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Inhibitory Effects of Etomidate and Ketamine on Adenosine Triphosphate–Sensitive Potassium Channel Relaxation in Canine Pulmonary Artery

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Background: **The authors recently demonstrated that etomidate and ketamine attenuated endothelium-dependent pulmonary vasorelaxation mediated by nitric oxide and Ca^{2+}-activated K^+ channels. In the current study, they tested the hypothesis that these intravenous anesthetics inhibit pulmonary vasorelaxation mediated by adenosine triphosphate–sensitive potassium (K^+_{ATP}) channel activation.**

Methods: **Endothelium intact and denuded pulmonary arterial rings were suspended in organ chambers for isometric tension recording. The effects of etomidate (5×10^{-6} and 5×10^{-5} M) and ketamine (5×10^{-5} and 10^{-4} M) on vasorelaxation responses to lemakalim (K^+_{ATP} channel activator), prostacyclin, and papaverine were assessed in phenylephrine-precontracted rings. The effect of cyclooxygenase inhibition with indomethacin was assessed in some protocols.**

Results: **Etomidate (5×10^{-6} M) only inhibited the vasorelaxant response to lemakalim in endothelium intact rings, whereas a higher concentration of etomidate (5×10^{-5} M) inhibited relaxation in both intact and endothelium-denuded rings. Pretreatment with indomethacin abolished the endothelium-dependent attenuation of lemakalim-induced relaxation caused by etomidate. Ketamine (5×10^{-5} and 10^{-4} M) inhibited the relaxation response to lemakalim to the same extent in both endothelium-intact and -denuded rings, and this effect was not prevented by indomethacin pretreatment. Etomidate and ketamine had no effect on the relaxation responses to prostacyclin or papaverine.**

Conclusions: **These results indicate that etomidate, but not ketamine, attenuates the endothelium-dependent component of lemakalim-induced pulmonary vasorelaxation *via* an inhibitory effect on the cyclooxygenase pathway. Both anesthetics inhibit K^+_{ATP}-mediated pulmonary vasorelaxation *via* a direct effect on pulmonary vascular smooth muscle.**

ADENOSINE triphosphate-sensitive potassium (K^+_{ATP}) channels have been identified in pulmonary arterial smooth muscle.[1] K^+_{ATP} channel activation results in membrane hyperpolarization and a decrease in Ca^{2+} influx through voltage-dependent Ca^{2+} channels, which in turn reduces vascular smooth muscle tone. K^+_{ATP} channels are known to be activated by hyperpolarizing agonists, such as diazoxide, pinacidil, cromakalim, and its enantiomer, lemakalim.[2,3] K^+_{ATP} channel agonists are used as therapeutic agents in a variety of cardiovascular diseases, including essential hypertension, angina pectoris, myocardial ischemia, and cerebral vascular disease.[4] Investigators in our laboratory and others have demonstrated that K^+_{ATP} channel agonists can cause marked pulmonary vasodilation, which is reversed by glibenclamide, a specific K^+_{ATP} channel inhibitor.[5–13] Moreover, endogenous K^+_{ATP} channel–induced vasodilation is functionally significant, because it has been shown to modulate the pulmonary vasoconstrictor response to hypoxia[14] and systemic hypotension.[7]

K^+_{ATP} channels are present not only in vascular smooth muscle cells, but also have been demonstrated in systemic vascular endothelial cells.[15,16] In endothelial cells, K^+_{ATP} channel activation results in hyperpolarization, an increase in Ca^{2+} influx, and the production of endothelium-derived vasodilators, such as nitric oxide and prostacyclin.[17,18] We have recently demonstrated that lemakalim-induced pulmonary vasorelaxation involves both endothelium-dependent and vascular smooth muscle components.[19] The endothelium-dependent component of lemakalim-induced vasorelaxation is mediated by a vasodilator metabolite of the cyclooxygenase pathway.[19] Moreover, K^+_{ATP} channels mediate a synergistic interaction between nitric oxide and prostacyclin in response to some endothelium-dependent agonists (*e.g.*, bradykinin).[20]

Etomidate and ketamine are intravenous anesthetics that are recognized to have minimal effects on clinically measured endpoints of cardiovascular function. Thus, they are often used as induction agents in patients with hemodynamic instability. Our laboratory has recently demonstrated that etomidate and ketamine attenuated pulmonary vasorelaxant responses to bradykinin and acetylcholine by inhibiting both nitric oxide and Ca^{2+}-activated potassium channel (K^+_{Ca})-mediated components of the response.[21] However, the effects of these anesthetics on K^+_{ATP}-mediated pulmonary vasorelaxation have not been investigated. An anesthesia-induced attenuation of pulmonary vasodilator mechanisms could increase right ventricular afterload, which could adversely affect patients with right ventricular dysfunction.

The first goal of this *in vitro* study was to investigate the effects of etomidate and ketamine on the pulmonary vasorelaxant response to the K^+_{ATP} agonist lemakalim. Based on our previous results,[19,21] we tested the hypothesis that these anesthetics would attenuate the endothelium-dependent component of lemakalim-induced relaxation. We also tested the hypothesis that the inhibitory effects of etomidate and ketamine involved a decrease in

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the production or activity of vasodilator metabolites of the cyclooxygenase pathway.

Material and Methods

All experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee of the The Cleveland Clinic Foundation.

