Etomidate attenuates phenylephrine-induced contraction in isolated rat aorta

[L'étomidate atténue la contraction induite par la phényléphrine dans des aortes isolées de rats]

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Purpose: A previous study has shown that etomidate inhibits the angiotensin II-induced calcium influx in rat aortic smooth muscle cells. The goals of our current *in vitro* study were to investigate the effect of etomidate on phenylephrine-induced contraction in rat aorta, and to elucidate the associated signalling pathway.

Methods: Endothelium-denuded aortic rings were suspended for isometric tension recording. Concentration-response curves for phenylephrine (10^{-9} to 10^{-6} M), 5-hydroxytryptamine (10^{-7} to 10^{-4} M) and potassium chloride (10 to 60 mM) were generated in the presence and absence of etomidate (5×10^{-6}, 3×10^{-5}, 5×10^{-5} M). For the rings pretreated with verapamil (10^{-5} M), the phenylephrine concentration-response curves were generated in the presence and absence of etomidate (5×10^{-5} M). In the rings exposed to calcium-free isotonic depolarizing solution, the contractile response induced by the addition of calcium was assessed in the presence and absence of etomidate (5×10^{-5} M).

Results: Etomidate (5×10^{-5} M) produced a significant rightward shift in the concentration-response curves for phenylephrine, 5-hydroxytryptamine and potassium chloride. Etomidate (5×10^{-5} M) did not alter phenylephrine-induced contraction in the rings pretreated with verapamil. Etomidate (5×10^{-5} M) significantly attenuated the contractile response induced by the addition of calcium in the calcium-free isotonic depolarizing solution.

Conclusion: The results suggest that etomidate, which exceeds the clinically relevant concentration, attenuates the phenylephrine-induced contraction by having an inhibitory effect on the calcium influx by blocking the L-type calcium channels in the rat aortic vascular smooth muscle.

***Objectif** : Une étude précédente a montré que l'étomidate inhibe le flux calcique entrant induit par l'angiotensine II dans les cellules musculaires lisses de l'aorte de rat. Les buts de notre étude actuelle in vitro étaient de chercher l'effet de l'étomidate sur la contraction de l'aorte de rat, induite par la phényléphrine, et de clarifier le mécanisme associé de transmission des signaux.*

***Méthode** : Des anneaux aortiques dépourvus d'endothélium ont été suspendus pour enregistrer la tension isométrique. Les courbes de concentration-réponse pour la phényléphrine (10^{-9} à 10^{-6} M), la 5-hydroxytryptamine (10^{-7} à 10^{-4} M) et le chlorure de potassium (10 à 60 mM) ont été générées avec et sans étomidate (5×10^{-6}, 3×10^{-5} 5×10^{-5} M). Pour les anneaux prétraités avec du vérapamil (10^{-5} M), les courbes de concentration-réponse de la phényléphrine ont été produites avec et sans étomidate (5×10^{-5} M). Dans les anneaux exposés à des solutions isotoniques dépolarisantes sans calcium, la réponse contractile induite par l'ajout de calcium a été évaluée avec et sans étomidate (5×10^{-5} M).*

***Résultats** : L'étomidate (5×10^{-5} M) produit un déplacement significatif vers la droite des courbes de concentration-réponse pour la phényléphrine, la 5-hydroxytryptamine et le chlorure de potassium. L'étomidate (5×10^{-5} M) n'a pas altéré la contraction induite par la phényléphrine dans les anneaux prétraités au vérapamil. L'étomidate (5×10^{-5} M) a significativement atténué la réponse contractile induite par l'ajout de calcium dans la solution isotonique dépolarisante sans calcium.*

***Conclusion** : Les résultats permettent de dire que l'étomidate, qui excède la concentration cliniquement significative, atténue la contraction induite par la phényléphrine par son effet inhibiteur sur le flux calcique entrant en bloquant les canaux calciques de type L dans le muscle lisse vasculaire de l'aorte de rat.*

