Endothelium-dependent relaxation induced by etomidate in the aortas of insulin-resistant rats

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Abstract

Introduction: Few reports have mentioned the effect of etomidate on the aortas of insulin-resistant (IR) rats. In this study, we investigated the effect of etomidate on isolated IR aortas of rats, and explored its underlying mechanism.

Material and methods: The IR rat model was established through feeding with a high-fructose diet. The systolic blood pressure (SBP) was measured by the tail-cuff method before grouping and at the end of the 8-week feeding; blood samples were also obtained for analysis. Thoracic aorta rings of IR rats were isolated and suspended in a tissue bath. The tensile force was recorded isometrically. The effect of etomidate on provoked contraction of the rings was assessed with or without a potassium channel blocker or NO synthase inhibitor.

Results: Etomidate-induced relaxation in IR rings was greater than normal control (NC) rings (all \( p < 0.001 \) with etomidate log M of \(-4 \) to \(-6\)). NG-nitro-L-arginine methyl ester (L-NAME, an NO synthase inhibitors) inhibited etomidate-induced relaxation in NC rings, but had no effect on the IR rings (all \( p < 0.001 \) with etomidate log M of \(-4 \) to \(-6\)). Pre-incubation with glibenclamide (Gli, a potassium channel blocker) significantly inhibited etomidate-induced relaxation in NC and IR rings (all \( p < 0.001 \) with etomidate log M of \(-4 \) to \(-6\)), and had no inhibited effect on endothelial denuded aortic rings.

Conclusions: Insulin resistance increased etomidate-induced relaxation in rat aortas. Etomidate causes vasodilation in IR rat aortas via both endothelium-dependent and independent ways; impaired NO-mediated relaxation was disrupted and ATP-sensitive potassium (\( K_{ATP} \)) channel-mediated relaxation may be involved in the endothelium-dependent relaxation of etomidate in IR rats.

Key words: insulin resistance, etomidate, vascular relaxation, endothelial function, ATP-sensitive potassium (\( K_{ATP} \)) channel.

Introduction

Insulin resistance (IR) is a physiological condition in which cells have lower sensitivity or a reduced response to insulin. Insulin resistance is a risk factor associated with cardiovascular diseases including hypertension, dyslipidemia, type 2 diabetes, and obesity [1, 2]. Insulin resistance has profound negative effects on artery function throughout the body [3]. Studies have demonstrated that IR impairs vascular function of the aorta and coronary artery [4, 5], mesenteric function and cerebral vascular beds [6]. Recent studies showed that IR is not only associated with the occurrence
and development of vascular lesions, but is also associated with the surgical stress response and anesthesia safety. Insulin resistance patients have poor tolerance of surgery. In addition, the anesthetic pharmacokinetic characteristics of IR patients are also different from those of non-IR patients.

The increasing number of patients with diabetes mellitus (DM) makes the work of the anesthetist even more challenging during the perioperative period: hyperglycemia might occur in the perioperative period due to a combination of tissue insulin resistance and decreased insulin secretion [7]. In addition, general anesthesia might cause a dangerous increase or decrease of systemic or pulmonary pressure because of variations in heart rate and pulmonary flows. Such variations during catheterizing would make the procedure useless. Exploring the effects of anesthetics on vascular responsiveness could provide useful information for anesthesia of IR patients. Etomidate is an anesthetic often used in inpatients with valvular or ischemic heart disease. Etomidate does not inhibit sympathetic tone or myocardial function. Typical anesthesia induction doses of etomidate induce only minimal changes in blood pressure and heart rate [8]. Ouedraogo et al. [9] reported that etomidate exerts relaxant effects on the pulmonary artery (PA) in chronically hypoxic (CH) rats, and etomidate could inhibit KATP-mediated relaxation on the thoracic isolated IR aortas interfered with by different drugs [18].

