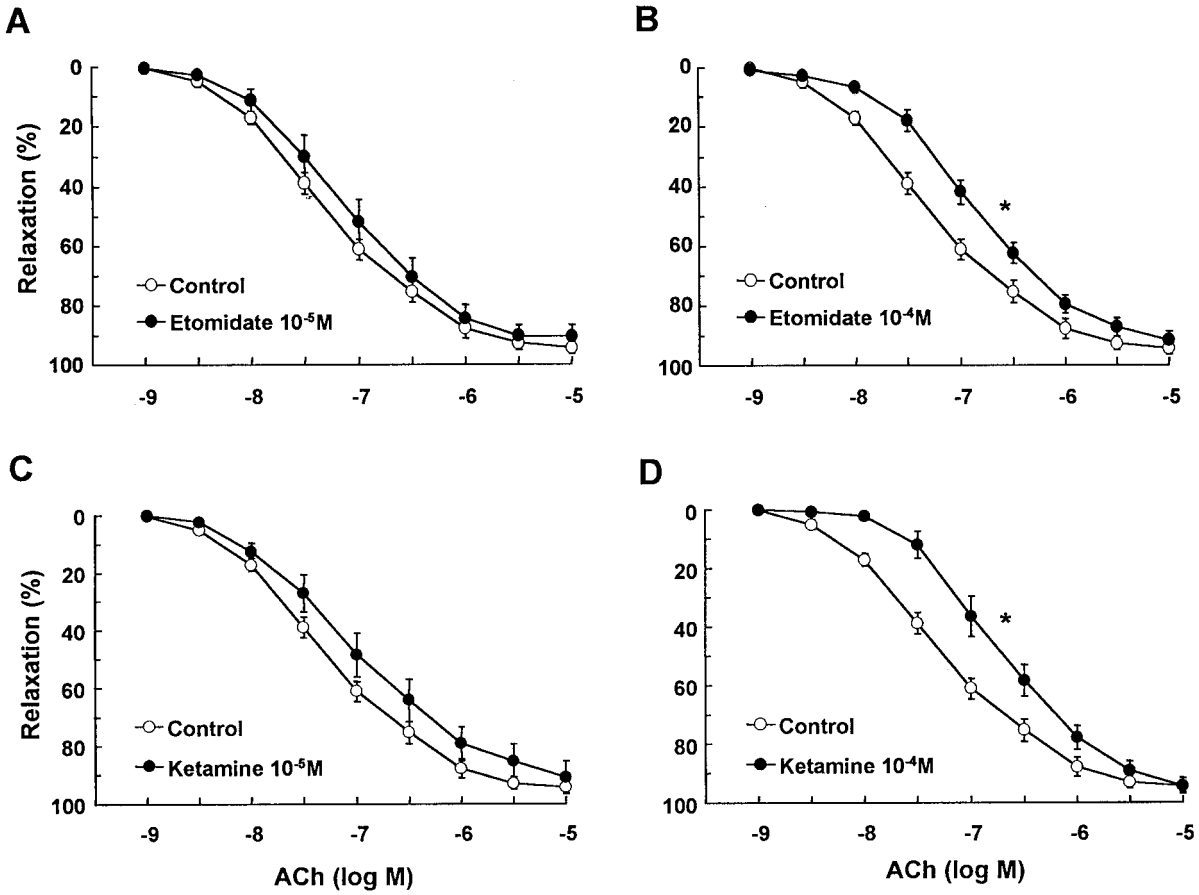

Fig. 1. Effects of etomidate (*A* and *B*) and ketamine (*C* and *D*) on the endothelium-dependent relaxation induced by acetylcholine in pulmonary arteries. Arterial rings were precontracted to the ED_{50} level of tension with phenylephrine. Relaxation responses are expressed as the percentage of phenylephrine precontraction. Etomidate (10^{-5} M) had little effect (*A*), whereas etomidate (10^{-4} M) significantly attenuated (*$P < 0.05$) the relaxation induced by acetylcholine (*B*). Ketamine also inhibited (*$P < 0.05$) acetylcholine-induced relaxation at a concentration of 10^{-4} M (*D*) but not at 10^{-5} M (*C*).

with induced ventricular fibrillation (Grass Instrument Co., Quincy, MA). The heart and lungs were removed from the thorax *en bloc*, and the lower right and left lung lobes were dissected free. Intralobar pulmonary arteries (2–4-mm ID) were carefully dissected and immersed in cold modified Krebs-Ringer bicarbonate solution composed of 118.3 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 0.016 mM Ca-EDTA, and 11.1 mM glucose. The arteries were cleaned of connective tissue and cut into ring segments 4–5 mm in length with special care taken not to damage the endothelium. In some rings, the endothelium was denuded by gently rubbing the intimal surface with a cotton swab. The integrity of the endothelium was verified by assessing the vasorelaxant response to acetylcholine (10^{-6} M).

Isometric Tension Experiments

Pulmonary arterial rings were vertically mounted between two stainless steel hooks in organ baths filled with 25 ml Krebs-Ringer bicarbonate solution (37°C) gassed with 95% air and 5% CO_2. One of the hooks was anchored and the other was connected to a strain gauge to measure isometric force. The rings were stretched at 10-min intervals in increments of 0.5 g to achieve opti-

Table 1. Effects of Etomidate and Ketamine on the Relaxant Response to Acetylcholine, Bradykinin, and A23187 in Canine Pulmonary Artery

	n	IC_{50}	R_{max} (%)
Acetylcholine			
Control	8	−7.31 ± 0.04	94.4 ± 2.0
10^{-5} M Etomidate	8	−7.13 ± 0.17	90.6 ± 3.9
10^{-4} M Etomidate	8	−6.89 ± 0.06*	91.6 ± 2.8
Vehicle	8	−7.27 ± 0.07	94.7 ± 2.3
10^{-5} M Ketamine	6	−7.04 ± 0.14	91.0 ± 5.6
10^{-4} M Ketamine	6	−6.76 ± 0.12*	94.3 ± 2.7
Bradykinin			
Control	6	−8.42 ± 0.07	90.2 ± 5.1
10^{-4} M Etomidate	6	−7.99 ± 0.13*	77.1 ± 4.3
10^{-4} M Ketamine	6	−8.02 ± 0.12*	79.6 ± 6.2
A23187			
Control	6	−7.19 ± 0.13	90.0 ± 4.2
10^{-4} M Etomidate	6	−7.21 ± 0.13	87.9 ± 3.8
10^{-4} M Ketamine	6	−6.93 ± 0.13	91.5 ± 4.4

* Significantly different from control in each group ($P < 0.05$).

