

# Differential Effects of Etomidate and Midazolam on Vascular Adenosine Triphosphate-sensitive Potassium Channels

## Isometric Tension and Patch Clamp Studies

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**Background:** The aim of this study was to investigate the effects of two imidazoline-derived intravenous anesthetics, etomidate and midazolam, on vascular adenosine triphosphate-sensitive potassium ( $K_{ATP}$ ) channel activity.

**Methods:** In isolated rat aorta, isometric tension was recorded to examine the anesthetic effects on vasodilator response to levromakalim, a selective  $K_{ATP}$  channel opener. Using the patch clamp method, the anesthetic effects were also examined on the currents through (1) native vascular  $K_{ATP}$  channels, (2) recombinant  $K_{ATP}$  channels with different combinations of various types of inwardly rectifying potassium channel (Kir6.0 family: Kir6.1, 6.2) and sulfonylurea receptor (SUR1, 2A, 2B) subunits, (3) SUR-deficient channels derived from a truncated isoform of Kir6.2 subunit (Kir6.2 $\Delta$ C36 channels), and (4) mutant Kir6.2 $\Delta$ C36 channels with reduced sensitivity to adenosine triphosphate (Kir6.2 $\Delta$ C36-K185Q channels).

**Results:** Etomidate ( $\geq 10^{-6}$  M), but not midazolam (up to  $10^{-6}$  M), inhibited the levromakalim-induced vasodilation, which was sensitive to glibenclamide ( $IC_{50}$ :  $7.21 \times 10^{-8}$  M; maximum inhibitory concentration:  $1.22 \times 10^{-4}$  M). Etomidate ( $\geq 3 \times 10^{-6}$  M), but not midazolam (up to  $10^{-4}$  M), inhibited the native  $K_{ATP}$  channel activity in both cell-attached and inside-out configurations with  $IC_{50}$  values of  $1.68 \times 10^{-5}$  M and  $1.52 \times 10^{-5}$  M, respectively. Etomidate ( $10^{-5}$  M) also inhibited the activity of various types of recombinant SUR/Kir6.0 $K_{ATP}$  channels, Kir6.2 $\Delta$ C36 channels, and Kir6.2 $\Delta$ C36-K185Q channels with equivalent potency.

**Conclusions:** Clinical concentrations of etomidate, but not midazolam, inhibit the  $K_{ATP}$  channel activity in vascular smooth muscle cells. The inhibition is presumably through its effects on the Kir6.0 subunit, but not on the SUR subunit, with the binding site different from adenosine triphosphate at the amino acid level.

ADENOSINE triphosphate-sensitive potassium ( $K_{ATP}$ ) channels, which are widely distributed in many tissues, respond to alterations in the metabolic activity of the cell and thereby act as sensors of glucose and oxygen availability.<sup>1-4</sup> In vascular smooth muscle, opening of  $K_{ATP}$

channels leads to membrane hyperpolarization, resulting in muscle relaxation and vasodilation.<sup>4</sup> This activation also plays an important role in regulating perfusion of various tissues during pathophysiologic events such as ischemia, hypoxia, and vasodilatory shock.<sup>4,5</sup>

Intravenous anesthetics, including barbiturates, propofol, and ketamine, have been shown to reduce vasodilation induced by  $K_{ATP}$  channel openers.<sup>6,7</sup> Our previous patch clamp studies also showed that these intravenous anesthetics directly inhibit  $K_{ATP}$  channel activity.<sup>8-10</sup> Etomidate and midazolam are intravenously administered imidazoline-derived anesthetics. It has been reported that structurally related imidazoline compounds, including phentolamine, clonidine, and cibenzoline, inhibit  $K_{ATP}$  channel activity.<sup>11-13</sup> These previous reports suggest that both etomidate and midazolam, like other intravenous anesthetics and imidazoline compounds, might similarly inhibit  $K_{ATP}$  channel activity. However, Choi *et al.*<sup>14</sup> compared the effects of etomidate (0.2-0.3 mg/kg) and midazolam (2-4 mg) on arterial blood pressure when used as an induction agent for rapid sequence intubation in emergency situations. A 10% decrease in mean systolic blood pressure was observed within 5 min after intubation in the midazolam group, but there was no significant change in the etomidate group. They also reported that hypotension developed in 19.5% of patients given midazolam but in only 3.6% of patients given etomidate, suggesting that midazolam, even at a low dose, was more likely than etomidate to cause significant hypotension. From these results, they concluded that etomidate is a better alternative.<sup>14</sup> In addition, Canessa *et al.*<sup>15</sup> reported that when etomidate, midazolam, and other intravenous anesthetic agents were used for elective cardioversion, etomidate was the only agent that did not decrease arterial blood pressure. On the basis of this report,<sup>14,15</sup> we hypothesized that the effects of etomidate and midazolam on vascular  $K_{ATP}$  channel activity might differ. Therefore, in the current study, we examined whether clinically relevant concentrations of etomidate or midazolam affect the vascular response to  $K_{ATP}$  channel opener levromakalim in isolated rat thoracic aorta. In addition, we used patch clamp techniques to examine the electrophysiologic effects of these anesthetics on native vascular and recombinant  $K_{ATP}$  channels and the molecular mechanisms underlying these effects.

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## Materials and Methods

The study was approved by the Animal Investigation Committee of Tokushima University (Tokushima, Japan) and was conducted according to the animal use guidelines of the American Physiologic Society (Bethesda, Maryland).

