原 著

Etomidate Produces Vasodilation by Mixed Endothelium Dependent and Independent Mechanisms

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ABSTRACT

Etomidate has been used in clinical amesthesia because of its little effect on cardiovascular and central nervous system. There were few reports about the direct effect of etomidate on the vessels, and we investigated the vasodilatory action of etomidate on rat thoracic aortic rings with and without endothelium. Sixteen male Sprague-Dawley rats (BW 353 ± 9 gm ; mean ± SEM) were used to collect thoracic aortic rings, cut into 3 mm width segments, which were immersed in the muscle baths contained Krebs-Henseleit solution and aerated with 95 % O_2-5 % CO_2. Randomly selected rings were denuded. Phenylephrine (3×10^{-7} M for endothelium intact rings, 1×10^{-7} M for denuded ones) or KCI (40 mM for intact, 30 mM for denuded) was added to contract the preparations, then etomidate (3×10^{-6} M to 3×10^{-4} M) was cumulatively added to the baths. In KCl contracted rings, etomidate produced more relaxation on endothelium intact rings than denuded ones significantly at 3×10^{-6} M, 1×10^{-5}M, 3×10^{-5}M, 6×10^{-5}M and 1×10^{-4}M (4.2±1.4 % vs 0.0 ±1.1 %, 15.0±2.5 % vs 4.0±1.3 %, 37.1±3.5 % vs 20.3±3.3 %, 58.8±3.3 % vs 43.3±4.4 % and 74.8±2.6 % vs 64.0±4.4 %, respectively ; mean ± SEM). In phenylephrine contracted rings, etomidate showed more relaxation on endothelium intact rings than denuded ones at 3×10^{-6}M, 1×10^{-5}M, 3×10^{-5}M and 6×10^{-5}M (7.5±1.8 % vs 2.2 ±0.9 %, 17.5±2.7 % vs 7.8±1.7 %, 37.4±3.4 % vs 20.4±2.3 % and 54.7±4.3 % vs 41.7± 4.1 %, respectively).

In conclusion, etomidate produced mixed endothelium-dependent and independent relaxation on rat thoracic aorta by nonspecific mechanism. Because of less relaxation in endothelium denuded rings, etomidate could be available safely even for patients with diabetes, hypertension or atherosclerosis.

Key words : Etomidate, Endothelium, Rat, Thoracic aorta, Vascular smooth muscle

Introduction

Normal vascular endothelium produces and releases both endothelium-derived relaxing factor (EDRF) for vasodilation and endothelium-derived contracting factor (EDCF) for vasoconstriction[1~5]. Many factors and mechanisms affecting vascular tone are identified[6~11]. Recently cultured vascular smooth muscle cells and endothelial cells have been used to investigate the interaction with nitric oxide[12,13]. Some drug-mediated vasodilation depends upon EDRF release (i.e., acethylcholine) while other drugs act directly on the smooth muscle (i.e., nitroprusside)[1,2]. Vascular ring has been reported to constrict by receptor-mediated intracellular Ca^{2+} release (i.e., phenylephrine) or by voltage-gated

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extracellular Ca^{2+} influx (i.e., KCl).

Some diseases such as essential hypertension, diabetes mellitus and atherosclerosis alter the normal endothelial function[14~17]. Recently the number of patients sufferring for these diseases is increasing and the opportunity of anesthesia for these patients is also increasing year by year. Because of these interactions, it is important to understand the direct vascular effects of anesthetic drugs that may be administered to patients with potentially abnormal endothelial function. Etomidate is a relatively new intravenous anesthetic which has a little effect on hemodynamics and central nervous system[18~20]. On the other hand, etomidate has reported to produce a cardiovascular instability following a bolus administration, but it mostly might be contributed to its vehicle, propylene glycol[21]. In this study, we examined the vascular response to etomidate in order to answer whether etomidate produces direct vasodilation, whether the vasodilation is endothelium dependent, and whether the etomidate vehicle, propylene glycol, contributes to the vasodilation.