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ETOMIDATE has only a minimal effect on cardiovascular function, and it has been used for anesthetizing patients whose hemodynamic stability must be maintained.[1] An induction dose of 0.3 $mg \cdot kg^{-1}$ of etomidate, when given to cardiac patients for noncardiac surgery, results in almost no hemodynamic change.[2] For patients with mitral or aortic valvular disease, etomidate produces a modest decrease in the mean arterial pressure.[3,4] Etomidate, when given as an electroencephalographic burst suppression dose (0.73 ± 0.49 $mg \cdot kg^{-1}$), decreases the mean arterial blood pressure in anesthetized patients undergoing cerebral aneurysm surgery.[5]

Etomidate alters the calcium mobilization that is induced by angiotensin II in rat aortic smooth muscle cells.[6] However, the effects of etomidate on contractions induced by contractile agonists (phenylephrine and 5-hydroxytryptamine) have not been investigated previously. The goals of this *in vitro* study were to investigate the effects of etomidate on the phenylephrine-induced contractions in rat aorta, and to elucidate the associated signalling pathway.

Methods

All the experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee of Gyeongsang National University Hospital.

Preparation of aortic rings for tension measurement

Male Sprague Dawley rats weighing 250 to 350 g each were anesthetized with an *ip* administration of pentobarbital sodium (50 $mg \cdot kg^{-1}$). The descending thoracic aorta was dissected free, and the surrounding connective tissue and fat were removed under a microscope while the blood vessel was bathed in Krebs solution of the following composition: 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.4 mM $CaCl_2$, 25 mM $NaHCO_3$, 11 mM glucose and 0.03 mM EDTA. The aorta was then cut into 2.5-mm rings, and these rings were suspended on Grass isometric transducers (FT-03, Grass Instrument, Quincy, MA, USA) at 2.0 g resting tension in 10 mL temperature-controlled baths (37°C) containing Krebs solution that was continuously gassed with 95% O_2 and 5% CO_2. The rings were equilibrated at 2.0 g resting tension for 120 min, during which time the bathing solution was changed every 15 min. Only one concentration-response curve elicited by the contractile agonists (phenylephrine, 5-hyroxytryptamine), KCl (potassium chloride) and calcium (Ca^{2+}) was made for each ring in all the experiments. In all the aortic rings, the endothelium was intentionally removed by inserting a 25-gauge needle tip into the lumen of ring and gently rolling the ring for a few seconds. The contractile response induced by isotonic 60 mM KCl was measured in all the aortic rings.

Experimental protocol

The first series of this *in vitro* experiment was conducted to assess the effect of etomidate on contractile responses induced by the α-1 adrenoceptor agonist phenylephrine in endothelium-denuded rings. Etomidate was added directly to the organ bath 20 min before a cumulative phenylephrine-induced contraction. The effect of etomidate on the concentration-response curve for phenylephrine (10^{-9} to 10^{-6} M) was assessed by comparing the contractile response in the presence and absence of etomidate (5×10^{-6} and 5×10^{-5} M). In addition, the effect of lipofundin medium-chain triglyceride/long-chain triglyceride (MCT/LCT) 20% (the vehicle for etomidate), at a dose equivalent to that administered with the highest concentration of etomidate, on the phenylephrine concentration-response curve was also assessed.

The second series of experiments was designed to assess the effect of etomidate on the serotonin receptor agonist 5-hydroxytryptamine-induced contraction in the endothelium-denuded aortic rings. The effect of etomidate on the concentration-response curve for 5-hydroxytryptamine (10^{-7} to 10^{-4} M) was assessed by comparing the contractile response in the presence and absence of etomidate (5×10^{-6}, 3×10^{-5} and 5×10^{-5} M). The etomidate was added directly to the organ bath 20 min before the 5-hydroxytryptamine-induced contraction.

In the third series of experiments, the effect of etomidate on the contractile response induced by KCl (potassium chloride) was assessed by comparing the KCl dose (10 to 60 mM)-response curves obtained in the presence and absence of etomidate (5×10^{-6}, 5×10^{-5} M). The etomidate was added directly to the organ bath 20 min before the KCl-induced contraction.