Preparation of Pulmonary Arterial Rings

Thirty healthy mongrel dogs weighing 20–30 kg were anesthetized with intravenous pentobarbital sodium (30 mg/kg) and intravenous fentanyl citrate (15 μg/kg). After tracheal intubation, the lungs were mechanically ventilated. A catheter was placed in the right femoral artery, heparin (8,000 units) was administered intravenously, and the dogs were exsanguinated by controlled hemorrhage. A left lateral thoracotomy was performed through the fifth intercostal space, and the heart was arrested 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 intentionally 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% O_2 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 optimal resting tension. Optimal resting tension was defined as the minimum amount of stretch required to achieve the largest contractile response to 40 mM KCl and was determined in preliminary experiments to be 5 g for the size of the 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. This allowed us to calculate the dose required to achieve 50% of the maximum response to phenylephrine (ED_{50}) in each ring. 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, incremental concentrations of

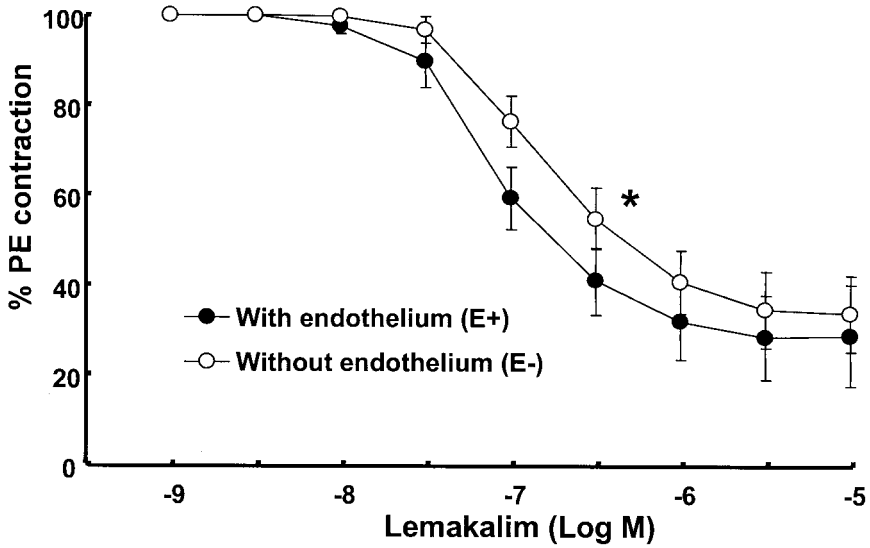

Fig. 1. Effect of endothelial denudation on lemakalim-induced vasorelaxation in isolated canine pulmonary arteries. In this and other figures, vasorelaxation in response to lemakalim is expressed as a percentage of precontraction to 50% of the maximal contractile response to phenylephrine (ED_{50} level). Lemakalim-induced vasorelaxation was attenuated (*$P < 0.05$) in endothelium-denuded rings compared with endothelium-intact rings. Values are presented as mean ± SD in all figures.

Table 1. Effects of Etomidate and Ketamine on Lemakalim-induced Vasorelaxation in Isolated Canine Pulmonary Arterial Rings

	Endothelium	N	Log IC_{50}	Maximal Relaxation, %
No drug	+	24	−7.04 ± 0.10	71.7 ± 9.4
	−	19	−6.74 ± 0.10*	66.5 ± 8.5
Etomidate, 5×10^{-6} M	+	8	−6.91 ± 0.08†	67.6 ± 13.3
	−	8	−6.76 ± 0.22	65.3 ± 10.2
Etomidate, 5×10^{-5} M	+	8	−6.89 ± 0.06†	52.8 ± 11.5†‡
	−	6	−6.75 ± 0.17	53.1 ± 8.3†‡
Ketamine, 5×10^{-5} M	+	16	−6.66 ± 0.20†	62.1 ± 17.0
	−	19	−6.53 ± 0.17†	51.3 ± 17.5†
Ketamine, 10^{-4} M	+	6	−6.59 ± 0.13†	50.2 ± 14.5†
	−	7	−6.43 ± 0.13†	45.1 ± 14.9†

Values are mean ± SD.

$P < 0.05$ *versus* * endothelium (+) in the no-drug condition; † no drug; ‡ etomidate 5×10^{-6} M.

lemakalim (10^{-9} to 10^{-5} M) were added to the organ bath to generate the concentration–response relations for both endothelium-intact and -denuded rings. The effects of etomidate (5×10^{-6} and 5×10^{-5} M) and ketamine (5×10^{-5} and 10^{-4} M) on the concentration–response curves for lemakalim were assessed by comparing vasorelaxant responses in the presence and absence of the anesthetics. The anesthetics were added directly to the organ bath 15 min before phenylephrine contraction. The effect of the vehicle for etomidate (propylene glycol), at a dose (27×10^{-3} M) equivalent to that administered with the highest concentration of etomidate, on the lemakalim concentration–response relation was also assessed.

To investigate the role of the cyclooxygenase pathway on lemakalim-induced vasorelaxation, the lemakalim concentration–response relation in endothelium-intact rings was assessed 30 min after the cyclooxygenase inhibitor indomethacin (10^{-5} M) was added to the bath, either alone or following combined pretreatment with etomidate (5×10^{-6} M). In a similar fashion, lemakalim concentration–response curves were generated in endothelium-intact and -denuded rings following indomethacin pretreatment alone or following combined pretreatment with ketamine (5×10^{-5} M).

Finally, the effects of etomidate and ketamine on the concentration–response relations for prostacyclin (cyclooxygenase metabolite: 10^{-9} to 10^{-6} M) and papaverine (nonspecific vasorelaxant: 10^{-7} to 5×10^{-5} M) were investigated in endothelium-intact or -denuded rings precontracted with phenylephrine.