Methods

After obtaining the approval of the institutional animal research committee, 16 male Spregue-Dawley rats (body weight 353 ± 9 gm ; mean ± SEM) were used. Rats were anesthetized with isoflurane. The thoracic aorta was removed from diaphragm to heart then placed in oxygenated Krebs-Henseleit (K-H) solution and dissected free of fat and connective tissue carefully not to damage the endothelial cell layers or stretch the vessels. Aortic rings, cut into 3 mm width segments, were mounted between 2 stainless steel wires and placed in 20 ml muscle baths containing a modified K-H solution of the following composition (mM): KCl 4.75, KH_2PO_4 1.19, $MgSO_4 \cdot 7H_2O$ 1.19, $CaCl_2 \cdot 2H_2O$ 2.54, NaCl 119, $NaHCO_3$ 25 and glucose 11, pH 7.4. The solution was continously aerated with a gas mixture of 95 % O_2 and 5 % CO_2, and maintained at 37 ℃. Tissues were equilibrated for 2h under a resting tension of 2 gram with changing of bath fluids every 15 min. Randomly selected rings were denuded of endothelium by gently rubbing the surface with forceps without damaging the smooth muscle. Isometric tension was measured with a Grass FT .03 force-displacement transducer (Quincy, MA, USA) and recorded with a Grass polygraph (Quincy, MA, USA)[22].

After a 2h equilibration period, the preparation was contracted with 3×10^{-7}M phenylephrine for the intact endothelium, 1×10^{-7}M for the denuded rings to test the integrity of the endothelium. This concentration of phenylephrine gives a submaximal tone (50-70 % of maximum) as previously determined in our laboratory. Acethylcholine (10^{-5}M) was then added to the bath. If the relaxation was more than 50 %, the ring was considered to be endothelium intact. Nitroprusside (10^{-8} to 10^{-7} M) was added endothelium denuded rings to test whether vascular smooth muscle was damaged (Fig. 1).

After this verification of the preparation, phenylephrine (3×10^{-7} M for endothelium intact, 1×10-7 M for endothelium denuded rings) or KCI (40 mM for endothelium intact, 30 mM for endothelium denuded rings) was added to precontract the rings. Then etomidate, from 3×10^{-6} M to 3×10^{-4} M concentrations, or propylene glycol, the same concentration as etomidate's vehicle included, were cumulatively added to the bath. At the end of the experiments the tissues were blotted and weighed. Relaxation responses were expressed as percent of decreased tension produced by etomidate or propylene glycol per contractile force elicited by either phenylephrine or KCl. All data were expressed as mean ± SEM. Differences between mean values were assessed by the analysis of variance followed by the unpaired Student's t-test. The paired t-test was used for paired comparison. A P value less than 0.05 was considered statistically significant.

Results

EFFECT OF ETOMIDATE

The tension developed after administration of submaximal concentration of phenylephrine and KCl is shown in Table. Although similar tension was developed with phenylephrine and KCl in aortic rings with denuded endothelium, tension with KCl was greater than that with phenylephrine in intact endothelium. Tension developed after phenylephrine and KCl was greater in denuded than in intact preparations. The weight of KCl group was heavier than that of phenylephrine group in intact rings.

The actual tracings of which demonstrate concentration dependent relaxing response for etomidate

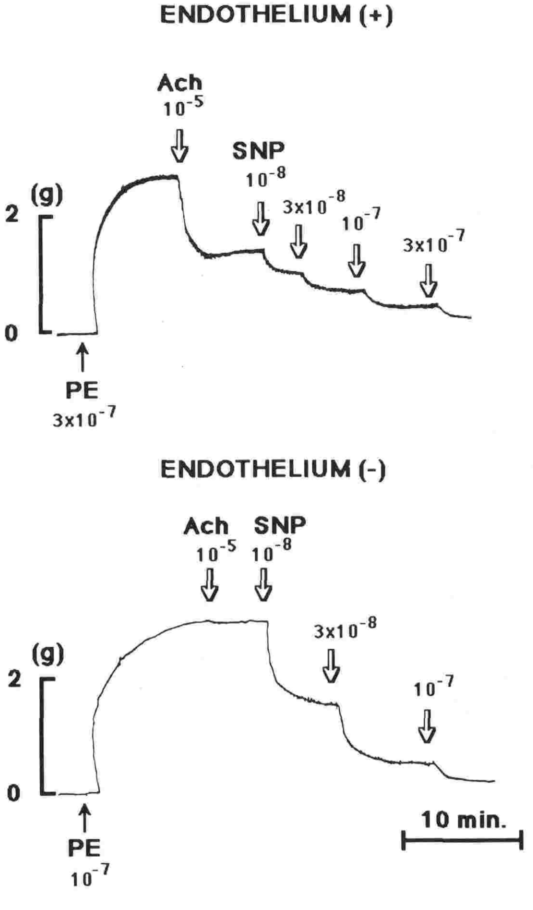


Figure 1. Verification of integrity of endothelium The upper column showes an actual change of vascular contractility in endothelum intact preparation after precontracted with 3×10^{-7}M phenylephrine (PE). The preparation was relaxed more than 50% with 10^{-5}M acethylcholine (Ach) administration. The lower column showes a change in endothelium denuded preparation and it was not relaxed by Ach, but was relaxed more than 70% with 3×10^{-7}M sodium nitroprusside (SNP) administration.