### *Isometric Tension Experiments*

Isometric tension experiments were performed on 2.5-mm thoracic aortic rings obtained from male Wistar rats (250–300 g) anesthetized with ether. Each ring was placed in modified Krebs-Ringer's bicarbonate solution (control solution) of the following composition: 118.3 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl<sub>2</sub>, 1.2 mM MgSO<sub>4</sub>, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 25.0 mM NaHCO<sub>3</sub>, 0.026 mM calcium EDTA, and 11.1 mM glucose. The endothelium of all rings was removed mechanically, because vasorelaxation in response to levromakalim is augmented in the presence of functional endothelium. The removal was confirmed by the absence of relaxation in response to acetylcholine (10<sup>-5</sup> M). Several rings cut from the same artery were studied in parallel, with each ring connected to an isometric force transducer (Micro Easy Magnus UC-2A; Kishimoto Medical Instruments Co., Ltd., Kyoto, Japan) and suspended in an organ chamber filled with 2 ml control solution (37°C, pH 7.4) bubbled with 95% oxygen and 5% carbon dioxide. Arteries were gradually stretched to the optimal point of the length-tension curve, as determined by contraction with phenylephrine (3 × 10<sup>-7</sup> M). In most of the arteries studied, optimal tension was achieved at approximately 1.0 g. Preparations were equilibrated for 90 min. During submaximal contractions produced in response to phenylephrine (3 × 10<sup>-7</sup> M), relaxation after administration of levromakalim (10<sup>-8</sup> to 10<sup>-5</sup> M) and a nonselective vasodilator, papaverine (10<sup>-7</sup> to 10<sup>-4</sup> M), was observed. Etomidate (10<sup>-6</sup>, 10<sup>-5</sup>, 10<sup>-4</sup> M), midazolam (10<sup>-8</sup>, 10<sup>-7</sup>, 10<sup>-6</sup> M), or glibenclamide (10<sup>-8</sup> to 10<sup>-5</sup> M) was applied to the preparations 15 min before the addition of phenylephrine. Vasorelaxation was expressed as a percentage of the maximum relaxation in response to papaverine (3 × 10<sup>-4</sup> M), which was added at the end of the experiments to produce maximum relaxation (100%) of the arteries. Concentration-response curves were obtained in a cumulative manner. Only one concentration-response curve was made for each ring.

### *Native Vascular Smooth Muscle Cells*

A cell line of A10 vascular smooth muscle cells, derived from the thoracic aorta of fetal rats, was obtained from the American Type Culture Collection (Manassas, VA). The cells were incubated in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (Life Technologies; Rockville, MD), 3.7 mg/ml NaHCO<sub>3</sub>, and 100 μg/ml gentamicin at 37°C in a humidified atmo-

sphere of 95% air and 5% CO<sub>2</sub>. The medium was changed initially at 48 h and then every 2–3 days. When the cells had formed a confluent monolayer after 7–9 days, they were made quiescent by incubation in serum-free medium for 24 h. They were then harvested by the addition of 0.05% trypsin and 0.1% fetal bovine serum. Passages 5–12 were used for experimental purposes. Cultured A10 cells were stimulated with etomidate (10<sup>-6</sup> to 10<sup>-4</sup> M) or midazolam (10<sup>-8</sup> to 10<sup>-4</sup> M).

### *Molecular Biology and Transfection*

Details of the recombinant experimental design were similar to those of our previous studies.<sup>8–10</sup> In brief, K<sub>ATP</sub>-deficient COS-7 cells were transiently cotransfected with two K<sub>ATP</sub> channel subunits, sulfonylurea receptor (SUR) and inwardly rectifying K<sup>+</sup> channel (Kir) subunits, which comprise specific tissue-type K<sub>ATP</sub> channels, with Lipofectamine, and with Opti-MEM1 reagents. A truncated form of human Kir6.2 lacking the last 36 amino acids at the C-terminus (Kir6.2ΔC36) was obtained by polymerase chain reaction amplification. Mutagenesis complementary DNA (cDNA) of Kir6.2ΔC36 was performed with a site-directed mutagenesis kit (Invitrogen Corp., Carlsbad, CA). All DNA products were sequenced with a BigDye terminator cycle sequencing kit and an ABI PRISM 377 DNA sequencer (Applied Biosystems, Foster City, CA) to confirm the sequence. For electrophysiologic recordings, COS-7 cells were plated onto dishes with glass coverslips, and Kir and SUR subunits were cotransfected with green fluorescent protein cDNA (pEGFP) as a reporter gene. After transfection, cells were cultured for 48–72 h before being subjected to electrophysiologic recordings.

### *Electrophysiologic Measurements*

Cell-attached and inside-out patch configurations were used to record the current through single channels *via* a patch clamp amplifier, as described previously.<sup>16</sup> For cell-attached configurations, the bath solution consisted of 140 mM KCl, 10 mM HEPES, 5.5 mM dextrose, and 1 mM EGTA. The pipette solution contained 140 mM KCl, 10 mM HEPES, and 5.5 mM dextrose. For inside-out configurations, the bath solution (intracellular solution) consisted of 140 mM KCl, 10 mM HEPES, 5.5 mM dextrose, 1 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.5 mM magnesium adenosine diphosphate, and 0.5 mM magnesium adenosine triphosphate. The pipette solution (extracellular medium) was of the same composition as that used in cell-attached experiments. The pH of all solutions was adjusted to 7.3–7.4 with potassium hydroxide. Recordings were made at 36 ± 0.5°C. Patch pipettes were pulled with an electrode puller (PP-830; Narishige, Tokyo, Japan). The resistance of pipettes filled with internal solution and immersed in Tyrode solution was 5–7 MΩ. The sampling frequency of the single-channel data was 5 KHz with a low-pass filter (1 KHz).