**Material and methods**

**Animal and ethical considerations**

All procedures and experimental protocols were carried out in accordance with EU Directive 2010/63/EU for animal experiments. Thoracic arteries were taken from male Sprague-Dawley rats (3–4 months, supplied by the Animal Center of Shanxi Medical University).

After feeding for 2 weeks with the normal diet, the rats were randomly assigned to the control diet group (NC, continued to be fed with normal control diet) or the fructose diet group (IR, fed with a diet containing 60% fructose, 11% fat, and 29% protein for 8 additional weeks). All rats were given free access to food and water and maintained in a 12-h light/dark cycle. Rats were housed in cages to collect 24-h urine samples and the food and water consumption were recorded. The systolic blood pressure (SBP) was measured by a tail-cuff method before grouping and the end of the 8-week period; the blood samples were also obtained for analysis.

**Drugs and reagents**

Etomidate was purchased from Nhwa (Nhwa Pharmaceutical, Jiangsu, CHN). Intralipid was purchased from SSSP (Sino-Swed Pharmaceutical, Jiangsu, CHN). Phenylephrine hydrochloride (PE), KC1, acetylcholine chloride (ACH), sodium nitroprusside (SNP), L-NAME, indomethacin (Indo), iberiotoxin, 4-aminopyridine (4-AP) and glibenclamide (Gli) were purchased from Sigma (St. Louis, MO, USA). Gli was dissolved in ethanol (final concentration of ethanol in a tissue bath ≤ 0.5%, with no influence on PE-induced contraction). Phenylephrine hydrochloride, etomidate, ACh, SNP, 4-AP and L-NAME were dissolved in distilled water. Indo was dissolved in 4% (wt/vol) NaHCO3.

**Systolic blood pressure measurement**

Systolic blood pressure (SBP) was measured by the tail-cuff method with an electrophysymograph (PE-300, Narco Bio-Systems, Austin, TX, USA). Before the initiation of fructose feeding, rats were trained and accustomed to daily measurement of SBP by the tail-cuff method. Briefly, rats were placed (9 a.m.) in their maintenance cages and SBP measurements were performed. SBP was measured on 2 consecutive days at the same time (11 a.m.). Measurements were carried out 8 times in each rat per day; the maximum and the minimum values were rejected.

**Biochemical measurements**

Serum insulin levels were measured by radioimmunoassay method. Blood glucose levels were determined by the colorimetric analysis method (Roche Hitachi 717 Chemistry Analyzer, GMI, Inc., USA). Insulin sensitivity index (ISI) is calculated by...
the formula ISI = –ln (FBS × fasting serum insulin (FSI)); when ISI ≤ –4.88, the IR model is considered as successful.

Preparation of aortic rings for tension measurement

After 8 weeks of fructose diet feeding, rats were sacrificed by cervical dislocation then exsanguinated. The thoracic aorta was isolated and transferred into HEPES solution (pH 7.4, 4°C) immediately with the following components: NaCl 144 mM, KCl 5.8 mM, CaCl₂ 2.5 mM, MgCl₂ 1.2 mM, HEPES 5 mM, and D-glucose 11.0 mM. The aortas were cut into rings with 3 mm length. The rings were suspended on 2 wire hooks in a water-bath tube containing HEPES solution. 95% O₂ and 5% CO₂ mixed gas was maintained in the tube at 37°C. The upper hook was connected with a force transducer, and the changes in isometric force were recorded by PowerLab Chart 5.4 system (AD Instruments, Bel-Vista, New South Wale, AUS). The lower hook was fixed. Each ring was equilibrated in 10 ml of bathing solution for 120 min before the experiment. The resting tension was adjusted to 2 g (this preload was found to be optimal for force development in preliminary studies).