IC_{50} = drug concentration eliciting 50% of the maximum relaxation response; R_{max} = maximum relaxant response.

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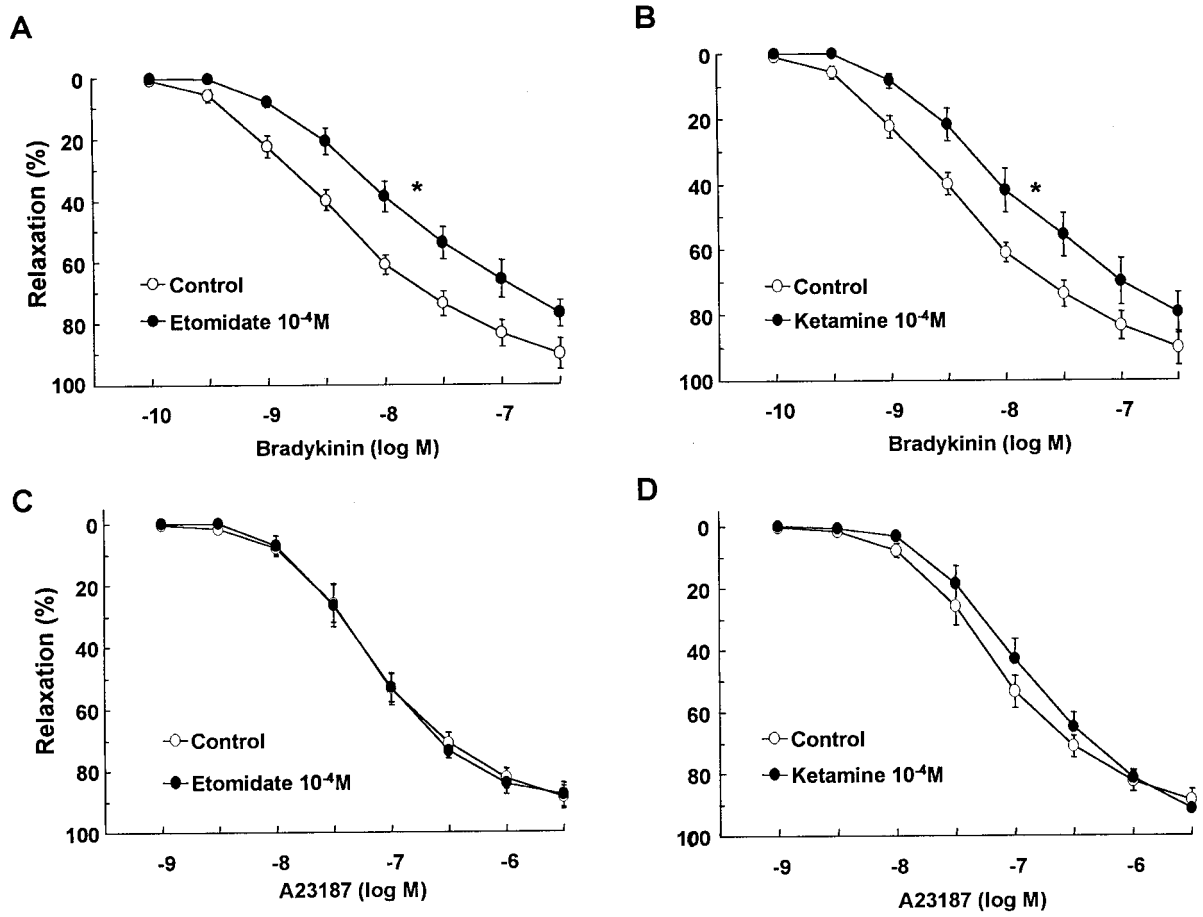

Fig. 2. Effects of etomidate and ketamine on relaxation induced by bradykinin (*A* and *B*) and A23187 (*C* and *D*). Pulmonary arterial rings were precontracted to the ED_{50} level of tension with phenylephrine. Etomidate (10^{-4} M) and ketamine (10^{-4} M) attenuated (*$P < 0.05$) bradykinin-induced relaxation but had no effect on A23187-induced relaxation.

mal resting tension. Optimal resting tension was defined as the minimum amount of stretch necessary to achieve the largest contractile response to 40 mM KCl and was determined in preliminary experiments to be 5 g for the size of arteries used in these experiments. After the arterial rings had been stretched to their optimal resting tension, the contractile response to 60 mM KCl was measured. After washing out KCl from the organ bath and the return of isometric tension to prestimulation values, a cumulative concentration–response curve to phenylephrine was performed in each ring. This was achieved by increasing the concentration of phenylephrine in half-log increments (from 10^{-8} M to 3×10^{-5} M) after the response to each preceding concentration had reached a steady state. All rings were pretreated with the β-adrenoreceptor antagonist, propranolol (5×10^{-6} M, incubated for 30 min), before phenylephrine administration in all protocols to avoid the β-agonist effect of phenylephrine.

Experimental Protocols

After washout of phenylephrine from the organ bath and return of isometric tension to baseline values, the rings were again pretreated with propranolol and precontracted to 50% of the maximal response to phenylephrine (ED_{50} level of tension). When the contractile response was stabilized, concentration–response curves to the endothelial cell activators, acetylcholine (10^{-9} to 10^{-5} M), bradykinin (10^{-10} to 3×10^{-7} M) and A23187 (10^{-9} to 3×10^{-6} M) were obtained. Each ring was exposed to only one endothelial activator. Responses to the endothelium-independent NO donor, *S*-nitroso-*N*-acetylpenicillamine (SNAP: 10^{-9} to 3×10^{-6} M), were also investigated in endothelium-denuded rings.

The effects of etomidate (10^{-5} and 10^{-4} M) and ketamine (10^{-4} and 10^{-5} M) on the concentration–response curves for these vasodilators were assessed by comparing vasorelaxant responses in the presence and absence of the anesthetics. The anesthetics were directly added to the organ bath 15 min before phenylephrine contraction.