Channel currents were recorded with a patch clamp amplifier (CEZ 2200; Nihon Kohden, Tokyo, Japan) and stored on a personal computer (Aptiva; International Business Machine Corp., Armonk, NY) with an analog-to-digital converter (DigiData 1200; Axon Instruments, Foster City, CA). pClamp version 7 software (Axon Instruments) was used for data acquisition and analysis. The open probability ( $P_o$ ) was determined from current amplitude histograms and was calculated as follows:

$$P_o = \frac{\left( \sum_{j=1}^N t_j \cdot j \right)}{T_d \cdot N},$$

where  $t_j$  is the time spent at current levels corresponding to  $j = 0, 1, 2, N$  channels in the open state;  $T_d$  is the duration of the recording; and  $N$  is the number of channels active in the patch. Recordings of 2–3 min were analyzed to determine  $P_o$ . Channel activity was expressed as  $NP_o$ . Changes in channel activity in the presence of drugs were calculated as the relative channel activity, *i.e.*, the ratio between values obtained before and after drug treatment. When the concentration-dependent effects of etomidate and midazolam were studied, increasing concentrations of these drugs were injected into the cell bath with a glass syringe (total volume injected was approximately 10–20  $\mu$ l).

Drug concentrations needed to induce half-maximum inhibition of the channels ( $IC_{50}$ ) and the Hill coefficient were calculated as follows:

$$y = \frac{1}{1 + ([D] / K_i)^H},$$

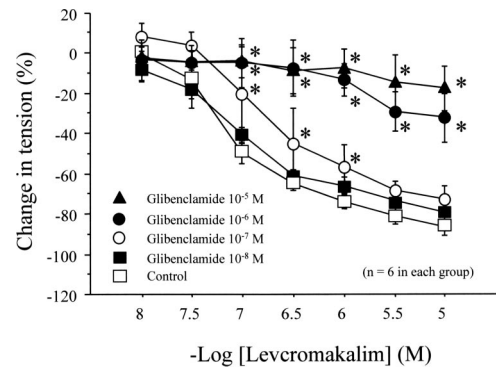
where  $y$  is the relative  $NP_o$ ,  $[D]$  is the concentration of drug,  $K_i$  is the  $IC_{50}$ , and  $H$  is the Hill coefficient.

### Drugs

The following pharmacologic agents were used: midazolam, dimethyl sulfoxide, glibenclamide, papaverine hydrochloride, phenylephrine, pinacidil (Sigma-Aldrich Japan, Tokyo, Japan), levcromakalim, and etomidate (Tocris, Ellisville, MO). Drugs were dissolved in distilled water such that volumes of less than 15  $\mu$ l were added to the organ chamber. Stock solutions of levcromakalim, glibenclamide, and pinacidil were prepared in dimethyl sulfoxide. The final concentration of dimethyl sulfoxide in the bath solution never exceeded 0.01%; dimethyl sulfoxide at a twofold higher concentration was shown not to affect  $K_{ATP}$  channel currents. The concentrations of drugs are expressed as final molar (M) concentrations.

### Statistical Analysis

All data are presented as mean  $\pm$  SD. Differences between data sets were evaluated either by repeated-measures one-way analysis of variance followed by the



**Fig. 1.** Concentration–response curves to levcromakalim ( $10^{-8}$  to  $10^{-5}$  M) in the absence and presence of glibenclamide ( $10^{-8}$  to  $10^{-5}$  M) obtained in rat thoracic aortas without endothelium. Data are shown as mean  $\pm$  SD and expressed as the percentage of maximum relaxation induced by papaverine ( $3 \times 10^{-4}$  M). \*  $P < 0.05$  between control rings and rings treated with glibenclamide. The number of rats from which the aortas were obtained is shown.

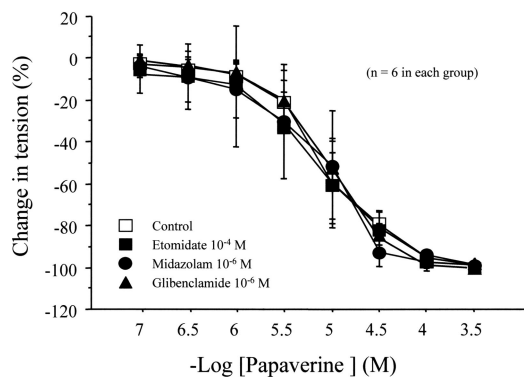
Scheffé F test or by Student  $t$  test with Welch correction.  $P < 0.05$  was considered statistically significant.