Drugs and Solutions

All drugs were of the highest purity commercially available: lemakalim (BRL 38227, a gift from SmithKline Beecham, Herts, United Kingdom), indomethacin, phenylephrine HCl, acetylcholine chloride, propranolol HCl, papaverine, propylene glycol (Sigma Chemical, St. Louis, MO), etomidate (Bedford Laboratories, Bedford, OH), ketamine HCl (Parke-Davis, Morris Plains, NJ), and prostacyclin (Cayman Chemical, Ann Arbor, MI). All concentrations are expressed as the final molar concentration in the organ chamber. Lemakalim was dissolved in 95% ethanol and diluted in distilled water (final organ chamber ethanol concentration, 0.054%). Indomethacin was dissolved in $NaHCO_3$ and diluted in distilled water (final organ chamber $NaHCO_3$ concentration, 2×10^{-4} M). The vehicles had no effect on relaxation responses at the concentrations used in these studies.[22] All other drugs were dissolved in distilled water.

Data Analysis

Values are expressed as means ± 1 SD. Vasorelaxant responses to lemakalim, prostacyclin, and papaverine are expressed as the percentage relaxation of the precontraction induced by the phenylephrine ED_{50} dose. The logarithm of drug concentration eliciting 50% of the maximum relaxation response (IC_{50}) was calculated by nonlinear regression analysis by fitting the dose–response relation for each vasorelaxant to a sigmoidal curve using commercially available software (Prism Version 3.02; Graph Pad Software, San Diego, CA). The maximum relaxant response (R_{max}) was measured as the maximal response to each vasorelaxant, with R_{max} = 100% indicating complete reversal of the phenylephrine ED_{50} contraction. Statistical analysis was performed using Student *t* test for paired samples or one-way analysis of variance followed by Scheffé F test. Differences were considered statistically significant at $P < 0.05$. N refers to the number of dogs from which pulmonary arterial rings were studied in each protocol. Multiple rings from the same dog were averaged, so that all dogs were weighted equally.

Results

Effect of Endothelial Denudation on Lemakalim-induced Pulmonary Vasorelaxation

To confirm our previous findings,[19] we assessed the effects of removing the endothelium on the lemakalim concentration–response relation. Endothelial denudation was verified by the absence of a pulmonary vasorelaxant response to acetylcholine (10^{-6} M). As summarized

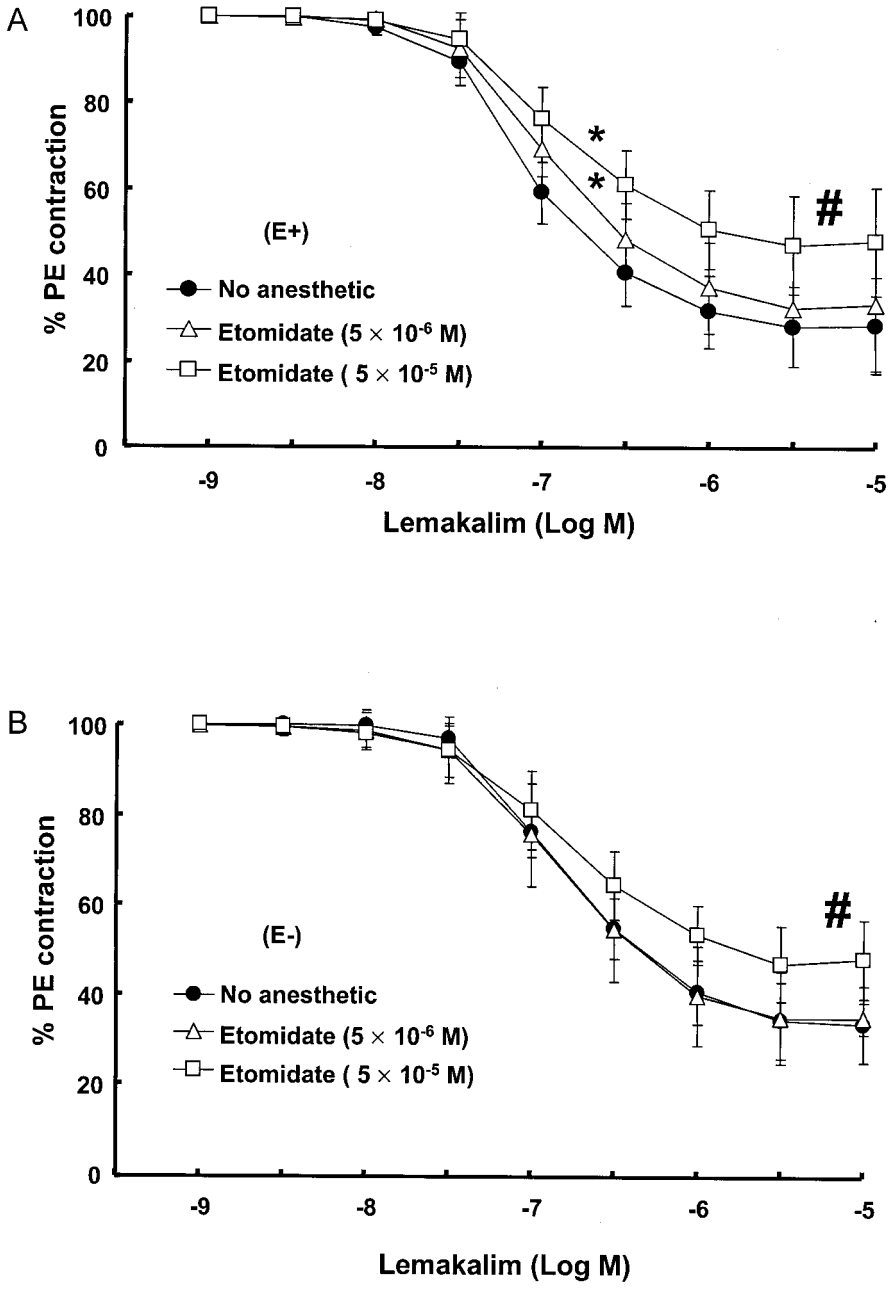

Fig. 2. (*A*) Effect of etomidate on lemakalim-induced vasorelaxation in endothelium-intact (E+) pulmonary arterial rings. Etomidate attenuated (P* < 0.05) lemakalim-induced vasorelaxation compared with rings without etomidate. Etomidate (5×10^{-5} M) also attenuated (#*P* < 0.05) the maximal relaxation response (R_{max}) to lemakalim. (*B*) Effect of etomidate on lemakalim-induced relaxation in endothelium-denuded (E−) pulmonary arterial rings. Etomidate (5×10^{-6} M) did not alter lemakalim-induced vasorelaxation (IC_{50}) compared with rings without etomidate, but etomidate (5×10^{-5} M) attenuated (#*P* < 0.05) maximal relaxation induced by lemakalim.**