After the preparatory work, the rings were exposed to KCl solution (120 mM). Vasorelaxation was terminated 30 min after equilibration [19]. The rings were rinsed with fresh HEPES solution and allowed to equilibrate for an additional 60 min before the application of phenylephrine (PE) (10 μM). The rings were activated 2 times with KCl (120 mM) or PE (10 μM). The integrity of the endothelium was presumed by the observation that the relaxation induced by acetylcholine (ACh, 10 μM) on the contraction was greater than 70%. When the contraction induced by PE and the relaxation induced by ACh were reproducible, the effects of the drugs were tested.

To assess the role of the endothelium in the vascular response of etomidate, endothelium was deprived in some thoracic aortic rings through rubbing of the luminal surface with a string. The removal of the endothelium was tested by the relaxation induced by ACh (10 μM) on the contraction being less than 10%. For the relaxation studies, the submaximal contraction was provoked with PE (1 μM). When the contraction was steady (30 min after equilibration), etomidate or ACh was added cumulatively to the tissue bath. Response results were expressed as the tension change percentage on PE-induced pre-contraction.

Endothelium and the effect of etomidate

To determine the extent of endothelium-dependent vasodilation, the following drugs were used for measurement of tension in endothelial-intact and denuded arteries after PE-induced pre-contraction: 1) etomidate alone (10⁻⁶ to 10⁻⁴ M); 2) lipid emulsion (Intralipid, 20%, the vehicle control for etomidate, 10⁻⁶ to 10⁻⁴ M) and 3) ACh (10⁻⁹ to 10⁻⁴ M) following 10 min pretreatment with and without etomidate (5 × 10⁻⁶ and 5 × 10⁻⁵ M). The etomidate doses used in this study are referenced from other research [18].

Nitric oxide synthase (NOS) inhibitor, epoxy synthase inhibitor and potassium channel blockers and the effect of etomidate

L-NAME, a nitric oxide synthase inhibitor, and indomethacin (Indo), an epoxy synthase inhibitor, were also used to evaluate the interaction effect. L-NAME or Indo was added to the tissue bath 10 min before different concentrations of etomidate were added. To determine the possible effects of K⁺ channels on the action of etomidate, potassium channel blockers, Gli (10 μmol/l), 4-AP (1 mmol/l), BaCl₂ (1 mmol/l) and iberiotoxin (100 nmol/l) were added to the tissue bath 10 min before etomidate was added.

Statistical analysis

Numerical values were expressed as mean ± SEM. Vasorelaxation-response results were expressed as the percentage relaxation of the pre-contraction induced by PE. The maximum relaxant response (R_max) was determined as R_max = 100%, which indicates complete reversal of PE contraction. Statistical analysis was performed using Student’s t test for unpaired comparisons. Value of p < 0.05 was considered as statistically significant.

Results

Laboratory metabolic parameters

After 8 weeks of feeding, the SBP, fasting blood glucose (FBG), serum insulin, and ISI in the IR group all increased significantly compared with normal controls (Table I, p < 0.05). The insulin resistant rats were successfully cultured.

Effects of etomidate on aortic rings pre-contracted by PE

The relaxation of etomidate to endothelial-intact and denuded aortic rings in response to PE contraction were compared in normal control (NC) and IR rats. The tension increments induced by PE (1 μM) in IR and NC rings with intact endothelium were 2.13 ±0.42 g and 1.95 ±0.38 g, respectively. Etomidate and Intralipid with different concentrations induced different relaxations (Figure 1). The induced relaxation increased with the increasing concentration. The induced relaxations...
in endothelial-intact rings were higher than endothelial-denuded rings at the same NC (24.18% in endothelial-intact ring and 16.66% in endothelial-denuded ring at the highest concentration of Intralipid, 10^{-4} mol/l, log M-4) or IR (95.15% in endothelial-intact ring and 86.48% in endothelial-denuded ring at the highest concentration of etomidate, log M-4) rings. In addition, the induced relaxations in endothelial-intact rings treated with etomidate were higher than endothelial-denuded rings (both NC and IR rings, log M-5.5 to log M-4 concentration of etomidate, p < 0.01, Figure 1).