To characterize the relative contribution of NO- and EDHF-mediated components to the total relaxation responses to these endothelial activators, arterial rings were pretreated with the combination of ibuprofen (10^{-5} M), a cyclooxygenase inhibitor, and *N*-nitro-L-argi-

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Table 2. Effects of Inhibitors on the Endothelium-dependent Relaxant Responses to Acetylcholine, Bradykinin, and A23187 in Canine Pulmonary Artery

	n	IC_{50}	R_{max} (%)
Acetylcholine			
Control	8	−7.31 ± 0.04	94.4 ± 2.0
+ L-NAME + ibuprofen	8	−6.65 ± 0.20*	35.3 ± 6.8*
+ TBA + ibuprofen	6	−6.42 ± 0.11*	74.2 ± 2.9†
+ L-NAME + ibuprofen + TBA	6	NC	−7.2 ± 2.2‡
+ L-NAME + ibuprofen + 30 mM KCl	6	NC	−1.9 ± 0.8‡
Bradykinin			
Control	6	−8.42 ± 0.07	90.2 ± 5.1
+ L-NAME + ibuprofen	6	−7.51 ± 0.05*	40.7 ± 3.5*
+ TBA + ibuprofen	6	−7.72 ± 0.08*	59.4 ± 6.9*
+ L-NAME + ibuprofen + TBA	6	NC	6.5 ± 4.4‡
+ L-NAME + ibuprofen + 30 mM KCl	6	NC	1.5 ± 0.6‡
A23187			
Control	6	−7.19 ± 0.13	90.0 ± 4.2
+ L-NAME + ibuprofen	6	−6.44 ± 0.06*	51.9 ± 5.0*
+ TBA + ibuprofen	6	−6.98 ± 0.08*	51.1 ± 6.9*
+ L-NAME + ibuprofen + TBA	6	NC	5.5 ± 2.9‡
+ L-NAME + ibuprofen + 30 mM KCl	6	NC	3.0 ± 2.7‡

* $P < 0.01$, † $P < 0.05$ *versus* control in each group. ‡ $P < 0.01$ *versus* + L-NAME + ibuprofen in each group.

IC_{50} = drug concentration eliciting 50% of the maximum relaxation response; R_{max} = maximum relaxant response; L-NAME = N^G-nitro-L-arginine methylester; TBA = tetrabutylammonium hydrogen sulfate; NC = IC_{50} value not calculated because the relaxation response was too small.

nine methylester (L-NAME: 10^{-4} M), an inhibitor of NO synthase, or tetrabutylammonium hydrogen sulfate (TBA: 10^{-3} M), an inhibitor of calcium-activated potassium channels (KCa). Previous studies have shown that EDHF-mediated relaxation can be inhibited by either membrane depolarization (30 mM KCl) or by inhibitors of KCa.[4,5] After incubation with these inhibitors for 20 min, the rings were contracted to the ED_{50} level of tension with phenylephrine. The inhibitors remained in the organ bath throughout the experiment. The relaxant responses to the endothelial activators in inhibitor-treated rings were compared with responses in untreated rings. The effects of etomidate (10^{-4} M) and ketamine (10^{-4} M) on the NO-mediated component of the relaxant response to acetylcholine and bradykinin were examined in rings pretreated with the combination of ibuprofen and TBA. The effects of these anesthetics on the EDHF-mediated component of the relaxant response to acetylcholine and bradykinin were examined in rings pretreated with the combination of ibuprofen and L-NAME.

Cell Culture of Pulmonary Artery Endothelial Cells

Bovine pulmonary artery endothelial cells were purchased from the American Type Culture Collection (CRL-1733, Rockville, MD). The cells were cultured in Ham F-12K medium (American Type Culture Collection) supplemented with 1.5% $NaHCO_3$, 10% horse serum, and 1% antibiotic–antimycotic mixture solution (10,000 units/ml penicillin, 10,000 μg/ml streptomycin, 25 μg/ml amphotericin B) in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. Cells were allowed to grow until they formed a confluent monolayer. The cells were then subcultured nonenzymatically to 35-mm glass dishes designed for fluorescence microscopy (Bioptechs, Inc., Butler, PA). Twenty-four hours before the experiment, the culture medium was replaced with a serum-free medium to arrest cell growth and allow for establishment of steady state cellular events independent of cell division.

Intracellular Ca^{2+} Measurements

Bovine pulmonary artery endothelial cells were washed twice in Krebs-Ringer-HEPES buffer, which contained 125 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 1.0 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 11 mM glucose, and 25 mM HEPES at pH 7.4 adjusted with NaOH. The cells were then incubated in Krebs-Ringer-HEPES containing 2 μM acetoxylmethyl ester of fura 2 (fura-2—AM; TEF Labs, Austin, TX) at room temperature for 40 min. After the 40-min loading period, the cells were washed twice in Krebs-Ringer-HEPES buffer. Culture dishes containing fura-2–loaded endothelial cells were placed in a temperature-regulated (37°C) chamber (Bioptechs, Inc.) mounted on the stage of an Olympus IX-70 inverted fluorescence microscope (Lake Success, NY). The volume of the culture dish was 1.5 ml, and temperature was maintained at 37°C. Fluorescence measurements were performed using a dual-wavelength spectrofluorometer (Deltascan RFK6002, Photon Technology International, South Brunswick, NJ) at excitation wavelength of 340 and 380 nm and an emission wavelengths of 510 nm. The 340 to 380 fluorescence ratio was used as an indicator of $[Ca^{2+}]_i$. Solution changes were accomplished by rapidly aspirating the buffer in the dish and adding 1.5 ml of the new buffer. Before data acquisition, back-