In the fourth series of experiments, the involvement of L-type calcium channels in the etomidate-induced attenuation of contractile response induced by phenylephrine was examined. For the endothelium-denuded rings pretreated with the L-type calcium channel blocker verapamil (10^{-5} M), the effect of etomidate (5×10^{-5} M) on the concentration-response curve for phenylephrine was assessed by comparing the contractile response in the presence and absence of etomidate (5×10^{-5} M). The incubation period for the verapamil (10^{-5} M) plus etomidate (5×10^{-5} M) or verapamil (10^{-5} M) alone was 20 min before the phenylephrine-induced contraction.

TABLE I Effect of etomidate on phenylephrine-induced contraction in isolated endothelium-denuded aortic rings

	N	*Contraction by 60 mM KCl (g)*	*Log ED_{50}*	*Maximal contraction (%)*
No drug	11	2.53 ± 0.50	-8.28 ± 0.27	127 ± 12
Etomidate 5×10^{-6} M	8	2.27 ± 0.46	-8.13 ± 0.19	128 ± 16
Etomidate 5×10^{-5} M	7	2.44 ± 0.40	-7.95 ± 0.31*	118 ± 15
Vehicle	6	2.68 ± 0.16	-8.20 ± 0.11	119 ± 7

N: number of rats. Contraction by 60 mM KCl (g): contractile response to isotonic 60 mM KCl. Maximal contraction (%): phenylephrine-induced maximal contraction as the percentage of isotonic 60 mM KCl-induced contractile response. *$P < 0.05$ *vs* no drug.

TABLE II Effect of etomidate on 5-hydroxytryptamine-induced contraction in isolated endothelium-denuded aortic rings

	N	*Contraction by 60 mM KCl (g)*	*Log ED_{50}*	*Maximal contraction (%)*
No drug	8	2.69 ± 0.50	-6.09 ± 0.28	122 ± 10
Etomidate 5×10^{-6} M	6	2.68 ± 0.37	-6.01 ± 0.27	125 ± 7
Etomidate 3×10^{-5} M	6	2.74 ± 0.38	-5.80 ± 0.30	120 ± 10
Etomidate 5×10^{-5} M	6	2.65 ± 0.50	-5.51 ± 0.07*	109 ± 7*

N: number of rats. Contraction by 60 mM KCl (g): contractile response to isotonic 60 mM KCl. Maximal contraction (%): 5-hydroxytrytamine-induced maximal contraction as the percentage of isotonic 60 mM KCl-induced contractile response. *$P < 0.05$ *vs* no drug.

In the final experiment, the participation of a decreased calcium influx from the extracellular to the intracellular space during the etomidate-induced attenuation of the contractile response induced by the contractile agonists (phenylephrine and 5-hydroxytryptamine) was examined. The denuded aortic rings were exposed to a calcium-free Kreb solution containing 2 mM of ethylene glycol-bis (ß-aminoethyl ether)-N,N,N',N',-tetraacetic acid (EGTA) for ten minutes. This solution was then replaced with a calcium-free isotonic depolarizing solution containing a high concentration of K^+ (100 mM KCl). The etomidate was added directly to the calcium-free isotonic depolarizing solution containing a high concentration of KCl (100 mM) 15 min before the calcium (Ca^{2+})-induced contraction. Finally, the calcium was added cumulatively to achieve a final bath concentration (from 0.5 to 2.5 mM).[7] The effect of etomidate on the concentration-response curve for calcium was assessed by comparing the contractile response induced by the addition of calcium in the presence and absence of etomidate (5×10^{-5} M).

Drugs and solutions

All drugs were of the highest purity commercially available: phenylephrine HCl, 5-hydroxytryptamine, verapamil hydrochloride, EGTA (Sigma Chemical, St. Louis, MO, USA), etomidate (Etomidate Lipuro, B. Brown, Melsung, German), lipofundin MCT/LCT 20% (B. Brown, Melsung, German). Etomidate was dissolved in lipofundin MCT/LCT 20% and diluted in distilled water, and it was tested at several concentrations (5×10^{-6}, 3×10^{-5} and 5×10^{-5} M). All drug concentrations were expressed as the final molar concentration in the organ bath. All other drugs were dissolved and diluted in distilled water.