## Results

### *Effects of Etomidate and Midazolam on Levcromakalim-induced Vasorelaxation*

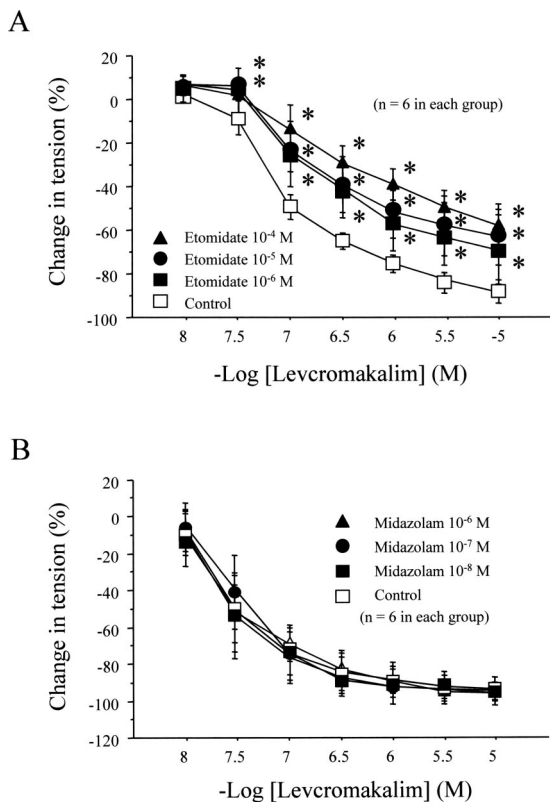
First, we examined the effects of various concentrations of selective  $K_{ATP}$  channel antagonist glibenclamide on relaxation produced by levcromakalim to show whether this potassium channel opener selectively produced relaxation mediated by  $K_{ATP}$  channels. Concentration–response curves for levcromakalim in the absence and presence of glibenclamide ( $10^{-8}$  to  $10^{-5}$  M) are shown in figure 1. During submaximum contractions induced by phenylephrine ( $3 \times 10^{-7}$  M), selective  $K_{ATP}$  channel opener levcromakalim ( $10^{-8}$  to  $10^{-5}$  M) induced concentration-dependent relaxation. Selective ATP-sensitive potassium channel antagonist glibenclamide eliminated this relaxation in a concentration-dependent manner with an  $IC_{50}$  value of  $(7.21 \pm 7.72) \times 10^{-8}$  M and a maximally inhibitory concentration of  $(1.22 \pm 1.62) \times 10^{-4}$  M. The  $IC_{50}$  and maximally inhibitory concentration of glibenclamide obtained in the current study were consistent with previously reported values.<sup>17</sup> Glibenclamide ( $10^{-6}$  M) did not affect relaxation produced by the nonselective vasodilator papaverine ( $10^{-7}$  to  $10^{-4}$  M) (fig. 2).

The effects of etomidate and midazolam on relaxation produced by levcromakalim and papaverine were then examined to determine whether these agents selectively modified relaxation mediated by  $K_{ATP}$  channels. Etomidate significantly impaired relaxation produced by levcromakalim in a concentration-dependent manner (fig. 3A). The effect of etomidate is rather modest in comparison to that of glibenclamide. However, midazolam did not significantly impair relaxation produced by



**Fig. 2.** Concentration–response curves to papaverine ( $10^{-7}$  to  $10^{-4}$  M) in the absence and presence of etomidate ( $10^{-4}$  M), midazolam ( $10^{-6}$  M), and glibenclamide ( $10^{-6}$  M) obtained in rat thoracic aortas without endothelium. Data are shown as mean  $\pm$  SD and expressed as the percentage of maximum relaxation induced by papaverine ( $3 \times 10^{-4}$  M). The number of rats from which the aortas were obtained is shown.

levcromakalim (fig. 3B). In contrast, even the highest concentrations of etomidate ( $10^{-4}$  M) and midazolam ( $10^{-6}$  M) did not affect relaxation produced by the non-selective vasodilator papaverine (fig. 2).