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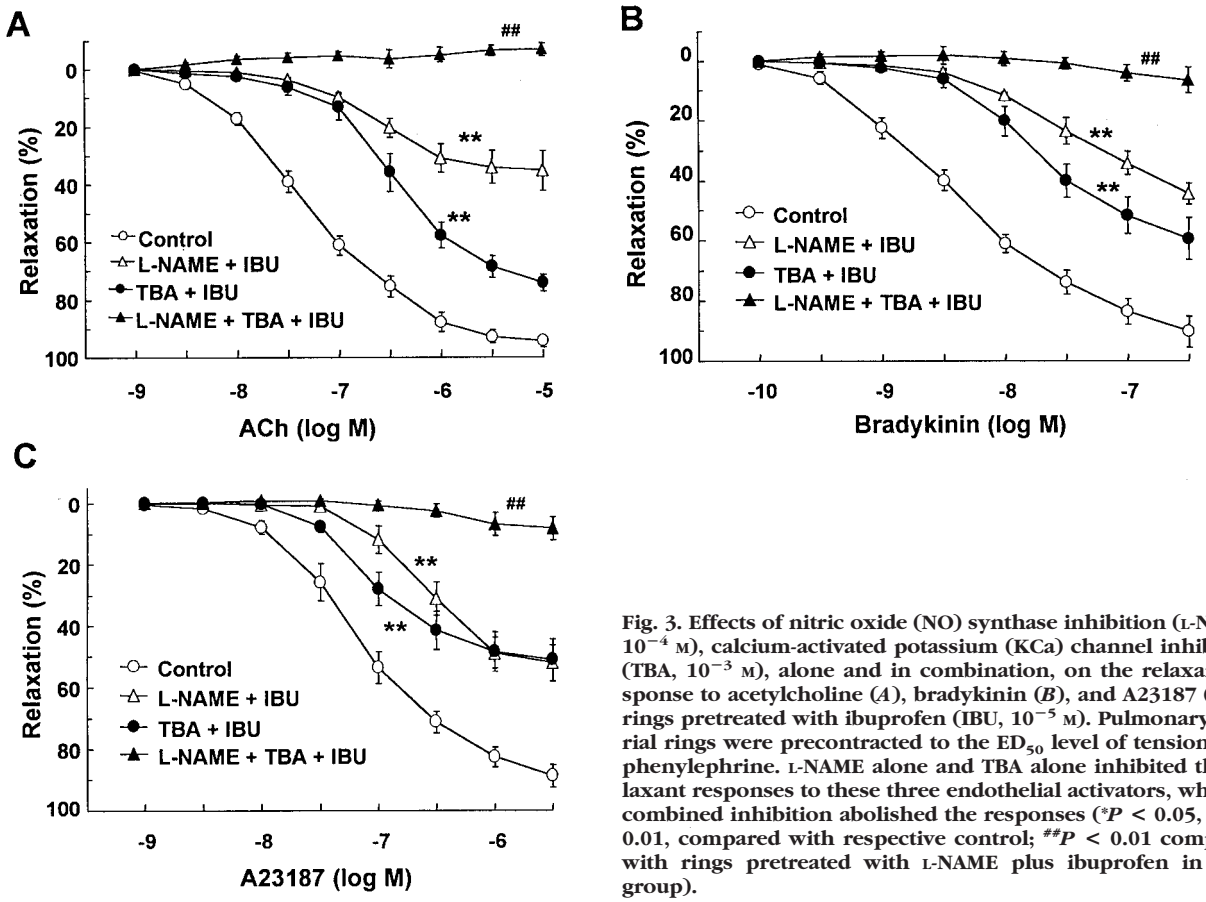

Fig. 3. Effects of nitric oxide (NO) synthase inhibition (L-NAME, 10^{-4} M), calcium-activated potassium (KCa) channel inhibition (TBA, 10^{-3} M), alone and in combination, on the relaxant response to acetylcholine (*A*), bradykinin (*B*), and A23187 (*C*) in rings pretreated with ibuprofen (IBU, 10^{-5} M). Pulmonary arterial rings were precontracted to the ED_{50} level of tension with phenylephrine. L-NAME alone and TBA alone inhibited the relaxant responses to these three endothelial activators, whereas combined inhibition abolished the responses (*$P < 0.05$, **$P < 0.01$, compared with respective control; ##$P < 0.01$ compared with rings pretreated with L-NAME plus ibuprofen in each group).

ground fluorescence was measured and subtracted automatically from the subsequent experimental measurements. Fura-2 fluorescence signals (340, 380, and 340/380 ratio) originating from bovine pulmonary artery endothelial cells were continuously monitored at a sampling frequency of 5 Hz and were collected using a software package from Photon Technology International.

Drugs and Solutions

All drugs were of the highest purity commercially available: acetylcholine chloride, A23187, bradykinin, L-NAME, phenylephrine HCl, propranolol HCl, SNAP, TBA (Sigma Chemical, St. Louis, MO), etomidate (Bedford Laboratories, Bedford, OH), ketamine HCl (Parke-Davis, Morris Plains, NJ), ibuprofen (Cayman Chemical, Ann Arbor, MI). All drug concentrations are expressed as the final concentration in the organ bath or cell dish. A23187 and ibuprofen were dissolved in dimethylsulfoxide and diluted with distilled water. The final concentration of dimethylsulfoxide in the organ bath or culture dish was less than 0.1 % (vol/vol). The vehicles have no effect on isometric tension or the 340/380 ratio at the concentrations used in these studies.

Data Analysis

All data are expressed as mean ± SEM. Vasorelaxant responses to the endothelium-dependent and -independent vasodilators are expressed as the percentage relaxation of the precontraction induced by phenylephrine. The logarithm of the drug concentration eliciting 50% of the maximum relaxation response (IC_{50}) was calculated by linear regression analysis, and the maximum relaxant response (R_{max}) was determined. R_{max} = 100% indicates complete reversal of phenylephrine contraction. Statistical analysis was performed using the Student *t* test for unpaired comparisons. When more than two means were compared, the Scheffé F test after one-way analysis of variance was used. A *P* value < 0.05 was considered significant. Sample size (n values) equals the number of dogs from which pulmonary arterial rings were taken in the isometric tension experiments, and the number of experiments in the endothelial cell studies.

Results

Effects of Etomidate and Ketamine on Pulmonary Vasorelaxation

Neither etomidate nor ketamine altered resting tension. Acetylcholine elicited endothelium-dependent relaxation of pulmonary arterial rings that was maximal at 10^{-5} M (fig. 1). Etomidate and ketamine had little effect on acetylcholine-induced relaxation at a concentration of 10^{-5} M, whereas a higher concentration of each anesthetic (10^{-4} M) increased the IC_{50} without changing

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the R_{max} for acetylcholine (table 1 and fig. 1). The vehicle for etomidate (35% propylene glycol) had no effect on the relaxant response to acetylcholine (table 1).

Bradykinin also induced receptor-mediated endothelium-dependent relaxation in pulmonary arterial rings precontracted with phenylephrine (figs. 2A and 2B). Etomidate and ketamine (10^{-4} M) caused rightward shifts ($P < 0.05$) in the bradykinin concentration–effect curve without affecting the R_{max} values (table 1 and figs. 2A and 2B). In contrast to these receptor-operated agonists, the vasorelaxant response to the non–receptor-mediated endothelium-dependent vasodilator, A23187, was not altered by etomidate or ketamine (table 1 and figs. 2C and 2D). Neither etomidate nor ketamine (10^{-4} M) altered SNAP-induced relaxation in endothelium-denuded rings. The log IC_{50} and R_{max} values in control, etomidate-treated, and ketamine-treated rings were -7.08 ± 0.12 and $100 \pm 3\%$, -7.02 ± 0.15 and $105 \pm 3\%$, and -7.06 ± 0.10 and $103 \pm 3\%$, respectively.