Table Absolute tension after precontraction and aortic ring weight

Group	Tension (mg)	Weight (mg)
ED(+) PE (n=12)	1479±119	3.63±0.17
ED(−) PE (n=10)	2403±101*	3.65±0.18
ED(+) KCl (n=12)	1813±156**	4.02±0.42**
ED(−) KCl (n=12)	2252±112*	3.66±0.36*

All date are expressed as average ± SEM.
ED(+): endothelium intact. ED(−): endothelium denuded.
PE : phenylephrine.
*$p<0.05$ vs ED(+), **$p<0.05$ vs PE.

in the KCl and phenylephrine contracted aorta with intact endothelium are shown in Fig.2. The rings began to relax at 10^{-5}M concentration.

Etomidate induced relaxation, expressed as percent relaxation of the KCl precontracted aorta, was compared in relation to etomidate dose in the presence and absence of endothelium. Etomidate produced concentration dependent relaxation that was significantly attenuated in the endothelium denuded rings compared to the endothelium intact rings at 3×10^{-6}, 10^{-5}, 3×10^{-5}, 6×10^{-5} and 10^{-4}M concentrations (Fig.3). Similarly, in the phenylephrine precontracted aorta, etomidate produced less relaxation in the endothelium denuded preparation when compared to the endothelium intact preparation at 3×10^{-6}, 10^{-5}, 3×10^{-5} and 6×10^{-5}M concentrations (Fig.3). There was no difference when the degree of relaxation by etomidate was compared in the aortic rings precontracted with either KCl or phenylephrine.

EFFECT OF PROPYLENE GLYCOL

Propylene glycol produced concentration dependent relaxation only in the endothelium intact rings precontracted with KCl whereas there was no change in the endothelium denuded rings (Fig.4). On the other hand, propylene glycol produced concentration dependent relaxation both in the endothelium intact and denuded rings precontracted with phenylephrine (Fig.4).

Discussion

The verification of vascular endothelial integrity was performed by using acetylcholine after phenylephrine. A relaxant response of less than 50 % of phenylephrine-induced contraction was regarded as unsatisfactory for endothelium intact ring, and the relaxant response of more than 10 % was regarded as unsatisfactory for the endothelium denuded ring. A function of vascular smooth muscle in the denuded ring was also comfirmed by using sodium nitroprusside showing relaxation more than 70 % of phenylephrine-induced contraction.

Although the absolute precontraction in denuded rings was significantly stronger than that in intact rings, the difference was small because of administrating low concentrations of either phenylephrine or KCl. And the precontraction in both rings was aimed at 50 to 70 % of the maximum contraction observed in our primitive study.

Etomidate has been reported to possess minimum effect on the central nervous system and cardiovascular system. Etomidate protects brain cells by inhibition of abnormal excitation due to GABA-mimetic action, but it has little effect on peripheral GABA receptor. Recently it was suggested that etomidate depressed the myocardial cell contraction in vitro. This mechanism was related to inhibition of transsarcolemmal Ca^{2+} influx[23].

There has been no report concerning the direct effect of etomidate on vessels. In this study we found that etomidate possessed the vasodilatory action. These results indicated that etomidate-induced vasodilation was partly endothelium dependent and partly endothelium independent because the relaxation induced by etomidate was still remained after the removal of endothelium. These mechanisms were thought to be the increase of c-GMP following the release of EDRF from endothelial cells and/or to be the inhibition of Ca^{2+} release from intracellular sarcoplasmic reticulum. But this relaxation was considered to be more dependent on endothelial cells.