Data analysis

Values are expressed as mean ± SD. Contractile responses to phenylephrine, 5-hydroxytryptamine and calcium (Ca^{2+}) are expressed as the percentages of the maximum contraction to isotonic 60 mM KCl. The logarithm of the drug concentration (ED_{50}) eliciting 50% of the maximal contractile response was calculated by non-liner regression analysis by fitting the concentration-response relation for each drug (phenylephrine and 5-hydoxytryptamine) to a sigmoidal curve using commercially available software (Prism version 2.0; Graph Pad Software, San Diego, CA, USA). The contractile agonists (phenylephrine and 5-hydroxytryptamine)-induced maximal contractile response was measured as the percentage of the maximum contraction to isotonic 60 mM KCl. Statistical analysis for comparison of ED_{50} and contractions (maximal contraction or contractions at each agonist concentrations) between no drug and treated groups was performed using the Student's t test. Differences were considered statistically significant at P values < 0.05. N refers to the number of rats whose descending thoracic aortic rings were used in each protocol.

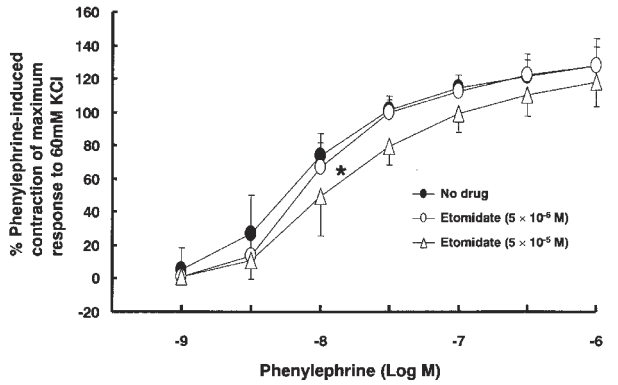

FIGURE 1 Effect of etomidate on the phenylephrine concentration-response curve in the endothelium-denuded rings. Low dose etomidate (5×10^{-6} M) had no significant effect on phenylephrine-induced contraction. However, high dose etomidate (5×10^{-5} M) produced (ED_{50}: $^*P <$ 0.05 *vs* no drug) a significant rightward shift in the phenylephrine concentration-response curve compared with the etomidate untreated rings.

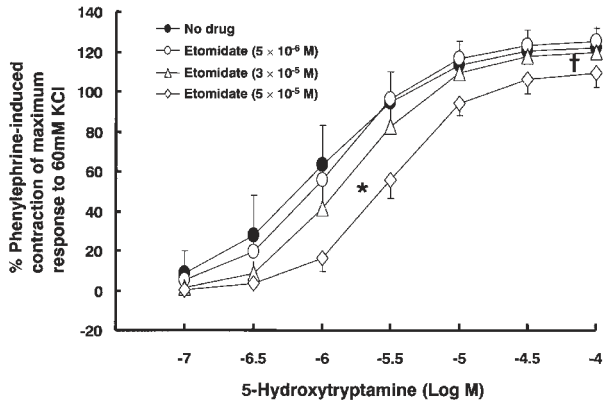

FIGURE 2 Effect of etomidate on the 5-hydroxytryptamine concentration-response curve in the endothelium-denuded rings. Low dose etomidate (5×10^{-6}, 3×10^{-5} M) had no significant effect on 5-hydroxytryptamine-induced contraction, whereas high dose etomidate (5×10^{-5} M) produced (ED_{50}: $^*P < 0.05$ *vs* no drug) a significant rightward shift in the 5-hydroxytryptamine concentration-response curve, and it attenuated ($\dagger P < 0.05$ *vs* no drug) the maximal contractile response compared with the etomidate untreated rings.

Results

Effects of etomidate on phenylephrine-induced contraction

In the endothelium-denuded rings, low dose etomidate (5×10^{-6} M) had no effect on the phenylephrine-induced contraction, whereas high-dose etomidate (5×10^{-5} M) significantly increased the ED_{50} ($^*P = 0.04$) for phenylephrine compared with the rings untreated with etomidate (Table I and Figure 1). Lipofundin MCT/LCT 20% (the vehicle for etomidate) at a concentration corresponding to the highest concentration of etomidate did not significantly alter the phenylephrine-induced contraction (Table I).