in figure 1 and table 1, lemakalim-induced pulmonary vasorelaxation was attenuated in endothelium-denuded rings compared with intact rings. These results indicate that the K^+_{ATP} agonist causes vasorelaxation that involves both endothelium-dependent and vascular smooth muscle components.

Effect of Etomidate on Lemakalim-induced Pulmonary Vasorelaxation

In endothelium-intact rings, etomidate caused a dose-dependent rightward shift in the lemakalim concentration–response relation (fig. 2A). Both concentrations of etomidate (5×10^{-6} and 5×10^{-5} M) increased the IC_{50} for lemakalim (table 1), and the highest concentration of etomidate decreased the maximum relaxation response (R_{max}). In endothelium-denuded rings (fig. 2B), low-dose etomidate had no effect, whereas high-dose etomidate attenuated lemakalim-induced relaxation, as reflected by a decrease in R_{max} (table 1). The vehicle for etomidate, propylene glycol, had no effect on the pulmonary relaxation response to lemakalim in either endothelium-intact or -denuded rings (table 2).

Effect of Ketamine on Lemakalim-induced Pulmonary Vasorelaxation

Figure 3 summarizes the effects of ketamine on lemakalim-induced relaxation. Ketamine caused a dose-

Table 2. Effects of Propylene Glycol, Indomethacin, and Indomethacin Plus Anesthetics on Lemakalim-induced Relaxation in Isolated Canine Pulmonary Arterial Rings

	Endothelium	N	Log IC_{50}	Maximal Relaxation, %
No drug	+	24	−7.04 ± 0.10	71.8 ± 9.4
	−	19	−6.74 ± 0.10*	66.5 ± 8.5
Propylene glycol, 27×10^{-3} M	+	6	−7.06 ± 0.10	69.0 ± 5.5
	−	6	−6.80 ± 0.06*	63.7 ± 10.5
Indomethacin, 10^{-5} M	+	6	−6.78 ± 0.12†	75.8 ± 9.0
	−	6	−6.85 ± 0.15	81.1 ± 8.6
Indomethacin (10^{-5} M) + etomidate (5×10^{-6} M)	+	6	−6.71 ± 0.12†	73.8 ± 8.1
Indomethacin, 10^{-5} M) + ketamine (5×10^{-5} M)	+	10	−6.44 ± 0.17‡	67.5 ± 8.9
	−	10	−6.38 ± 0.20‡	57.9 ± 10.4‡

Values are mean ± SD.

$P < 0.05$ *versus* * endothelium (+); † no drug; ‡ indomethacin.

dependent attenuation of lemakalim relaxation that was similar in magnitude in endothelium-intact (fig. 3A) and endothelium-denuded (fig. 3B) rings. The IC_{50} values for lemakalim were increased, and the R_{max} values were decreased in endothelium-intact and -denuded rings (table 1).

Effect of Etomidate and Ketamine on Papaverine-induced Pulmonary Vasorelaxation

We tested the hypothesis that the inhibitory effects of etomidate and ketamine on lemakalim-induced relaxation were caused by nonspecific effects of these anesthetics on pulmonary vasorelaxant activity. The effects of high-dose etomidate (5×10^{-5} M) and ketamine (10^{-4} M) on the pulmonary vasorelaxant response to the nonspecific vasodilator, papaverine, are summarized in figure 4. Neither anesthetic had an effect on papaverine-induced relaxation in either endothelium-intact (fig. 4A) or endothelium-denuded (fig. 4B) rings.

Effect of Cyclooxygenase Inhibition on Etomidate- and Ketamine-induced Changes in Lemakalim Vasorelaxation

We tested the hypothesis that low concentrations of etomidate (5×10^{-6} M) and ketamine (5×10^{-5} M) attenuated lemakalim-induced relaxation by inhibiting vasodilator metabolites of the cyclooxygenase pathway in endothelium-intact rings. Indomethacin alone caused a rightward shift in the lemakalim concentration–response relation, by increasing the IC_{50} for lemakalim with no effect on R_{max} (table 2). In indomethacin-pretreated rings, etomidate no longer had an inhibitory effect on lemakalim relaxation (fig. 5A and table 2). In contrast, ketamine continued to inhibit lemakalim-induced relaxation following cyclooxygenase inhibition (fig. 5B and table 2). Ketamine (5×10^{-5} M) also attenuated lemakalim-induced relaxation in endothelium-denuded rings pretreated with indomethacin (table 2).

Effect of Etomidate and Ketamine on Prostacyclin-induced Pulmonary Vasorelaxation

To further investigate the role of the cyclooxygenase pathway in mediating the etomidate-induced attenuation of lemakalim relaxation, we tested the hypothesis that etomidate inhibits the vasorelaxant activity of cyclooxygenase metabolites. The effect of etomidate (5×10^{-6} M) on the prostacyclin concentration–response relation is summarized in figure 6A). Etomidate had no effect on prostacyclin-induced pulmonary vasorelaxation. Ketamine also had no effect on the relaxation response to prostacyclin (fig. 6B).