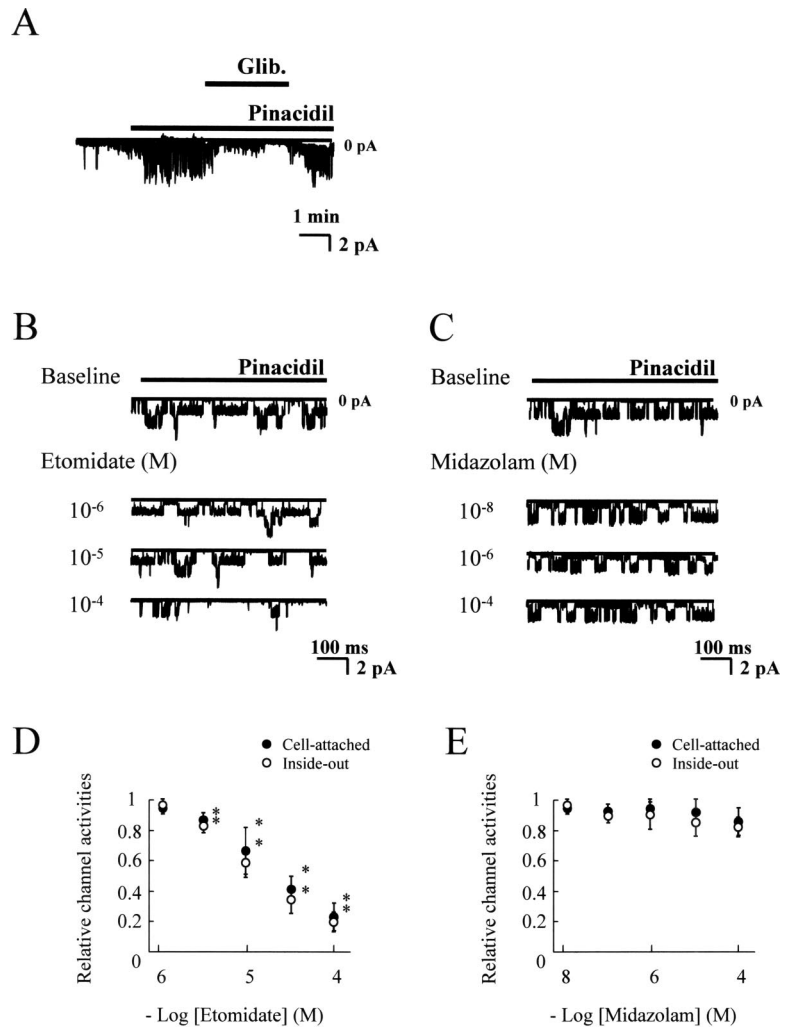


**Fig. 3.** Concentration–response curves to levcromakalim in the absence and presence of etomidate ( $10^{-6}$ ,  $10^{-5}$ ,  $10^{-4}$  M; A) or midazolam ( $10^{-8}$ ,  $10^{-7}$ ,  $10^{-6}$  M; B) obtained in rat thoracic aortas without endothelium. Data are shown as mean  $\pm$  SD and expressed as the percentage of maximum relaxation induced by papaverine ( $3 \times 10^{-4}$  M). \*  $P < 0.05$  between control rings and rings treated with etomidate or midazolam. The number of rats from which the aortas were obtained is shown.

### Effects of Etomidate and Midazolam on $K_{ATP}$ Channel Activity in Vascular Smooth Muscle Cells

To investigate whether etomidate and midazolam affect  $K_{ATP}$  channel activity in vascular smooth muscle cells, we measured single  $K_{ATP}$  channel currents by the patch clamp technique. As shown in figure 4A, spontaneous single channel activity was observed infrequently in the cell-attached configuration ( $NP_o < 0.01$ ,  $n = 10$ ). However, application of  $10^{-4}$  M pinacidil, a selective  $K_{ATP}$  channel opener, to the bath solution significantly activated  $K^+$ -selective channels ( $NP_o = 0.447 \pm 0.171$ ,  $P < 0.05$  vs. baseline,  $n = 12$ ). This channel activity was completely blocked by  $3 \times 10^{-6}$  M glibenclamide, a specific  $K_{ATP}$  channel blocker (fig. 4A;  $n = 12$ ). The channel showed a single channel conductance of  $29.4 \pm 4.9$  pS ( $n = 15$ ), as measured by the current–voltage relation between  $-80$  and  $+60$  mV membrane potential. These channel properties were consistent with those reported previously.<sup>18</sup> Representative examples of the effects of etomidate on pinacidil-induced  $K_{ATP}$  channel activity in the cell-attached configuration are shown in figure 4B. Application of  $10^{-6}$ ,  $10^{-5}$ , and  $10^{-4}$  M etomidate to the outside of the membrane surface inhibited pinacidil-induced  $K_{ATP}$  channel currents, with relative channel activity decreasing to  $0.96 \pm 0.07$  ( $n = 8$ ),  $0.67 \pm 0.22$  ( $n = 10$ ), and  $0.21 \pm 0.11$  ( $n = 9$ ), respectively. Representative examples of the effects of midazolam on pinacidil-induced  $K_{ATP}$  channel activity in the cell-attached configuration are shown in figure 4C. In contrast to etomidate, at  $10^{-8}$ ,  $10^{-6}$ , and  $10^{-4}$  M, midazolam did not inhibit pinacidil-induced  $K_{ATP}$  channel currents in the cell-attached configuration, with relative channel activity of  $0.98 \pm 0.04$  ( $n = 14$ ),  $0.96 \pm 0.15$  ( $n = 16$ ), and  $0.90 \pm 0.14$  ( $n = 12$ ), respectively.

Concentration-dependent effects of etomidate on pinacidil-induced  $K_{ATP}$  channel activity in the cell-attached and inside-out configurations are shown in figure 4D. Etomidate significantly inhibited  $K_{ATP}$  channel activity at concentrations of  $3 \times 10^{-6}$  M or greater in both the cell-attached and inside-out configurations. The  $IC_{50}$  values in the cell-attached and inside-out configurations were  $1.68 \times 10^{-5}$  M and  $1.52 \times 10^{-5}$  M, respectively. The Hill coefficients in the cell-attached and inside-out configurations were 1.23 and 1.44, respectively. Etomidate did not change the single channel conductance in either the cell-attached or inside-out configuration (data not shown). Concentration-dependent effects of midazolam on pinacidil-induced  $K_{ATP}$  channel activity in cell-attached and inside-out configurations are shown in figure 4E. Even high concentrations of midazolam had no significant inhibitory effect on  $K_{ATP}$  channel activity in either the cell-attached or inside-out configuration.



**Fig. 4.** Effects of etomidate and midazolam on adenosine triphosphate-sensitive potassium ( $K_{ATP}$ ) channel activity in vascular smooth muscle cells. (A) Single-channel characteristics of  $K_{ATP}$  channels in the cell-attached configuration. Membrane potentials were clamped at  $-60$  mV. Zero current levels are indicated by the horizontal lines marked "0 pA." Pinacidil ( $10^{-4}$  M) and glibenclamide (Glib.) ( $3 \times 10^{-6}$  M) were superfused into the bath solution as indicated by the horizontal solid bars. Effects of etomidate (B) and midazolam (C) on  $K_{ATP}$  channel activity in the cell-attached configuration. Concentration-dependent effects of etomidate (D) and midazolam (E) on the activity of  $K_{ATP}$  channels in cell-attached (●) and inside-out (○) configurations. Each vertical bar constitutes measurements from 18–20 patches (mean  $\pm$  SD). \*  $P < 0.05$  versus baseline.