Characterization of Relaxant Responses to Acetylcholine, Bradykinin, and A23187

The effects of the various inhibitors on the relaxant responses to each endothelial activator are summarized in table 2 and figure 3. None of the inhibitors had an effect on baseline tension. The combination of L-NAME plus ibuprofen, as well as the combination of TBA plus ibuprofen, attenuated ($P < 0.01$) the pulmonary vasorelaxant responses to acetylcholine, bradykinin, and A23187. Pretreatment with either TBA or 30 mM KCl (to depolarize pulmonary arterial smooth muscle) essentially abolished ($P < 0.01$) the L-NAME plus ibuprofen–resistant relaxation responses to these endothelial activators. These results indicate that in ibuprofen-pretreated rings, the pulmonary vasorelaxant responses to acetylcholine, bradykinin, and A23187 are mediated by both NO and an EDHF.

Effects of Anesthetics on Nitric Oxide– and Endothelium-derived Hyperpolarizing Factor–mediated Relaxation

The effects of the two intravenous anesthetics (10^{-4} M) on each component of the relaxation response to acetylcholine and bradykinin were investigated. In rings treated with L-NAME plus ibuprofen, etomidate ($P < 0.05$) and ketamine ($P < 0.01$) induced a rightward shift of the concentration–effect curve for acetylcholine without changing the R_{max} values (table 3 and figs. 4A and 4B), *i.e.*, both anesthetics inhibited the EDHF-mediated component of the vasorelaxant response to acetylcholine. These anesthetics also attenuated ($P < 0.05$) the TBA plus ibuprofen-resistant acetylcholine relaxation (table 3 and figs. 4C and 4D), *i.e.*, both anesthetics inhibited the NO-mediated component of the vasorelaxant response to acetylcholine. Etomidate and ketamine also inhibited ($P < 0.05$) the NO- and EDHF-mediated components of bradykinin-induced relaxation (table 3 and fig. 5).

Effects of Etomidate and Ketamine on Endothelial Intracellular Ca^{2+} Concentration in Response to Bradykinin and A23187

Bradykinin induced a rapid increase in endothelial $[Ca^{2+}]_i$ followed by a gradual decline to a sustained plateau phase above the resting level (fig. 6A, left side). After washing out with buffer for 15 min, a second bradykinin application produced a similar but slightly smaller response in the same cell (fig. 6A, right side). To account for this slight rundown effect, changes in the 340/380 ratio in response to the second exposure to bradykinin in the presence or absence (control) of anesthetics are expressed as a percentage of the first response to bradykinin. Pretreatment with ketamine (10^{-4} M) attenuated both the peak ($68 \pm 5\%$; $P < 0.05$) and sustained ($35 \pm 3\%$; $P < 0.01$) phases when compared with the control group ($92 \pm 8\%$ and $89 \pm 5\%$, respectively) (figs. 6B and 6C). Etomidate (10^{-4} M) also attenuated increases in $[Ca^{2+}]_i$ at the peak ($67 \pm 7\%$; $P < 0.05$) and sustained ($50 \pm 11\%$; $P < 0.01$) phases in response to bradykinin.

In contrast to bradykinin, A23187 (10^{-7} M) induced a monophasic increase in endothelial $[Ca^{2+}]_i$ that was sustained even after washing out with fresh medium (fig. 7). The increase in $[Ca^{2+}]_i$ induced by A23187 was $65 \pm 5\%$ of the sustained increase in $[Ca^{2+}]_i$ in response to bradykinin (10^{-8} M) in the same cell (n = 5). Neither etomidate nor ketamine (10^{-4} M) altered the A23187-induced $[Ca^{2+}]_i$ transient (etomidate = $63 \pm 8\%$; ketamine = $67 \pm 9\%$, n = 5 each).

Table 3. Effects of Anesthetics on Relaxation Responses to Acetylcholine and Bradykinin in the Presence of Inhibitors

	n	IC_{50}	R_{max} (%)
Acetylcholine			
L-NAME + ibuprofen	8	−6.65 ± 0.20	35.3 ± 6.8
+ 10^{-4} M etomidate	9	−5.90 ± 0.20*	36.4 ± 2.9
+ 10^{-4} M ketamine	7	−5.85 ± 0.19†	32.8 ± 6.1
Acetylcholine			
TBA + ibuprofen	6	−6.42 ± 0.11	74.2 ± 2.9
+ 10^{-4} M etomidate	6	−6.02 ± 0.07*	67.1 ± 6.0
+ 10^{-4} M ketamine	6	−6.04 ± 0.10*	58.3 ± 7.3
Bradykinin			
L-NAME + ibuprofen	6	−7.51 ± 0.05	49.7 ± 3.5
+ 10^{-4} M etomidate	6	−7.23 ± 0.15*	29.0 ± 3.9†
+ 10^{-4} M ketamine	6	−7.19 ± 0.05*	30.3 ± 4.4*
Bradykinin			
TBA + ibuprofen	6	−7.72 ± 0.08	59.4 ± 6.9
+ 10^{-4} M etomidate	6	−7.42 ± 0.08*	35.7 ± 7.2
+ 10^{-4} M ketamine	6	−7.34 ± 0.11*	38.1 ± 7.1

* $P < 0.05$, † $P < 0.01$ *versus* without anesthetic in each group.

IC_{50} = drug concentration eliciting 50% of the maximum relaxation response; R_{max} = maximum relaxant response; L-NAME = N^G-nitro-L-arginine methyl-ester; TBA = tetrabutylammonium hydrogen sulfate.