Discussion

Despite their widespread use as induction agents, we believe this is the first study to assess the effects of etomidate and ketamine on K^+_{ATP}-mediated pulmonary vasorelaxation. Our results indicate that etomidate, but not ketamine, attenuates the endothelium-dependent component of K^+_{ATP}-mediated pulmonary vasorelaxation *via* an inhibitory effect on the cyclooxygenase pathway. On the other hand, both anesthetics exert inhibitory effects on this important vasodilator pathway by directly altering pulmonary vascular smooth muscle function.

As in previous *in vivo*[5–7] and *in vitro*[19] studies, we used lemakalim to activate K^+_{ATP} channels in the current study. Lemakalim is the (−) enantiomer of cromakalim and is 100–200 times more potent and also more selective than cromakalim for K^+_{ATP} channel activation.[23] Lemakalim mimics the membrane hyperpolarizing effect of intracellu-

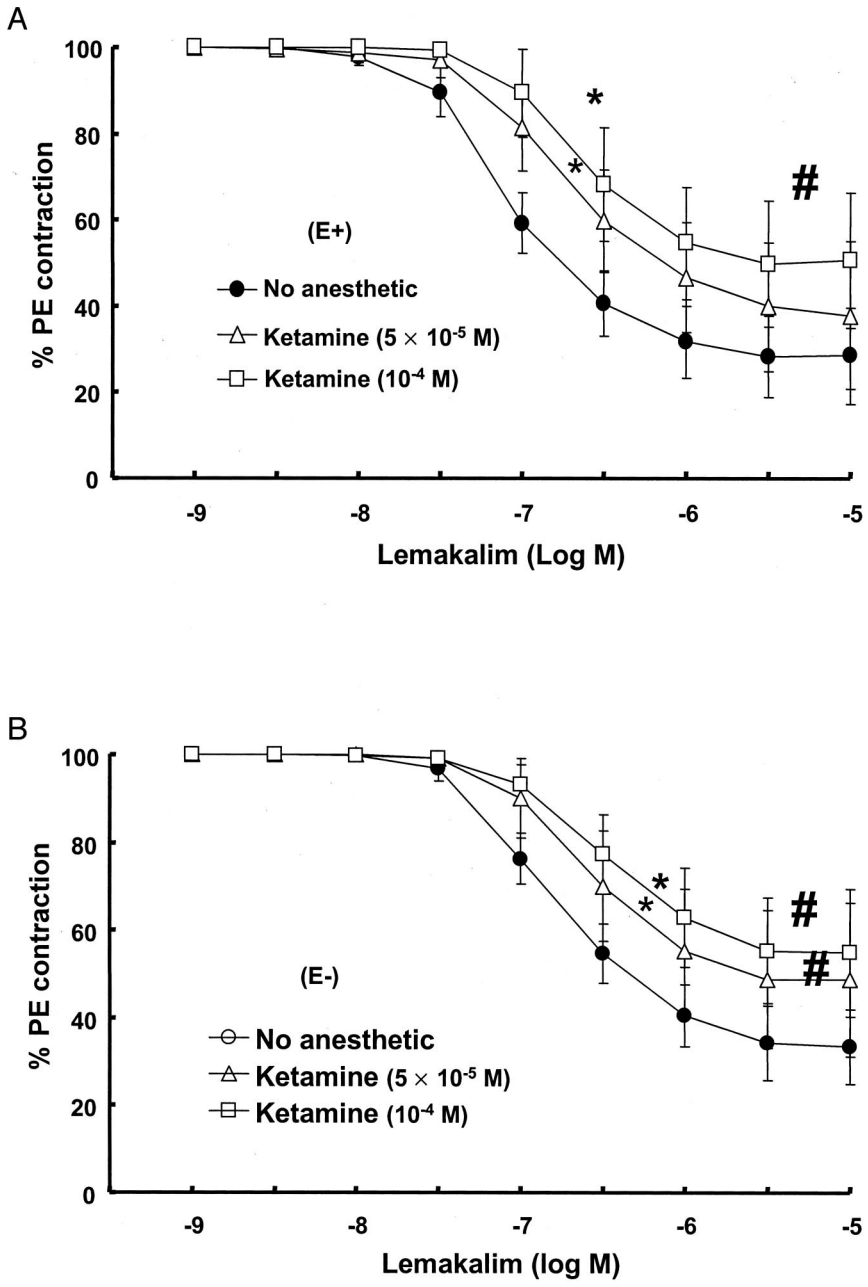

Fig. 3. (*A*) Effect of ketamine on lemakalim-induced vasorelaxation in endothelium-intact (E+) pulmonary arterial rings. Ketamine (5×10^{-5}, 10^{-4} M) attenuated (P* < 0.05) lemakalim-induced relaxation compared with rings without ketamine. Ketamine (10^{-4} M) also attenuated (#*P* < 0.05) maximal vasorelaxation induced by lemakalim. (*B*) Effect of ketamine on lemakalim-induced relaxation in endothelium-denuded (E−) pulmonary arterial rings. Ketamine (5×10^{-5}, 10^{-4} M) attenuated lemakalim-induced vasorelaxation (IC_{50}: **P* < 0.05; R_{max}: #*P* < 0.05).**

lar ATP depletion in pulmonary artery smooth muscle cells, and this effect is reversed by the K^+_{ATP} channel inhibitor, glybenclamide.[1] Lemakalim-induced pulmonary vasorelaxation is also inhibited by glybenclamide.[19] As we have previously reported,[19] the results shown in figure 1 clearly demonstrate that K^+_{ATP}-mediated pulmonary vasorelaxation involves both endothelium-dependent and vascular smooth muscle components.