#### Effects of Etomidate on Recombinant $K_{ATP}$ Channel Activity

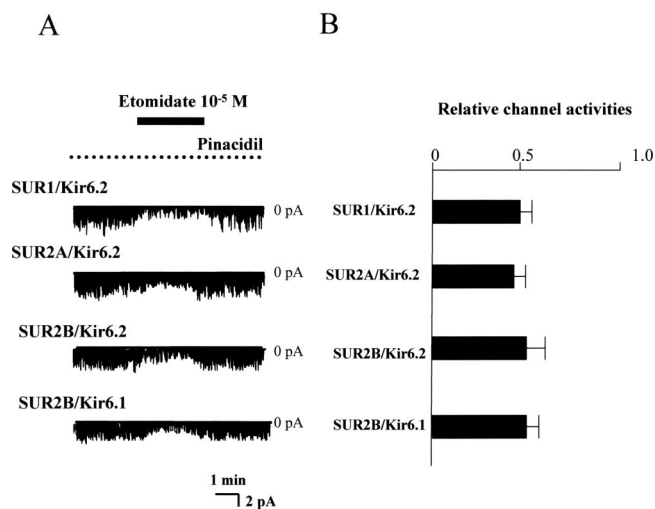
To determine the tissue-specific effects of etomidate on  $K_{ATP}$  channel activity, we used inside-out patch clamp configurations to investigate the effects of etomidate on the activities of various types of recombinant Kir6.0/SUR channels. Sarcolemmal  $K_{ATP}$  channels, SUR2A/Kir6.2 (cardiac type), SUR2B/Kir6.1 (vascular smooth muscle type), SUR2B/Kir6.2 (nonvascular smooth muscle type), and SUR1/Kir6.2 (pancreatic  $\beta$ -cell type) were heterologously expressed in COS-7 cells.<sup>19</sup> Our previously reported experiments showed that the single-channel characteristics of all types of expressed  $K_{ATP}$  channels are similar to those of native  $K_{ATP}$  channels.<sup>8–10</sup> As shown in figure 5A, application of  $10^{-5}$  M etomidate to the intracellular membrane surface inhibited pinacidil-induced SUR1/Kir6.2, SUR2A/Kir6.2, SUR2B/Kir6.2, and SUR2B/Kir6.1 channel currents with equivalent potency; relative channel activity decreased to  $0.47 \pm 0.11$ ,  $0.43 \pm 0.07$ ,  $0.58 \pm 0.14$ , and  $0.56 \pm 0.18$ , respectively (fig. 5B). The inhibitory effects of etomidate on recombinant  $K_{ATP}$  channel activity were

reversible; channel activity recovered after washout (fig. 5A).

#### Effects of Etomidate on Kir6.2 $\Delta$ C36 Channel Activity

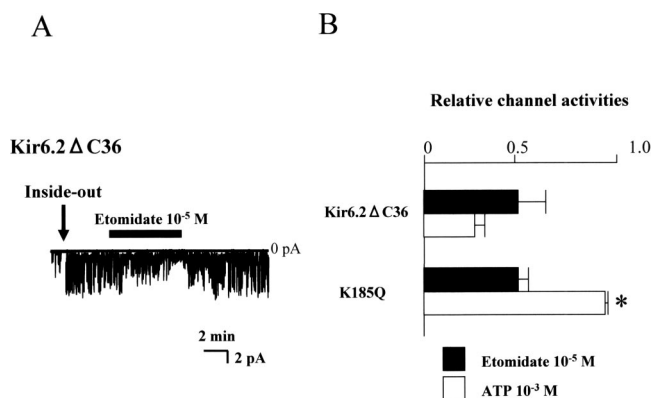
A C-terminal truncated pore-forming subunit of Kir6.2 (Kir6.2 $\Delta$ C36), lacking the last 36 amino acids, is capable of forming a functional channel in the absence of SUR.<sup>20</sup> This has proved to be a useful tool for discriminating the site of action of various agents on  $K_{ATP}$  channels. As seen in figure 6A, etomidate at  $10^{-5}$  M inhibited Kir6.2 $\Delta$ C36 channel currents, with relative channel activity decreasing to  $0.49 \pm 0.24$  ( $n = 8$ ). This result indicates that the Kir subunit, rather than SUR, is primarily responsible for the effects of etomidate on wild-type  $K_{ATP}$  channels. The inhibitory effects of etomidate on Kir6.2 $\Delta$ C36 channel activity were reversible; channel activity recovered after washout (fig. 6A).

We next used site-directed mutagenesis of Kir6.2 $\Delta$ C36 channels to examine whether the site at which etomidate mediates  $K_{ATP}$  channel inhibition was identical to that involved in adenosine triphosphate block. We used



**Fig. 5.** Effects of etomidate on the currents of different recombinant adenosine triphosphate-sensitive potassium channels in the inside-out configuration. Membrane potentials were clamped at  $-60$  mV. (A) Shown are representative examples of sulfonylurea receptor (SUR) 1/inwardly rectifying potassium channel (Kir)6.2, SUR2A/Kir6.2, SUR2B/Kir6.2, and SUR2B/Kir6.1 currents obtained before and after application of etomidate ( $10^{-5}$  M). The periods of etomidate administration are marked with *horizontal solid bars*. The periods of pinacidil administration are marked with *horizontal dotted bars*. (B) The percentage of inhibition of recombinant channel activity by intracellular etomidate ( $10^{-5}$  M). Each *horizontal bar* represents measurements from 12 patches (mean  $\pm$  SD).

a double-mutant form of Kir6.2 (Kir6.2 $\Delta$ C36-K185Q).<sup>21</sup> In this mutant, the inhibitory potency of adenosine triphosphate was significantly reduced, whereas  $10^{-5}$  M etomidate inhibited Kir6.2 $\Delta$ C36-K185Q currents as effectively as it inhibited Kir6.2 $\Delta$ C36 currents (fig. 6B).