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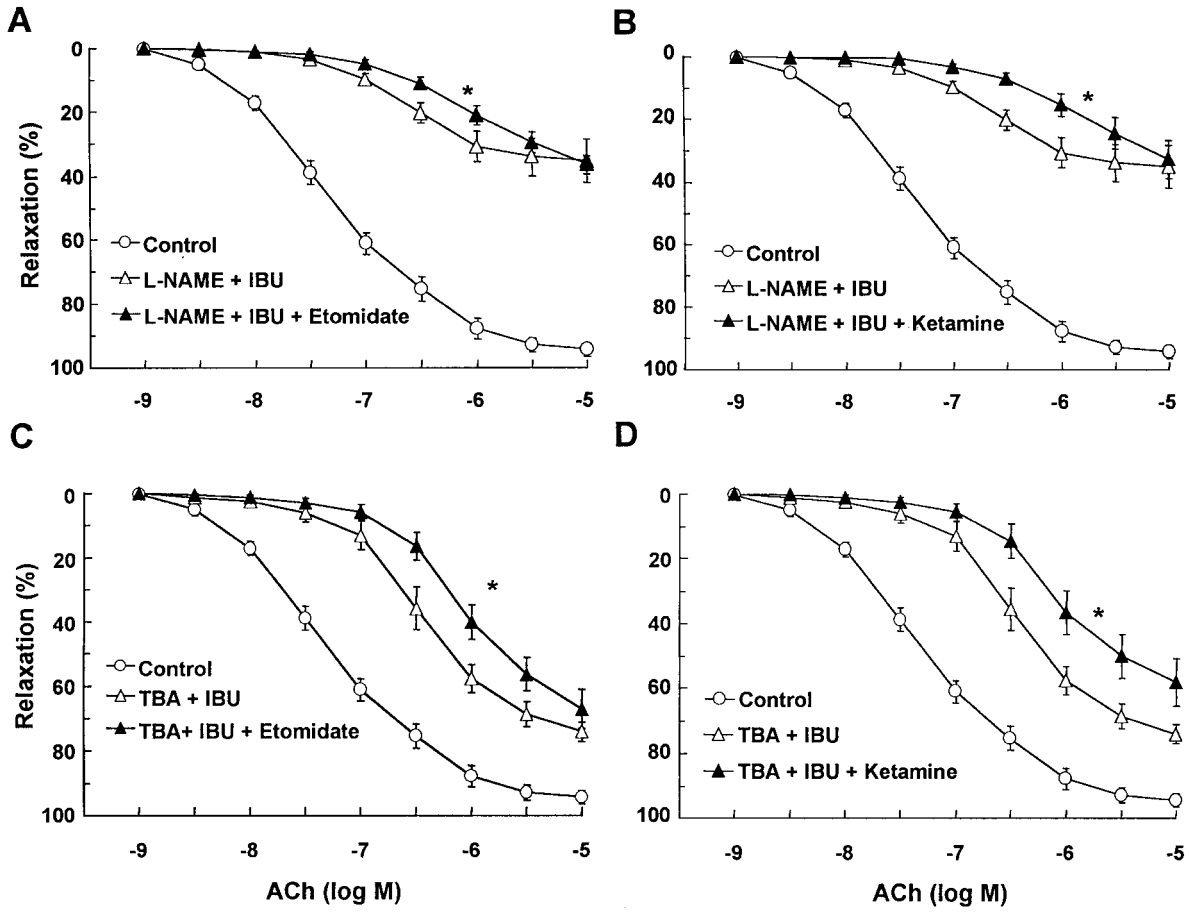

Fig. 4. Concentration–effect curves summarizing the effects of etomidate (10^{-4} M) and ketamine (10^{-4} M) on the L-NAME–resistant (*A* and *B*) and TBA-resistant (*C* and *D*) components of acetylcholine-induced relaxation in ibuprofen (IBU)-pretreated rings. Pulmonary arterial rings were precontracted to the ED_{50} level of tension with phenylephrine. Etomidate and ketamine attenuated (*$P < 0.05$) the L-NAME–resistant and TBA-resistant relaxation.

Discussion

The key findings of our study are as follows. Both etomidate and ketamine attenuated endothelium-dependent pulmonary vasorelaxation in response to the receptor-mediated agonists, acetylcholine and bradykinin, but not the receptor-independent agonist, A23187. The relaxation responses induced by all three of these endothelial activators consisted of NO- and EDHF-mediated components. Etomidate and ketamine attenuated the vasorelaxant responses to acetylcholine and bradykinin by inhibiting both the NO and EDHF components of the response. Finally, both of these anesthetics inhibited the increase in endothelial $[Ca^{2+}]_i$ induced by bradykinin, but not A23187.

The three primary endothelium-derived vasodilators are NO, prostacyclin, and EDHFs. We recently demonstrated that NO and EDHFs are the primary mediators of acetylcholine relaxation in canine pulmonary artery, with prostacyclin only playing a role at high concentrations of acetylcholine.[8] Prostacyclin also mediates a small component of bradykinin and A23187-induced pulmonary vasorelaxation.[9] To focus on the effects of the anesthetics on the NO- and EDHF-mediated components of these three endothelial activators, rings were pretreated with the cyclooxygenase inhibitor, ibuprofen, before administration of L-NAME or TBA. In ibuprofen-pretreated rings, L-NAME and TBA each attenuated the vasorelaxant responses to acetylcholine, bradykinin, and A23187, and combined inhibition abolished these vasorelaxant responses. Thus, in ibuprofen-pretreated rings, the vasorelaxant response to these endothelial activators that remains after TBA is mediated by NO, and the vasorelaxant response that remains after L-NAME is mediated by EDHFs.

Etomidate and ketamine inhibited receptor-mediated (acetylcholine and bradykinin) but not receptor-independent (A23187) endothelium-dependent vasorelaxation. These results indicate that the anesthetics do not exert an inhibitory effect on NO or EDHF activity, because NO and EDHF are common mediators for all three vasodilators. Moreover, the normal vasorelaxant response to SNAP indicates that these anesthetics do not inhibit NO-induced pulmonary vascular smooth muscle cyclic guanosine monophosphate production. Taken to-