Activation of vascular smooth muscle K^+_{ATP} channels with hyperpolarizing agents such as lemakalim results in membrane hyperpolarization, a consequent reduction in Ca^{2+} influx though voltage-dependent Ca^{2+} channels, and a decrease in vasomotor tone. K^+_{ATP} channels are also present in at least some systemic vascular endothelial cells,[15,16] although they have not been definitively identified in pulmonary arterial endothelial cells. In contrast to vascular smooth muscle cells, K^+_{ATP}-induced hyperpolarization of endothelial cells results in an increase in Ca^{2+} influx,[17,18] which could stimulate the production of endothelium-derived relaxing factors such as nitric oxide and prostacyclin. We previously demonstrated that the endothelium-dependent component of lemakalim-induced pulmonary vasorelaxation requires the activity of the cyclooxygenase pathway but is independent of nitric oxide synthase activity.[19] In the current study, cyclooxygenase pathway inhibition with indomethacin caused a rightward shift in the lemakalim concentration–response relation, which confirms that this pathway mediates the endothelium-dependent component of lemakalim-induced pulmonary vasorelaxation.

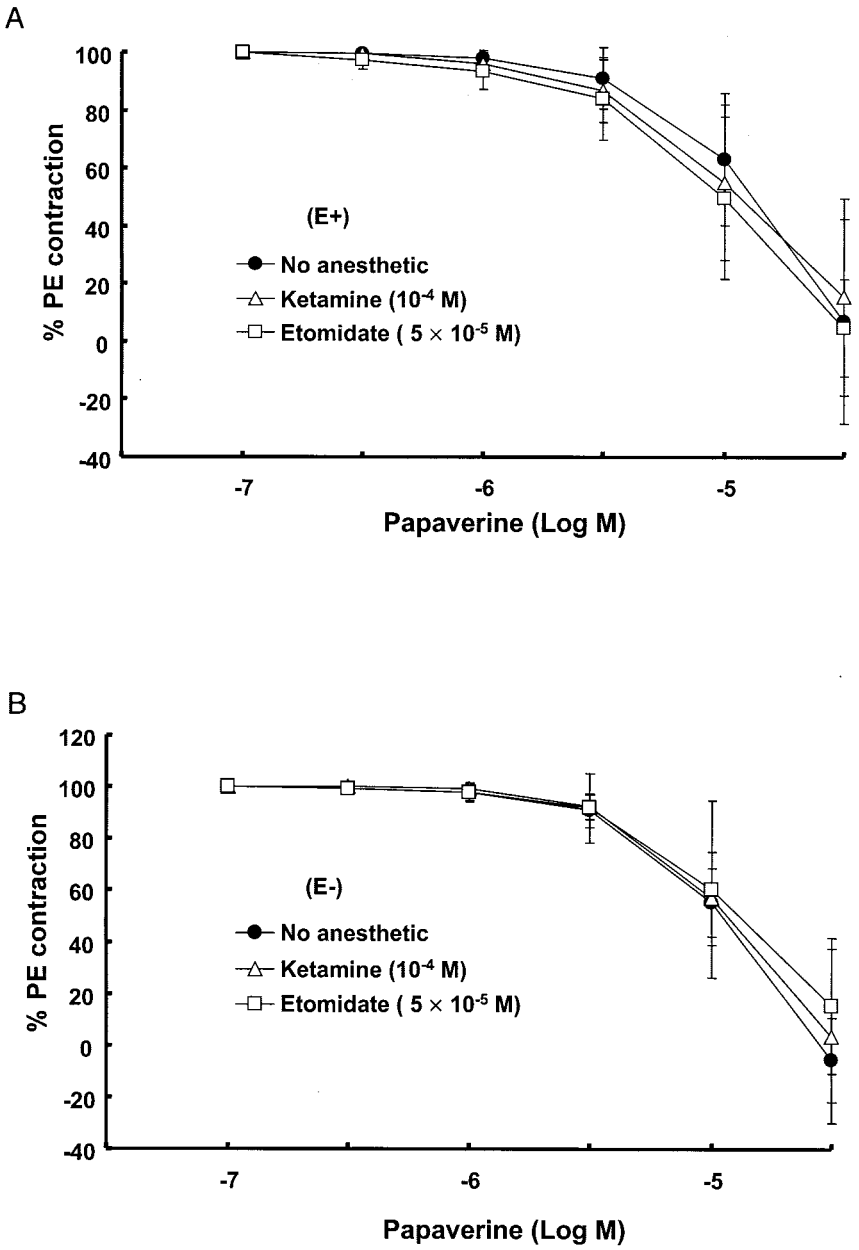

Fig. 4. Effect of etomidate and ketamine on papaverine-induced vasorelaxation in endothelium-intact (E+; *A*) and endothelium-denuded (E−; *B*) pulmonary arterial rings. Etomidate and ketamine did not alter papaverine-induced vasorelaxation compared with rings without anesthetic in either intact or denuded rings.

Both etomidate and ketamine attenuated the pulmonary vasorelaxant response to lemakalim. This inhibitory effect of the anesthetics was not caused by a nonspecific reduction in pulmonary vasorelaxant activity, because the vasorelaxant response to papaverine was not altered by either anesthetic. At a concentration of 5×10^{-6} M, etomidate only inhibited the vasorelaxant response to lemakalim in endothelium-intact rings, whereas 5×10^{-5} M etomidate inhibited relaxation in both intact and endothelium-denuded rings. Thus, the effects of etomidate on lemakalim-induced pulmonary vasorelaxation are concentration-dependent. The endothelium-dependent inhibitory effect of the low concentration of etomidate was abolished by indomethacin. It would appear that the low concentration of etomidate selectivity inhibits lemakalim vasorelaxation mediated by endothelium-derived metabolites of the cyclooxygenase pathway, whereas the higher concentration of etomidate also has an inhibitory effect on the vascular smooth muscle component of lemakalim vasorelaxation. Etomidate does not appear to exert its inhibitory effect on the activity of vasodilator metabolites of the cyclooxygenase pathway, because it had no effect on the pulmonary vasorelaxant response to prostacyclin in endothelium-denuded rings. The vehicle for etomidate, propylene glycol, also had no effect on the pulmonary vasorelaxant response to lemakalim in either endothelium-intact or -denuded rings.