**Fig. 6.** Effects of etomidate on the channel activities of the truncated isoform of inwardly rectifying potassium channel 6.2 (Kir6.2 $\Delta$ C36), which can form functional adenosine triphosphate (ATP)-sensitive potassium channels in the absence of sulfonylurea receptor molecules, in the inside-out configuration. Membrane potentials were clamped at  $-60$  mV. (A) Representative examples of Kir6.2 $\Delta$ C36 currents obtained before and after application of etomidate ( $10^{-5}$  M). The periods of etomidate treatment are marked with *horizontal bars*. (B) The percentage of inhibition of channel activity of Kir6.2 $\Delta$ C36 channel alone and Kir6.2 $\Delta$ C36 channel bearing the K185Q mutation by intracellular etomidate ( $10^{-5}$  M) and ATP ( $10^{-3}$  M). Each *horizontal bar* represents measurements from 8 patches (mean  $\pm$  SD). \*  $P < 0.05$  versus Kir6.2 $\Delta$ C36 channel alone.

## Discussion

The most important findings in the current study is that the representative imidazoline-derived anesthetics, etomidate and midazolam, have different effects on vascular  $K_{ATP}$  channels in both isometric tension and patch clamp experiments. The isometric tension experiment in rat aortic ring preparations showed that etomidate reduces relaxation produced by levromakalim, a selective  $K_{ATP}$  channel opener, in a concentration-dependent manner, whereas midazolam does not affect this vasorelaxation. Similarly, electrophysiologic study by the patch clamp method showed that etomidate inhibits native vascular  $K_{ATP}$  channel activity in a concentration-dependent manner, whereas midazolam did not significantly affect  $K_{ATP}$  channel activity in either the cell-attached or inside-out configuration. These results suggest that etomidate, but not midazolam, inhibits the activities of vascular  $K_{ATP}$  channels at clinical concentrations.

Etomidate is a rapid-acting sedative/hypnotic agent with little or no cardiovascular or respiratory effects.<sup>22</sup> Because of these properties, etomidate is widely used as an anesthetic induction agent in patients with poor cardiovascular reserve. Similarly, midazolam is used as a premedication, sedative, and anesthesia induction agent because it has only minimal effects on cardiovascular dynamics.<sup>23</sup> It was reported that induction of anesthesia with midazolam, even in patients with limited coronary flow, was accompanied by no change in cardiac output or central venous pressure and only a modest reduction in peripheral vascular resistance.<sup>24</sup> Structurally related imidazoline compounds may be closely related to  $K_{ATP}$  channels. Lee *et al.*<sup>12</sup> reported that imidazoline compounds inhibit  $K_{ATP}$  channel activity in guinea pig ventricular myocytes and in pancreatic  $\beta$  cells. However, Choi *et al.*<sup>14</sup> revealed that, when used as an induction agent for emergency department rapid sequence intubation, a 10% decrease in mean systolic blood pressure was observed within 5 min after intubation in the midazolam group but that no significant change was observed in the etomidate group. They also reported that hypotension developed in 19.5% of patients given midazolam but in only 3.6% of patients given etomidate, suggesting that midazolam, even at a low dose, was more likely than etomidate to cause significant hypotension. From these results, they concluded that etomidate is a better alternative.<sup>14</sup> In addition, Canessa *et al.*<sup>15</sup> reported that etomidate was the only agent that did not decrease arterial blood pressure comparing midazolam and other intravenous anesthetic agents for elective cardioversion. Systolic arterial blood pressure decreased significantly with midazolam (19%), whereas no significant change was evident in patients receiving etomidate. Based on these reports,<sup>14,15</sup> we hypothesized that the effects of etomidate and midazolam on  $K_{ATP}$  channel activity might differ.

Adenosine triphosphate-sensitive potassium channels are present in a wide variety of tissues and are believed to link cellular metabolic status and excitability.<sup>1-4,18</sup> In vascular smooth muscle cells,  $K_{ATP}$  channels regulate the membrane potential, which controls calcium entry through voltage-dependent calcium channels, and thereby contractility through changes in intracellular calcium.<sup>5,18</sup> Physiologic studies have suggested that opening  $K_{ATP}$  channels in vascular smooth muscle causes vasodilation; this occurs physiologically in response to certain neurotransmitters and hypoxia and pharmacologically during therapy with  $K_{ATP}$  channel openers.<sup>4,5</sup> A recent clinical study further suggested that prophylactic administration of  $K_{ATP}$  channel opener nicorandil proved useful for perioperative prevention of cardiac complications.<sup>25</sup> Previous studies, however, showed that some intravenous anesthetics, including barbiturates, propofol, and ketamine, reduced  $K_{ATP}$  channel opener-induced rat aortic vasodilation.<sup>6,7</sup> In a similar experimental system, the current study showed that etomidate also reduced this vasodilation (fig. 3A). This finding agrees in part with recently reported findings, that etomidate reduced  $K_{ATP}$  channel opener lemakalim-induced vasorelaxation in the canine pulmonary artery.<sup>26</sup> These results suggest, therefore, that the inhibitory effect of etomidate on  $K_{ATP}$  channel opener-induced vasorelaxation is independent of the specific vascular site. In contrast to other intravenous anesthetics, midazolam at the concentrations we used did not affect this vasorelaxation (fig. 3B).