Insulin resistance induced higher relaxations in aortic rings treated with etomidate whether the endothelium was intact or denuded: the induced relaxation were 95.15 ±3.09 in endothelial-intact IR rings and 86.49 ±2.23% in endothelial-denuded IR rings at the highest concentration of etomidate (log M-4). Comparison of results also showed that IR induced higher relaxation than NC arteries (p < 0.01) in the same etomidate treated rings when endothelium was intact (or denuded).

**Effects of potassium channel blockers on the action of etomidate**

Pre-incubation with 4-AP, Iber, or BaCl_2 had no effect on etomidate treated NC or IR rings (Figure 2 A). Pre-incubation with Glik significantly inhibited etomidate-induced relaxation in NC and IR rings (Figure 2 A) (log M-5.5 to log M-4 concentration of etomidate, both p < 0.01); while Gli had almost no inhibitory effect on endothelial-denuded rings of NC or IR rats (Figure 2 B).

**Effects of L-NAME and indomethacin on the action of etomidate**

Pre-incubation with L-NAME (0.1 mM) had no relaxation effect on IR rings, but pre-incubation with L-NAME significantly inhibited etomidate-induced relaxation in NC rings (log M-5.5 to log M-4 concentration of etomidate, p < 0.01, Figure 3). Pre-incubation with indomethacin (Indo, 10 μM) had no relaxation effect on NC or IR rings (Figure 3).

**Effects of etomidate on ACh-induced relaxation in rat thoracic aorta**

Etomidate significantly inhibited ACh-induced relaxation in NC rings. We observed significantly reduced relaxation caused by etomidate in ACh induced NC rings (maximal relaxation of 84.51% in NC ring, and 70.1% and 65.45% at 5 × 10^{-6} and 5 × 10^{-5} M of etomidate (3 × 10^{-7} to 3 × 10^{-5} concentration of Ach, both p < 0.01, Figure 4). In the aortic rings of IR rats, pre-incubation with different doses of etomidate had no significant effect on the relaxation caused by ACh. The ACh-induced relaxation had significant difference in IR rings whether etomidate treated or untreated when compared with NC untreated rings (3 × 10^{-8} to 3 × 10^{-5} concentration of Ach, p < 0.01, Figure 4).

**Discussion**

Etomidate is an imidazole derivative nonbarbiturate intravenous short acting anesthetic. It has no analgesic effect and is especially suitable for patients with shock, cardiovascular risk factors, hemodynamic instability, low blood volume and high airway reactivity. The GABA A receptors are

| Group | SBP [mm Hg] | FBS [mm] | FSI [mU/l] | ISI [–ln (FBS × FSI)] | TG [mm] |
|-------|-------------|---------|------------|----------------------|--------|
| NC    | 110.41 ±1.57| 2.79 ±0.35| 19.15 ±3.12| −3.98 ±0.07          | 0.88 ±0.17|
| IR    | 149.2 ±1.21**| 5.47 ±1.38**| 30.78 ±4.31**| −5.12 ±0.25**        | 2.55 ±0.21*|

*p < 0.05, **p < 0.01, compared with NC (normal control) group. IR – insulin resistance.
closely involved in the molecular mechanism and regulatory effect of etomidate [20]. Etomidate is also believed to cause generalized vascular relaxation. Shin et al. [17] reported that etomidate attenuates PE-induced contraction in rat aorta. There have been studies indicating that etomidate could inhibit vasorelaxation in canine pulmonary artery [18]. Kessler et al. [21] indicated that the relaxant response of human renal artery to acetylcholine was markedly reduced by the K+ Ca channel antagonist and the cytochrome P450 inhibitor. They concluded that etomidate selectively attenuated the relaxant response to acetylcholine, and K+ Ca channels might be involved in the action of etomidate. All these studies focused on the vascular effects of etomidate in normal blood vessels while few studies investigated the effect of etomidate in IR blood vessels. In this study, we found that etomidate could enhance aortic vasodilation of IR rings compared with NC rings. The present study also provides insight as to why enhanced vasodilation occurs in IR arteries at the vascular level.