In contrast to etomidate, ketamine attenuated lemakalim-induced pulmonary vasorelaxation to the same extent in both endothelium-intact and -denuded rings. Moreover, the ketamine-induced attenuation of lemakalim vasorelaxation was still observed following

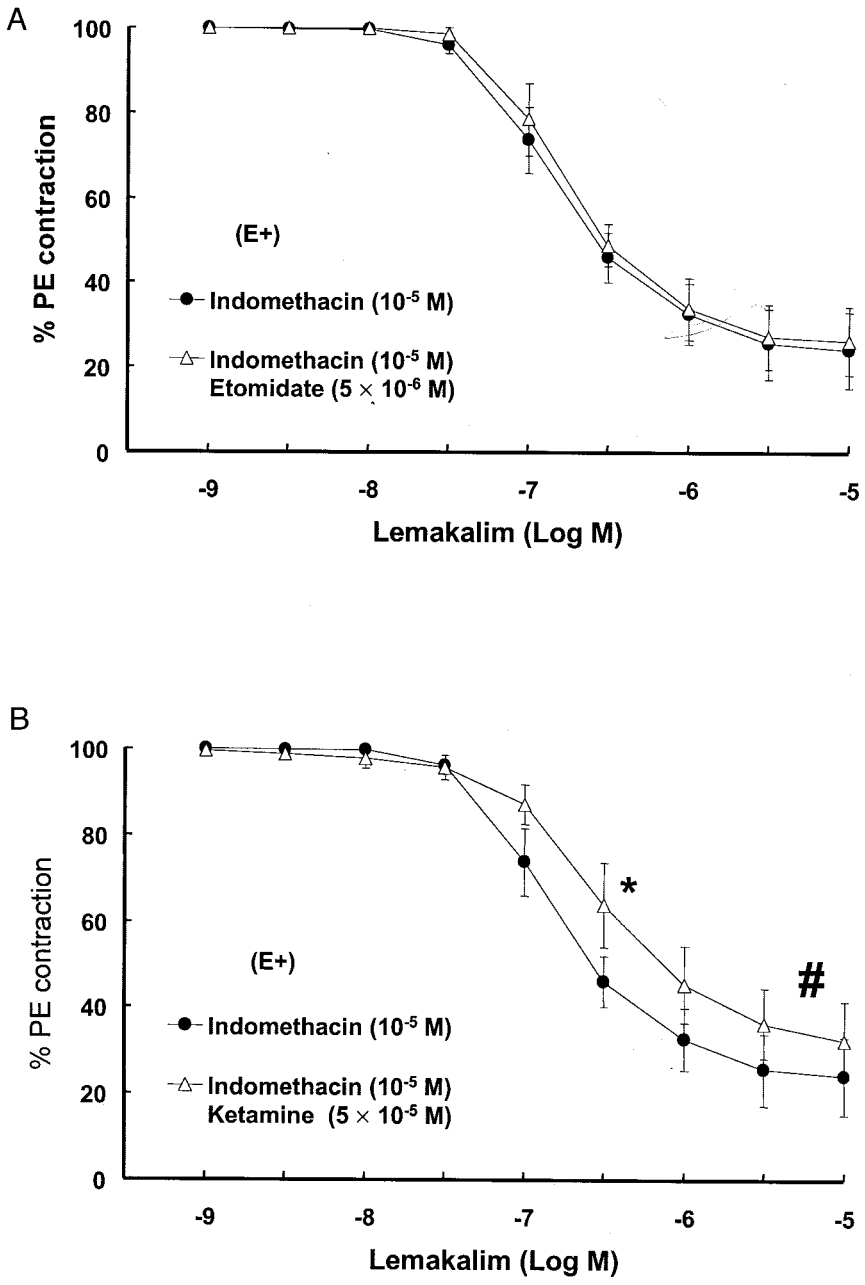

Fig. 5. (*A*) Effect of indomethacin on etomidate-induced attenuation of the pulmonary vasorelaxant response to lemakalim in endothelium-intact (E+) rings. Indomethacin pretreatment abolished the etomidate-induced attenuation of lemakalim vasorelaxation. (*B*) Effect of indomethacin on ketamine-induced attenuation of the pulmonary vasorelaxant response to lemakalim in endothelium-intact (E+) rings. The ketamine-induced attenuation (IC_{50}: $^*P < 0.05$; R_{max}: $\#P < 0.05$) of lemakalim vasorelaxation was still observed in indomethacin-pretreated rings.

cyclooxygenase inhibition in both endothelium-intact and -denuded rings. Ketamine also had no effect in the pulmonary vasorelaxant response to prostacyclin in endothelium-denuded rings. These results suggest that ketamine directly inhibits the vascular smooth muscle component of lemakalim-induced vasorelaxation, and this effect is independent of the cyclooxygenase pathway.