Effect of etomidate on 5-hydroxytryptamine-induced contraction

Low dose etomidate (5×10^{-6}, 3×10^{-5} M) did not significantly alter the 5-hydroxytryptamine-induced contraction. High dose etomidate (5×10^{-5} M) significantly increased the ED_{50} ($^*P = 0.0004$) for 5-hydroxytryptamine compared with the rings untreated with etomidate (Table II and Figure 2).

Effect of etomidate on the contractile response induced by KCl (potassium chloride)

Low dose etomidate (5×10^{-6} M) had no significant effect on the contraction induced by KCl, whereas high dose etomidate (5×10^{-5} M) significantly attenuated ($P < 0.05$) the contraction compared with the rings untreated with etomidate (Figure 3).

Effect of etomidate on phenylephrine-induced contraction in the rings pretreated with verapamil (10^{-5} M)

In endothelium-denuded rings pretreated with verapamil (10^{-5} M), high dose etomidate (5×10^{-5} M) had no effect on the phenylephrine-induced contraction compared with the rings untreated with etomidate (Table III and Figure 4).

Effect of etomidate on contractile response induced by the addition of calcium (Ca^{2+}) in the calcium-free isotonic depolarizing solution containing 100 mM KCl

In the calcium-free isotonic depolarizing solution containing 100 mM KCl, high dose etomidate (5×10^{-5} M) significantly attenuated ($P < 0.05$) the contraction induced by the cumulative addition of calcium (0.5 to 2.5 mM) compared with the rings untreated with etomidate (Figure 5).

Effect of verapamil on phenylephrine-induced contraction

Verapamil (10^{-5} M) significantly increased the ED_{50} ($^*P < 0.05$) for phenylephrine and decreased phenylephrine-induced maximal contraction compared with the verapamil untreated rings (Table IV, Figure 6).

TABLE III Effect of etomidate on phenylephrine-induced contraction in isolated endothelium-denuded aortic rings pretreated with verapamil

	N	*Contraction by 60 mM KCl (g)*	*Log ED_{50}*	*Maximal contraction (%)*
Verapamil 10^{-5} M	6	2.67 ± 0.48	-6.79 ± 0.24	77 ± 14
Verapamil 10^{-5} M + Etomidate 5×10^{-5} M	6	2.54 ± 0.62	-6.90 ± 0.20	78 ± 13

N: number of rats. Contraction by 60 mM KCl (g): contractile response to isotonic 60 mM KCl.
Maximal contraction (%): phenylephrine-induced maximal contraction as the percentage of isotonic 60 mM KCl-induced contractile response.

TABLE IV Effect of verapamil on phenylephrine-induced contraction in isolated endothelium-denuded aortic rings

	N	*Contraction by 60 mM KCl (g)*	*Log ED_{50}*	*Maximal contraction (%)*
No drug	11	2.53 ± 0.50	-8.28 ± 0.27	127 ± 12
Verapamil 10^{-5} M	6	2.67 ± 0.48	-6.79 ± 0.24*	77 ± 14*

N: number of rats. Contraction by 60 mM KCl (g): contractile response to isotonic 60 mM KCl.
Maximal contraction (%): phenylephrine-induced maximal contraction as the percentage of isotonic 60 mM KCl-induced contractile response. *$P < 0.05$ *vs* no drug.

Discussion

Despite the wide spread use of etomidate as an *iv* anesthetic that has minimal acute effects on hemodynamics, this is the first study to assess the effects of etomidate on phenylephrine-induced contraction in rat aortas. Our results suggest that high dose etomidate (5×10^{-5} M) attenuates phenylephrine-induced contraction by having an inhibitory effect on the calcium influx by blocking the L-type calcium channels in the rat aortic vascular smooth muscle.