There was no difference in the degree of relaxation by etomidate between contracted aortic rings with KCl and phenylephrine. These results suggest that etomidate produces vasodilation by a nonspecific mechanism because KCl and phenylephrine produce the contraction by different mechanisms : by extracellular Ca^{2+} influx through voltage gated Ca^{2+} channels for KCl and receptor operated in-

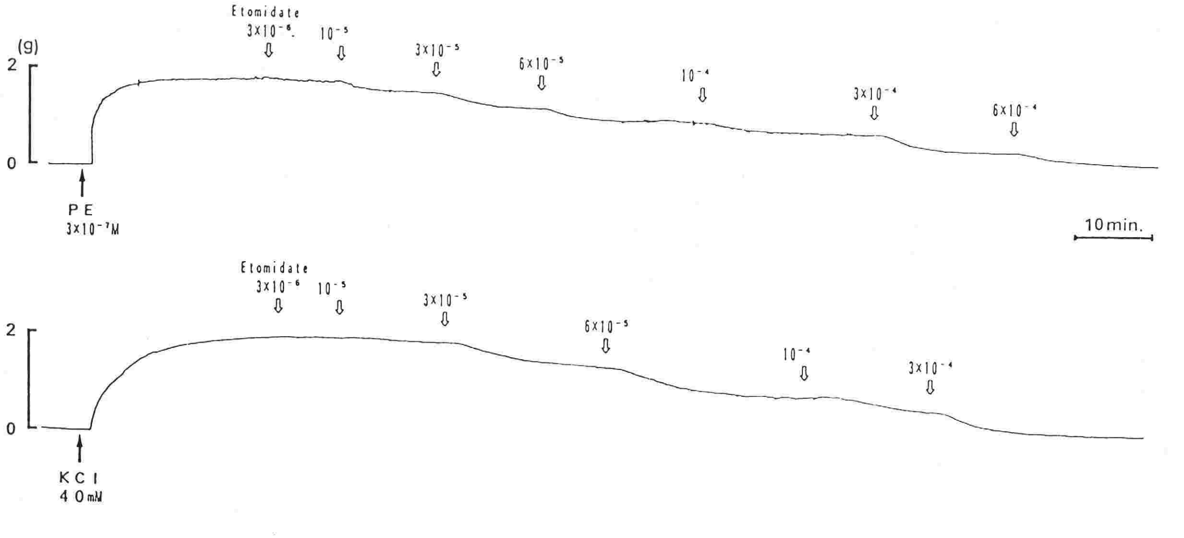
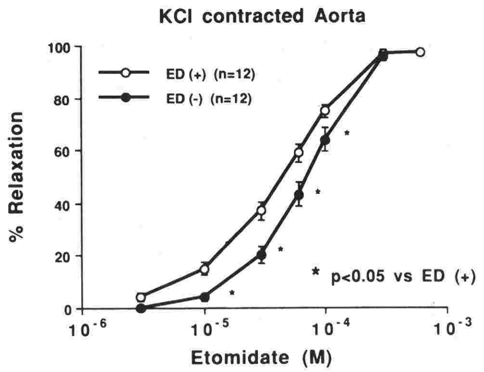


Figure 2. Actual recordings of the relaxation response for etomidate
The upper tracing showes an actual relaxation induced by cumulatively added etomidate in the phenylephrine contracted aorta. The lower tracing indicates in the KCl contracted aorta. Both recordings demonstrate concentration relaxation response for etomidate which began at 10^{-5}M. PE = phenylephrine.

tracellular Ca^{2+} release and Ca^{2+} channels for phenylephrine.

Etomidate is dissolved in 35 % propylene glycol, but propylene glycol has been reported to produce cardiovascular depression or pulmonary edema due to anaphylactic vasodilatory effects[24~26]. Accordingly, it might be possible that vasodilatory effect of etomidate may partly be due to the action of the vehicle. In this study, propylene glycol produced vasodilation by mixed endothelium-dependent and independent machanisms same as etomidate. The vasodilating effect of propylene glycol was about half of those by etomidate on the aortic rings with endothelium, and about one third effects of etomidate on the aortic rings without endothelium precontracted by phenylephrine, or nearly no effects on the rings precontracted by KCl. From these results, the solvent of etomidate, propylene glycol appeared to possess the vasodilating effects but not so strong as etomidate[27].