Recent patch clamp studies of vascular  $K_{ATP}$  channels showed that these channels are targets of a wide variety of vasodilators and constrictors, which act through multiple cellular signaling pathways, such as protein kinase A and protein kinase C.<sup>5</sup> In the current electrophysiologic study, however, etomidate inhibited native vascular  $K_{ATP}$  channel activity with similar potency in both cell-attached and inside-out configurations (fig. 4D). These results suggest that the inhibitory effect of etomidate on  $K_{ATP}$  channel activity may be due to direct binding to these channels rather than modulation of the cell-signaling pathway. However, in agreement with the results of our isometric tension study, midazolam had no effect on  $K_{ATP}$  channel activity in either the cell-attached or inside-out configuration (fig. 4E). These results indicate, therefore, that the differential effects of etomidate and midazolam on levromakalim-induced vasorelaxation were based on direct but different action on vascular smooth muscle  $K_{ATP}$  channel activity.

The  $K_{ATP}$  channel is a hetero-octamer composed of two subunits: a Kir6.0 family subunit (Kir6.1 or Kir6.2) and the SUR subunit (SUR1, SUR2A, or SUR2B).<sup>19</sup> SUR acts as a regulatory subunit, whereas Kir subunits form the ATP-sensitive channel pore. Different combinations of Kir and SUR subunits generate tissue-specific  $K_{ATP}$  channel subtypes.<sup>19</sup> Several  $K_{ATP}$  channel activators and

inhibitors show various tissue specificities, and different types of  $K_{ATP}$  channels exhibit different pharmacologic properties, which are mainly due to the SUR subunit.<sup>18,19</sup> In the current study, however, inhibitory potencies of etomidate on recombinant SUR/Kir6.0 channel activity were not influenced by the type of SUR subunit (fig. 5). This result suggests that the Kir6.0 subunit, rather than the SUR subunit, is primarily responsible for the effects of etomidate on  $K_{ATP}$  channels. This view is strongly supported by the fact that etomidate inhibited Kir6.2 $\Delta$ C36 channels with the same potency as for the recombinant SUR/Kir6.0 channels (fig. 6A). Furthermore, our results indicate that the binding site of etomidate is not identical to that of adenosine triphosphate at the amino acid level; mutation of K185Q markedly decreased adenosine triphosphate sensitivity but was without significant effects on etomidate inhibition (fig. 6B). Although the precise binding site of etomidate remains unclear, recent electrophysiologic studies showed that other imidazoline compounds, including phentolamine and cibenzoline, blocked recombinant  $K_{ATP}$  channels *via* the Kir6.2 subunit.<sup>11,13</sup> It is, therefore, possible that inhibitory action *via* Kir6.0 is a common feature of imidazoline compounds, which modulate  $K_{ATP}$  channel activity.

The recommended intravenous dose for inducing anesthesia is approximately 0.3 mg/kg for etomidate and 0.2-0.4 mg/kg for midazolam. Peak plasma concentrations during induction at these doses vary widely but are reported to be approximately  $3 \times 10^{-6}$  M for etomidate<sup>27</sup> and 0.5 to  $3 \times 10^{-6}$  M for midazolam.<sup>24,28</sup> Because approximately 75% of etomidate<sup>29</sup> and 96-97% of midazolam<sup>24</sup> binds to plasma proteins, the concentrations of etomidate and midazolam used in the current study are clinically relevant. Therefore, it is likely that the differential effects of etomidate and midazolam observed in the current experiments will be encountered in clinical settings. Recently reported physiologic studies showed that vascular  $K_{ATP}$  channels are involved in the maintenance of resting blood flow in a number of vascular beds, notably the coronary circulation, as well as in vasodilation in response to metabolic demand.<sup>5,18</sup> Thus,  $K_{ATP}$  channel openers might be advantageous in the treatment of ischemic conditions such as angina. Therefore, our results suggest that in clinical situations, etomidate, but not midazolam, inhibits vascular  $K_{ATP}$  channel activity, impairing the vasodilation mediated by  $K_{ATP}$  channel openers. However, there is increasing evidence that excessive activation of vascular  $K_{ATP}$  channels plays a role in the catastrophic vasodilation and vascular hyporeactivity of circulatory shock.<sup>30</sup> Under these conditions, the inhibitory action of etomidate on vascular  $K_{ATP}$  channels could be advantageous. Further studies are needed to clarify the clinically observed influence of etomidate and midazolam on vascular  $K_{ATP}$  channels.

In conclusion, our study showed that in the isolated rat

aorta, clinically relevant concentrations of etomidate, but not midazolam, inhibited relaxation induced by a  $K_{ATP}$  channel opener. Electrophysiologic patch clamp measurements indicated that the different actions of the two anesthetics are based on differential direct action on vascular smooth muscle  $K_{ATP}$  channel activity; etomidate directly inhibited  $K_{ATP}$  channel activity *via* the Kir6.0 subunit, whereas midazolam at clinically relevant concentrations had no effect on  $K_{ATP}$  channel activity.

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