The doses of etomidate used in the study are clinically relevant. It has been estimated that the

![Figure 2](image2.png)

**Figure 2.** Etomidate-induced relaxation on pre-contracted aortic rings with or without K+ channel inhibitors. *A* – Pre-incubation with Iber, 4-AP and BaCl2 had no effect on etomidate-treated NC or IR rings. Pre-incubation with Gli significantly inhibited etomidate-induced relaxation in NC and IR rings. *B* – Gli had no inhibitory effect on endothelial-denuded rings in NC or IR rats.

**p < 0.01 compared with same concentration of etomidate-treated NC rings.**

**Figure 3.** Pre-incubation with indomethacin (Indo, 10 μM) had no relaxation effect on NC or IR rings; pre-incubation with L-NAME significantly inhibited etomidate-induced relaxation in NC rings.

**p < 0.01 compared with same concentration of etomidate-treated NC rings.**

![Figure 4](image4.png)

**Figure 4.** Etomidate significantly inhibited ACh-induced relaxation in NC rings. The ACh-induced relaxation had a significant difference in IR rings whether etomidate treated or untreated when compared with NC untreated rings.

**p < 0.01 compared with same concentration of ACh-treated NC rings.**
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... that the induction effect of etomidate partially relies on $K_{ATP}$ channels from the endothelium but not other $K^+$ channels.

Deficiency of endothelium-derived NO is believed to be the primary defect that links insulin resistance and endothelial dysfunction [33]. We found that pre-incubation with L-NAME, an NO synthase inhibitor, had no relaxation effect on IR rings, but had an inhibitory effect on etomidate-induced relaxation in NC rings. Our results support this conclusion: relaxation of the aorta induced by etomidate was related to the synthesis of NO in normal rats. A possible explanation is that etomidate may increase NO synthesis and the bioavailability of NO in normal rats, while under the conditions of IR, the NO pathway is disrupted. Ogawa et al. [34] indicated that propofol inhibited the synthesis of prostacyclin in rabbit mesenteric resistance arteries. Horibe et al. [35] demonstrated that prostacyclin did not participate in ACh-induced pulmonary vasorelaxation. We did not find that etomidate has a relaxation effect on indomethacin treated IR rings. This suggests that the arachidonic acid-cyclooxygenase pathway was not involved in the etomidate-induced relaxation in IR aorta and the precise mechanism of the effect of etomidate in IR is unclear and needs further research.

It is believed that acetylcholine (ACh) could dilate normal blood vessels by promoting the release of a vasorelaxant substance from the endothelium, while it constricts blood vessels if the endothelium is removed experimentally [36]. Oyama et al. [37] reported that insulin resistance led to acetylcholine-induced microvascular constriction in a patient with vasospastic angina. Consistent with Oyama’s report, our research demonstrates that insulin resistance attenuates the relaxation caused by ACh, and administration of etomidate could partially reverse IR-induced vasoconstriction in ACh treated aorta. Another interesting finding in our study was that the increase of ACh-induced dilation by etomidate in IR rings did not have the same effect compared with ACh-induced NC rings treated with etomidate. Previous studies have indicated that as a result of endothelial dysfunction, activation of $K_{ATP}$ channels is impaired in IR rats [38]. Therefore, we speculate that etomidate’s effect on ACh-induced constriction in IR aorta may be associated with impaired activation of $K_{ATP}$ channels.

There are some deficiencies of our study: firstly, the effect of insulin resistance on different parts of the blood vessels may be different [39]. The study of etomidate in more parts of the vascular such as the coronary artery and renal artery is necessary. Secondly, the isolated environment is not equiv-
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