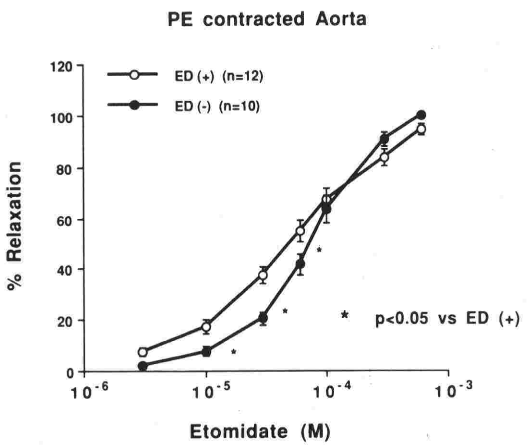


Figure 3. The effects of etomidate on aortic rings contracted with KCl or phenylephrine
The upper graph indicates concentration dependent relaxation of etomidate, which is significantly attenuated in the endothelium denuded rings compared to the endothelium intact rings. Also the lower graph showes same response. (PE = phenylephrine, ED (+) = endothelium intact, ED (−) = endothelium denuded)

In conclusion, etomidate produced mixed endothelium-dependent and independent relaxation on rat thoracic aorta by the nonspecific mechanism. Because etomidate produced less relaxation in endothelium denuded rings, one might expect a less pronounced depressor effect clinically in patient with diabetes, hypertension or atherosclerosis, which adversely affect endothelium function.

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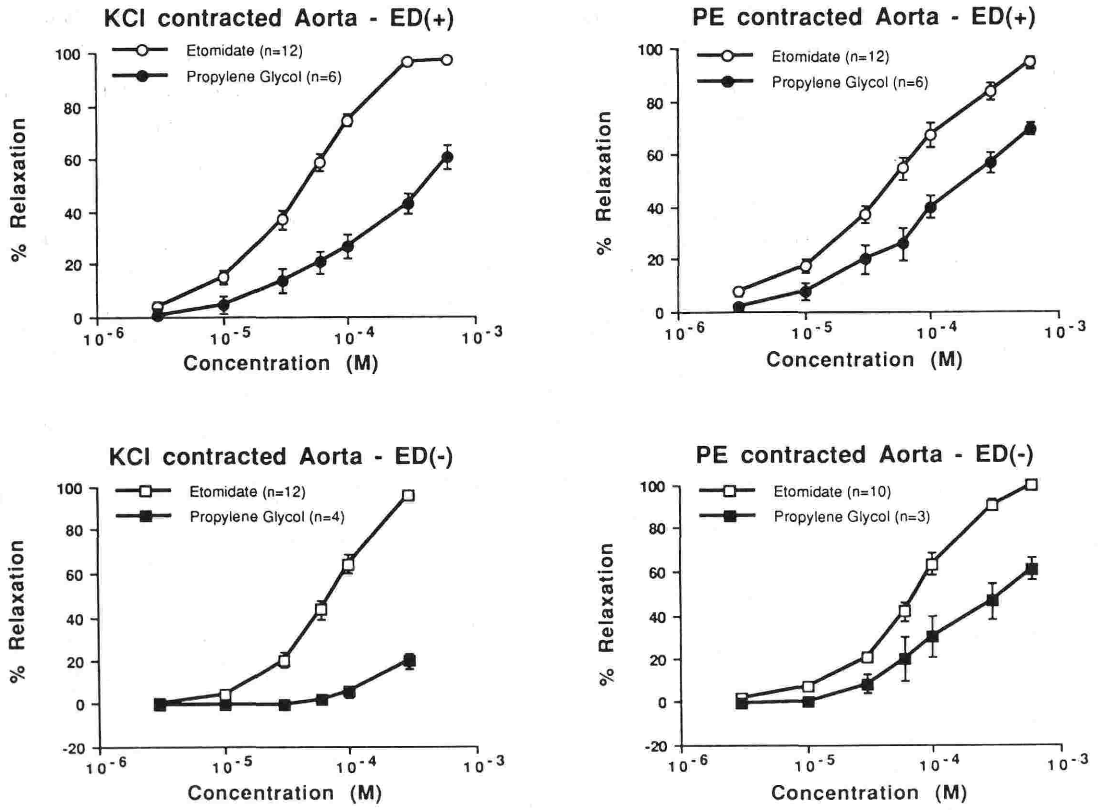

Figure 4. The effects of propylene glycol on aortic rings contracted with KCl or phenylephrine, and the comparisons with etomidate
Propylene glycol produced concentration dependent relaxation except in the endothelium denuded rings precontracted with KCl. Etomidate is appeared to have more vasodilatory property than propylene glycol. (PE=phenylephrine, ED(+)=endothelium intact, ED(−)=endothelium denuded)

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