Mechanism of etomidate-induced attenuation

The mechanism for the increase in the cytosolic Ca^{2+} level ($[Ca^{2+}]_i$) in vascular smooth muscle can be explained by two different calcium influx pathways: the receptor-linked calcium channels and the voltage-dependent calcium channels.[8] High K^+ levels induce membrane depolarization that in turn opens the voltage-dependent calcium channels.[8] The interaction of the contractile agonists (phenylephrine and 5-hdroxytryptamine) with their receptors induces the generation of inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) that activates protein kinase C.[8] This 1,4,5 IP_3 binds to its receptor in the sarcoplasmic reticulum, and this releases calcium from the sarcoplasmic reticulum to induce an initial transient contraction and subsequently opens the receptor-linked calcium channels, which may be activated by IP_3 and inositol 1,3,4,5-tetrakisphosphate.[9] Etomidate (5×10^{-5} M) attenuated the contractile response induced by the α-1 adrenoceptor agonist phenylephrine and the serotonin

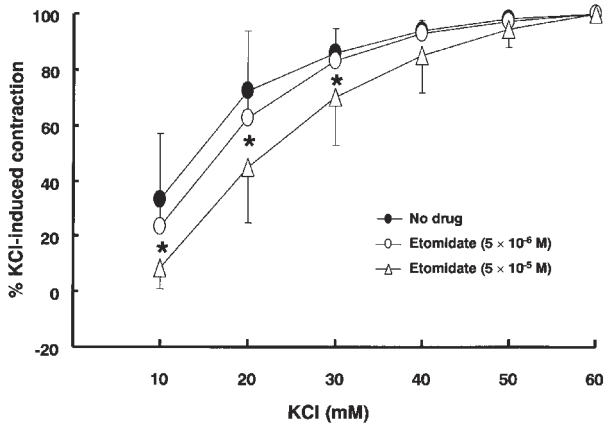

FIGURE 3 Effect of etomidate on the KCl (potassium chloride) dose-response curve in the endothelium-denuded rings. High dose etomidate (5×10^{-5} M) produced (*$P < 0.05$ *vs* no drug) a significant rightward shift in the KCl dose (10 to 60 mM)-response curve compared with the etomidate untreated rings. Data are shown as mean ± SD, and they are expressed as a percentage of the maximal contraction induced by 60 mM KCl (60 mM KCl-induced contraction: 100% = 2.73 ± 0.66 g [N = 8], 100% = 2.67 ± 0.57 g [N = 8], 100% = 2.94 ± 0.55 g [N = 8] for the etomidate untreated rings, the etomidate [5×10^{-6} M] and [5×10^{-5} M] pretreated rings, respectively).

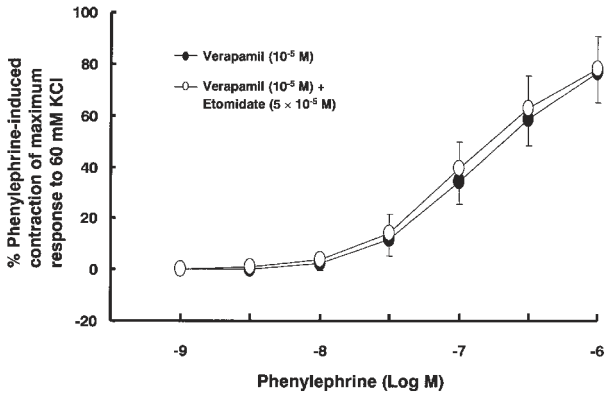

FIGURE 4 Effect of etomidate on the phenylephrine concentration-response curve in the endothelium-denuded rings pretreated with verapamil. In rings pretreated with verapamil (10^{-5} M), high dose etomidate (5×10^{-5} M) had no effect on the phenylephrine-induced contraction compared with the rings untreated with etomidate.