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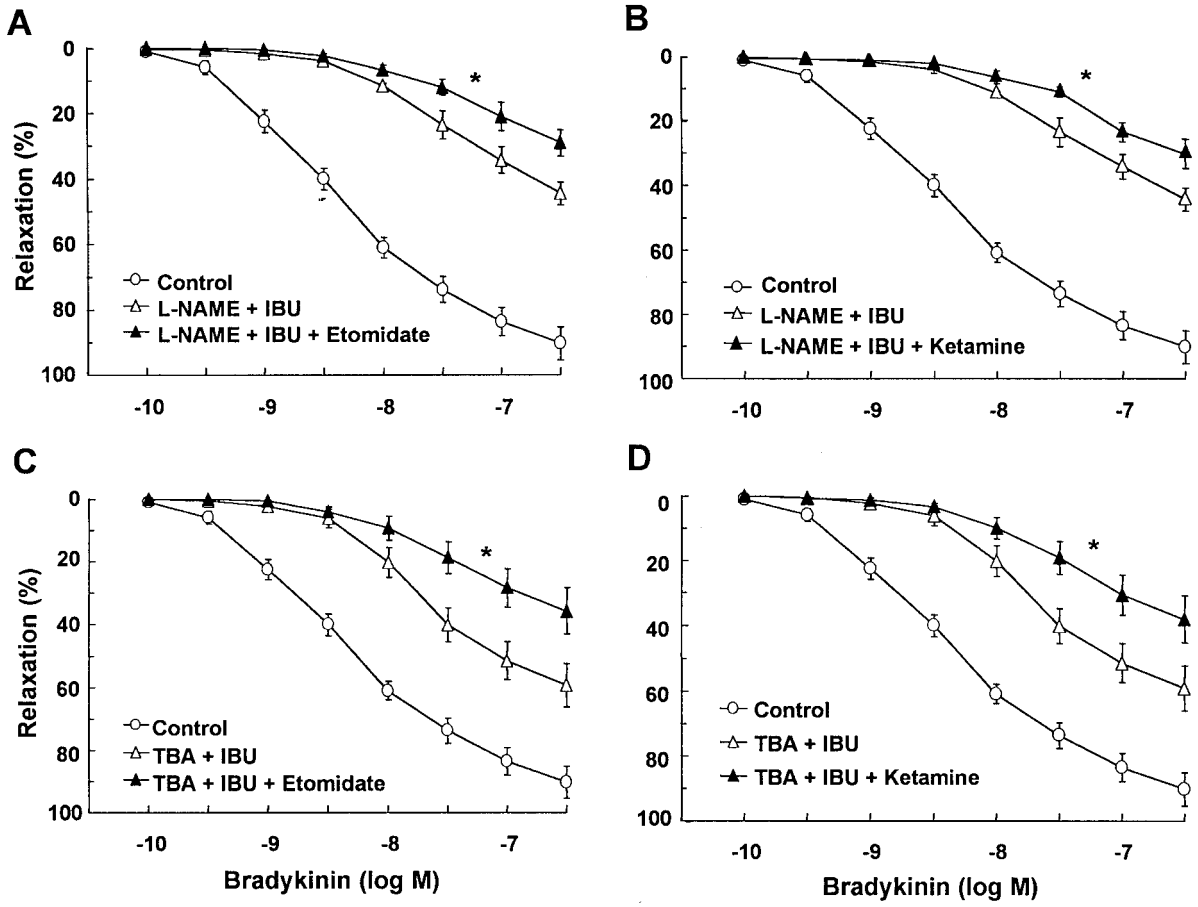

Fig. 5. Concentration–effect curves summarizing the effects of etomidate (10^{-4} M) and ketamine (10^{-4} M) on the L-NAME–resistant (*A* and *B*) and TBA-resistant (*C* and *D*) components of bradykinin-induced relaxation in ibuprofen (IBU)-pretreated rings. Pulmonary arterial rings were precontracted to the ED_{50} level of tension with phenylephrine. Etomidate and ketamine attenuated (*$P < 0.05$) the L-NAME–resistant and TBA-resistant relaxation.

gether, these results indicate that the inhibitory effects of etomidate and ketamine involve endothelial signaling pathways requiring receptor activation.

Etomidate and ketamine inhibited the TBA- and L-NAME–resistant vasorelaxant responses to acetylcholine and bradykinin, which indicates that the anesthetics inhibited both the NO- and EDHF-mediated components of the response, respectively. An increase in endothelial $[Ca^{2+}]_i$ is an essential step in the production of NO and EDHF.[10,11] Thus, we hypothesized that the anesthetics attenuated receptor-mediated relaxation by inhibiting increases in endothelial $[Ca^{2+}]_i$. To test this hypothesis, we assessed the effects of the anesthetics on changes in endothelial $[Ca^{2+}]_i$ induced by bradykinin and A23187. Surprisingly, we were not able to measure consistent changes in endothelial $[Ca^{2+}]_i$ in response to acetylcholine, perhaps reflecting phenotypic changes in receptor activity in these cultured cells. We used concentrations of bradykinin and A23187 that were close to the IC_{50} values calculated from the isometric tension measurements. Bradykinin activates endothelial BK_2 receptors, which stimulates the release of Ca^{2+} from intracellular stores *via* inositol 1,4,5-triphosphate production, as well as transmembrane Ca^{2+} influx from the extracellular space.[12,13] The bradykinin-induced increase in $[Ca^{2+}]_i$ consisted of two phases, an initial rapid increase followed by a gradual decrease to a sustained plateau (fig. 6). The initial phase is likely caused by intracellular Ca^{2+} release, and the sustained phase is caused by Ca^{2+} influx.[14,15] Although volatile anesthetics have been reported to inhibit Ca^{2+} mobilization in response to bradykinin in aortic endothelial cells,[14,16] the effects of intravenous anesthetics on $[Ca^{2+}]_i$ transients in endothelial cells have not been previously investigated. Our observation that etomidate and ketamine inhibited both the initial peak and sustained increases in $[Ca^{2+}]_i$ in response to bradykinin suggests that these anesthetics may alter inositol 1,4,5-triphosphate–mediated Ca^{2+} release from the sarcoplasmic reticulum and receptor-activated Ca^{2+} influx.

Receptor-activated Ca^{2+} influx is not mediated *via* voltage-gated Ca^{2+} channels, because vascular endothelial cells lack functional L-type Ca^{2+} channels.[17] It has been postulated that agonist-induced Ca^{2+} influx in endothelial cells is mediated by capacitative Ca^{2+} entry through nonselective cation channels, where the magni-

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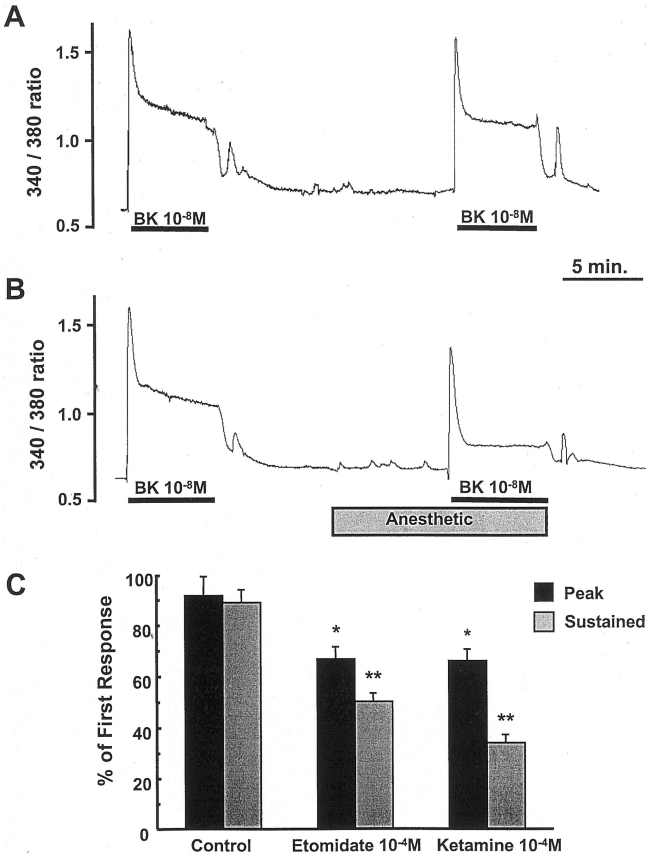