We recently reported that etomidate and ketamine attenuated pulmonary vasorelaxant responses to the endothelium-dependent agonists, bradykinin and acetylcholine, by inhibiting both the nitric oxide and EDHF components of the response.[21] In contrast, these anesthetics had no effect on the vasorelaxant response to the receptor-independent endothelial agonist, A23187, or the nitric oxide donor, SNAP.[21] Both anesthetics attenuated increases in endothelial intracellular Ca^{2+} concentration in response to bradykinin but had no effect on the response to A23187.[21] Taken together, these results imply that etomidate and ketamine attenuated the vasorelaxant responses to acetylcholine and bradykinin by inhibiting the production, but not the activity, of the endothelium-derived relaxing factors, nitric oxide and EDHF. In the current study, it is possible that etomidate inhibited a lemakalim-induced increase in endothelial Ca^{2+} concentration, which in turn decreased the production of a vasodilator metabolite of the cyclooxygenase pathway. One possible mechanism is an etomidate-

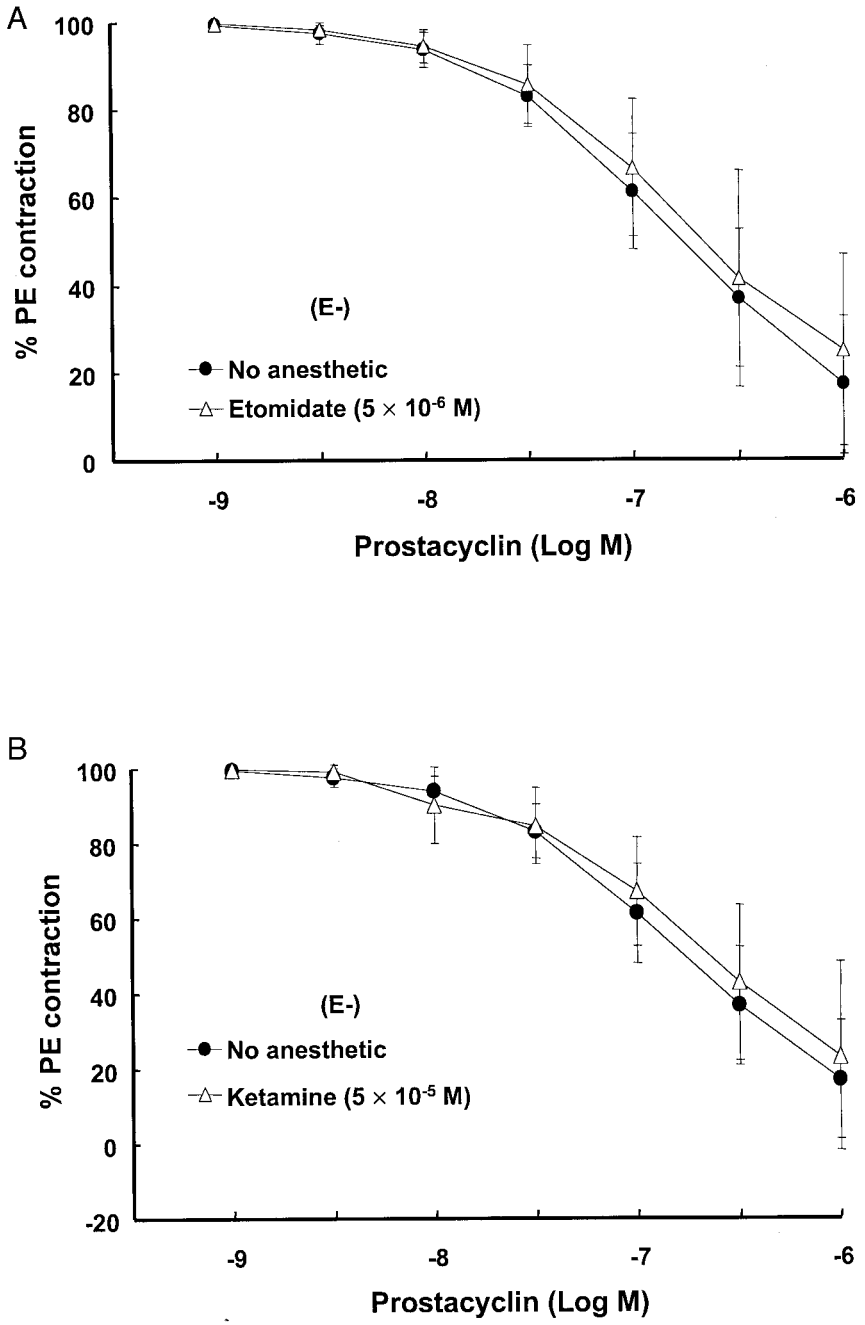

Fig. 6. Effect of etomidate (*A*) and ketamine (*B*) on prostacyclin-induced vasorelaxation in endothelium-denuded (E−) pulmonary arterial rings. Neither etomidate nor ketamine altered prostacyclin-induced vasorelaxation compared with rings with no anesthetic.

induced decrease in endothelial cell capacitative Ca^{2+} entry. We recently demonstrated that the intravenous anesthetic propofol inhibits capacitative Ca^{2+} entry in pulmonary arterial smooth muscle cells.[24] In addition, both etomidate and ketamine also appeared to exert a direct inhibitory effect on K^+_{ATP}-mediated changes in pulmonary vascular smooth muscle tone. Whether this effect involves a direct effect of the anesthetics on K^+_{ATP} channels or on the intracellular signaling pathway for K^+_{ATP} vasodilation remains to be elucidated.

The peak plasma concentration of etomidate during induction of general anesthesia is approximately 10^{-5} M,[25,26] whereas the free plasma concentration is likely to be less than 10^{-5} M because 75% of etomidate is bound to plasma protein.[27] The plasma concentration of ketamine following the intravenous administration of 2 mg/kg has been reported to be 1.1×10^{-4} M,[28] with 20% bound to plasma protein. Thus, the inhibitory effects of etomidate and ketamine on lemakalim-induced pulmonary vasorelaxation reported in this study appear to be significant at clinically relevant concentrations.

In summary, etomidate and ketamine inhibited K^+_{ATP}-induced pulmonary vasorelaxation. At low concentrations, etomidate inhibited the endothelium-dependent component of vasorelaxation in response to the K^+_{ATP} agonist lemakalim, and this effect was dependent on the

cyclooxygenase pathway. Etomidate and ketamine also inhibited the vascular smooth muscle component of lemakalim-induced vasorelaxation.

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