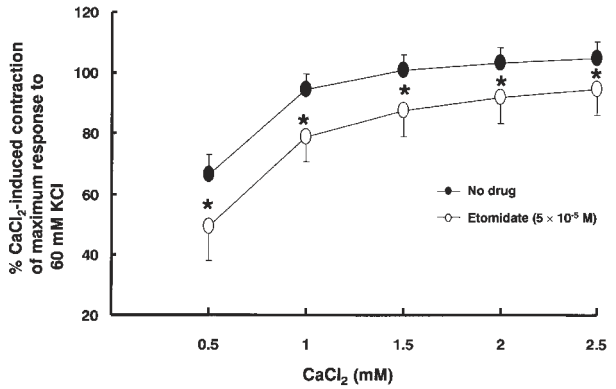

FIGURE 5 The effect of etomidate (5×10^{-5} M) on the contractile response in endothelium-denuded rings induced by the addition of calcium (0.5 to 2.5 mM) in a previously calcium-free isotonic depolarizing solution containing 100 mM KCl. Etomidate (5×10^{-5} M) attenuated (*$P < 0.05$ *vs* no drug) the contractile response induced by addition of calcium compared with the etomidate untreated rings. Data are shown as mean ± SD, and they are expressed as percentages of maximal contraction induced by isotonic 60 mM KCl (isotonic 60 mM KCl-induced contraction: 100% = 3.42 ± 0.52 g [N = 7], 100% = 3.18 ± 0.34 g [N = 7] for the etomidate untreated and etomidate [5×10^{-5} M] treated rings, respectively).

receptor agonist 5-hydroxytryptamine. This result suggests that etomidate-induced attenuation could be due to a nonspecific action on cellular calcium homeostasis in rat aorta rather than to an action via specific receptors for the contractile agonists.

A previous study has suggested that the norepinephrine-induced increase in $[Ca^{2+}]_i$ is due to Ca^{2+} influx through both the L-type and non-L-type calcium channels.[10] Some overlap between the electromechanical (via the voltage-dependent calcium channels) and pharmacomechanical (via the non-voltage-dependent calcium channels) coupling occurs.[11] The receptor-linked calcium channel is less sensitive to calcium channel blockers, including verapamil and nifedipine than is the voltage-dependent calcium channel.[8] In accordance with previous reports,[8,10] verapamil (10^{-5} M) attenuated ($P < 0.05$) the phenylephrine-induced contraction in this *in vitro* experiment (Table IV and Figure 6). Any inhibition of the receptor-mediated responses by calcium antagonists appears to depend on the transduction system and a specific cellular mechanism (e.g., the voltage-dependent calcium channel opening consequent to partial depolarization) activated by the receptor for the contractile agonists.[12] Noradrenaline induces contraction of rat aortic ring by activating calcium release and the subsequent calcium influx through the voltage-dependent calcium channels and the receptor-operated calcium channels.[13] Taking the above previous reports[8,10–13] into consideration, the verapamil-induced attenuation of the contractile response induced by phenylephrine that was observed in this *in vitro* experiment suggests that the voltage-dependent calcium channels are associated with the cellular signal transduction pathway for phenylephrine-induced contraction in rat aorta smooth muscle.

Etomidate (5×10^{-5} M) inhibited the KCl-induced contraction through the activation of voltage-dependent calcium channels. Verapamil (10^{-5} M) pretreatment abolished the etomidate (5×10^{-5} M)-induced attenuation of the contractile response induced by phenylephrine. Taken together, these results suggest that etomidate would act on vascular smooth muscle as a calcium channel blocker. Norepinephrine and other contractile agonists seem to open the same verapamil-sensitive, L-type Ca^{2+} channels as high concentrations of K^+, and this channel[8] may be the major Ca^{2+} influx pathway in smooth muscle. Reinforced with our results from previous *in vitro* protocols, etomidate attenuated the contractile response induced by the addition of calcium (Ca^{2+}) in the calcium-free isotonic depolarizing solution containing 100 mM KCl. Taken together, these results indicate that etomidate (5×10^{-5} M) attenuates the contractile response

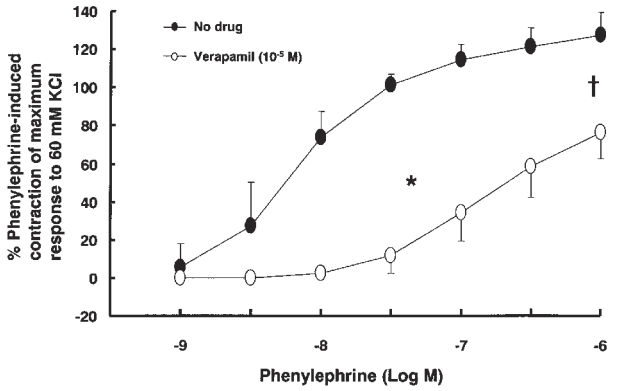

FIGURE 6 Effect of verapamil on the phenylephrine concentration-response curve in the endothelium-denuded rings. Verapamil (10^{-5} M) produced (ED_{50}: $^*P < 0.05$ *vs* no drug) a significant rightward shift in the phenylephrine concentration-response curve and attenuated ($\dagger P < 0.05$ *vs* no drug) the maximal contractile response compared with the verapamil untreated rings.