Fig. 6. Changes in intracellular calcium concentration ($[Ca^{2+}]_i$) (340/380 ratio) of cultured bovine pulmonary artery endothelial cells in response to consecutive applications of bradykinin (10^{-8} M) in the absence (*A*) or presence of anesthetic (*B*) (n = 6 each). Peak and sustained increases in $[Ca^{2+}]_i$ in response to the second application of bradykinin were expressed as a percentage of the first response. Etomidate (10^{-4} M) and ketamine (10^{-4} M) attenuated both the peak and sustained increases in endothelial $[Ca^{2+}]_i$ in response to bradykinin (*C*) (*$P < 0.05$, **$P < 0.01$, compared with control).

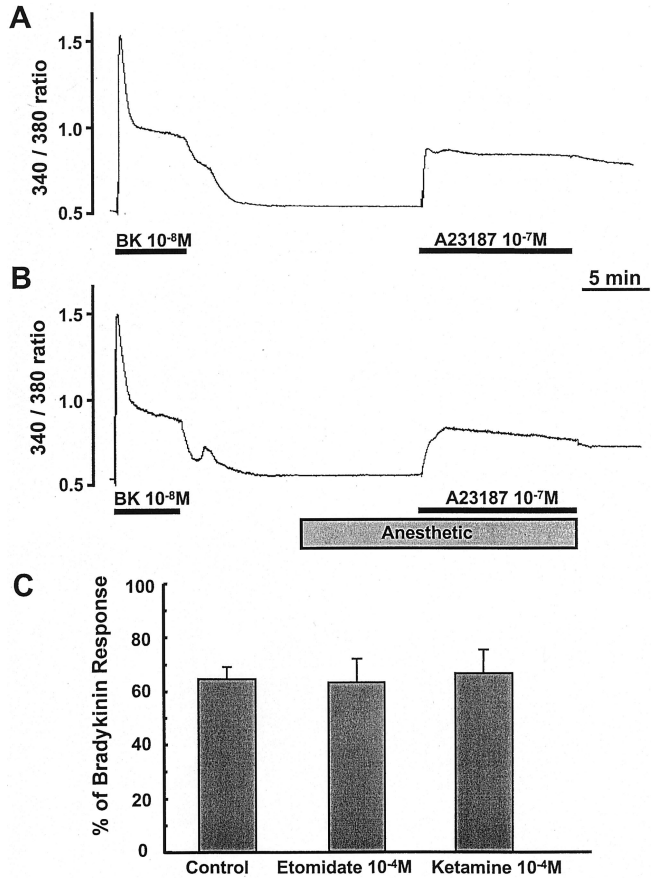

Fig. 7. Changes in $[Ca^{2+}]_i$ of cultured bovine pulmonary artery endothelial cells in response to the calcium ionophore, A23187 (10^{-7} M) in the absence (*A*) or presence of anesthetic (*B*) (n = 5 each). Changes in $[Ca^{2+}]_i$ induced by A23187 were expressed as a percentage of the sustained increase in $[Ca^{2+}]_i$ induced by bradykinin (10^{-8} M) in the same cell. Etomidate (10^{-4} M) and ketamine (10^{-4} M) had no effect on the increase in endothelial $[Ca^{2+}]_i$ in response to A23187 (*C*).

tude of Ca^{2+} entry is regulated by the extent of filling of intracellular Ca^{2+} stores.[17–20] The anesthetics clearly attenuated the initial peak $[Ca^{2+}]_i$ transient in response to bradykinin. This could result in less depletion of intracellular Ca^{2+} stores, thereby causing an indirect anesthesia-induced inhibitory effect on capacitative Ca^{2+} entry. Alternatively, the anesthetics could directly inhibit capacitative Ca^{2+} entry *via* effects on nonselective Ca^{2+} channels. In preliminary studies, we have observed that propofol attenuates capacitative Ca^{2+} entry in pulmonary artery smooth muscle cells.[21] In contrast to receptor activation, A23187 increases $[Ca^{2+}]_i$ by increasing the Ca^{2+} permeability of the cell membrane, as well as intracellular organelles containing Ca^{2+}. Neither anesthetic had an effect on the A23187 vasorelaxation response or the A23187-induced increase in $[Ca^{2+}]_i$. Taken together, it seems likely that etomidate and ketamine inhibited the pulmonary vasorelaxant response to acetylcholine and bradykinin by reducing the increase in endothelial $[Ca^{2+}]_i$ in response to receptor activation, and therefore reducing the synthesis of NO and EDHF.

Volatile anesthetics have been reported to inhibit both the NO and EDHF components of acetylcholine relaxation in rat[22] and rabbit[23] mesenteric artery and rabbit carotid artery.[24] Etomidate and thiopental attenuated the EDHF component of acetylcholine- and bradykinin-induced relaxation in human renal artery[25] and the rat coronary circulation,[26] whereas propofol and ketamine inhibited the NO component of acetylcholine relaxation in rat aorta.[27] In contrast to the systemic circulation, very little is known about the effects of intravenous anesthetics on endothelium-dependent relaxation in the pulmonary circulation. Our laboratory has reported that propofol attenuates the NO and EDHF components of acetylcholine relaxation, but not bradykinin relaxation, in canine pulmonary arterial rings.[8] The effects of etomidate and ketamine on endothelium-dependent relaxation in the pulmonary circulation have not been previously investigated.

The plasma concentration of ketamine after the intra-

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venous administration of 2 mg/kg has been reported to be 1.1×10^{-4} M.[28] Thus, the effects of ketamine on pulmonary vasorelaxation and the endothelial $[Ca^{2+}]_i$ transient were apparent at clinically relevant concentrations. The peak plasma concentration of etomidate during induction of general anesthesia is approximately 10^{-5} M.[29,30] Because 75% of etomidate is bound to plasma protein,[31] the free plasma concentration of etomidate is likely to be less than 10^{-5} M. Thus, the effects of etomidate reported in this study appear to be significant only at supraclinical concentrations.

In summary, etomidate and ketamine selectively attenuated receptor-mediated endothelium-dependent pulmonary vasorelaxation by inhibiting the NO and EDHF components of the response. These anesthetic effects appear to be caused by inhibition of the endothelial $[Ca^{2+}]_i$ transient in response to receptor activation.

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