induced by contractile agonists (phenylephrine and 5-hyroxytryptamine) via an inhibitory effect on calcium influx from the extracellular to the intracellular space through L-type calcium channels in vascular smooth muscle. High dose etomidate (10^{-5}, 10^{-4}, 1.2×10^{-4}, 9.3×10^{-5} M)[5,6,14,15] inhibits the angiotensin II-induced calcium influx and acetylcholine-induced calcium response, produces a half-maximal relaxation of potassium chloride- or norepinephrine-precontracted human internal mammary artery, and reduces the histamine-induced maximal contraction. In addition, etomidate (5×10^{-6}, 5×10^{-5} M) inhibits endothelium-dependent relaxation, and L-type calcium current in canine ventricular cells.[16,17] The etomidate-induced attenuation of the contractile response induced by agonists observed in this *in vitro* experiment was in agreement with other previous studies.[5,15,17] Further investigations are required to determine the effect of etomidate on G protein, phospholipase C, the coupling processes, IP_3 and DAG, as all of these factors are involved in the cellular signal transduction pathway.

Clinical applications

The peak plasma concentration of etomidate during induction of general anesthesia is approximately 10^{-5} M,[18,19] whereas the free plasma concentration is likely to be 2.5×10^{-6} M because about 76.5% of etomidate is bound to plasma protein.[20] Thus, etomidate (5×10^{-6} M) at a clinically relevant concentration in this *in vitro* experiment had no significant effect on the phenylephrine-induced contraction. In addition, the concentration of etomidate (5×10^{-5} M) that demonstrated an inhibitory effect on the phenylephrine-induced contraction would be higher than the expected free plasma concentration after an induction dose of etomidate. However, in the field of cerebral aneurysm surgery and neurointensive care, high dose etomidate (0.73 ± 0.49 mg·kg^{-1}) would be used to induce electroencephalographic burst suppression for cerebral protection.[5] The etomidate dose required to reach electroencephalographic burst suppression without other anesthetics would be 1.28 ± 0.11 mg·kg^{-1}, which is about four times more than the conventional induction dose of 0.3 mg·kg^{-1} etomidate.[21] Taking the above two factors[5,21] into consideration, 5×10^{-5} M etomidate required for an inhibitory effect on phenylephrine-induced contraction might be the concentration encountered in the clinical setting.

Bolus administration of etomidate (0.26 ± 0.06 mg·kg^{-1}) does not affect systemic vascular resistance during cardiopulmonary bypass (= constant pump flow).[22] In addition, etomidate has no effect on arterial pressure and systemic vascular resistance,[2] and does not alter systemic vascular resistance in elderly patients undergoing upper abdominal surgery.[23] In this *in vitro* study, etomidate (5×10^{-6} M) at a clinically relevant concentration had no significant effect on both phenylephrine-induced and KCl-induced contraction, whereas high dose etomidate (5×10^{-5} M) attenuated the contraction. Any clinical implication for etomidate on the regional hemodynamics must be tempered by the fact that a large conduit artery like the aorta was used in this *in vitro* study, whereas organ blood flow is controlled by diameter changes of the arterioles that have diameters less than 150 μm. In addition, a previous study[24] has shown differential responses for rat aorta and the mesenteric artery to norepinephrine and serotonin *in vitro*. Even with these limitations, however, our findings may help explain the minimal hemodynamic change that follows an induction dose of etomidate,[2,22,23] and also may help explain moderate hypotension following higher electroencephalographic burst suppression dose.[5]

Conclusion

In conclusion, our study has shown that 5×10^{-5} M etomidate, which exceeds the clinically relevant concentration, attenuates the contractile response induced by agonists (phenylephrine and 5-hydroxytryptamine) in rat aortic ring. This suggests that etomidate has an inhibitory effect on calcium influx from the extracel-

lular to the intracellular space by blocking the L-type calcium channels in rat aortic vascular smooth muscle.

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