Short communication

Involvement of mitochondrial ATP-sensitive potassium channels in etomidate preconditioning-induced protection in human myeloid HL-60 cells

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A B S T R A C T

Exposure of HL-60 cells, a human myeloid cell line, to 500 μM etomidate for 24 h reduced cell viability and increased nitric oxide production and mitochondrial permeability transition pore (mPTP) opening. Preconditioning (1 h) with 1 μM etomidate 4 h before exposure to the 500 μM dose of etomidate attenuated those detrimental effects. The mitochondrial ATP-sensitive potassium channel (mitoKATP channel) opener diazoxide attenuated the mPTP opening caused by the large dose of etomidate. Our results suggest that etomidate can induce a preconditioning effect that may involve mitoKATP channel activation.

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1. Introduction

Compared with other general anaesthetics, the intravenously applied agent etomidate has an advantage of minimally affecting patient hemodynamics. However, etomidate can affect immune functions (Patel, 2002). Propofol, another intravenous anaesthetic causes apoptosis of immunocytes, at high concentrations (Patel, 2002), It is not known whether etomidate can also induce immunocy

Preconditioning is a phenomenon in which a prior stimulus or application of a drug induces protection against a subsequent detrimental insult. Preconditioning-induced protection has been shown in various organs and tissues including skeletal muscle (Badhwar et al., 2004), heart (Ferrari et al., 1999), kidney (Cochrane et al., 1999), brain (Watanabe et al., 2008) and liver (Peralta et al., 2003). However, there is very little evidence on whether preconditioning can also induce protection in blood cells such as neutrophils.

Thus, we designed the current study using HL-60 cells, a human myeloid cell line, to determine whether etomidate can affect blood cell viability and whether preconditioning with a small dose of etomidate can reduce this effect. These cells are commonly used to study cell apoptosis. Mitochondrial ATP-sensitive K⁺ channels (mitoKATP) are involved in the mediation of preconditioning effects in several organs (Tomai et al., 1994; Watanabe et al., 2008) and the role of these channels was also evaluated during the investigation of etomidate-mediated preconditioning effects.

2. Materials and methods

2.1. Main reagents

Pure etomidate was donated by the Enhua Pharmacological Group (Jiangsu, China). Roswell Memorial Institute (RPMI) 1640 medium was obtained from Gibco (Carlsbad, CA, USA), the cell counting kit-8 (CCK-8) was from Dojindo Molecular Technologies, Inc. (Kumamoto, Japan) and the nitric oxide Assay Kit was procured from the Beyotime Institute of Biotechnology (Shanghai, China). Diazoxide (a mitoKATP channel activator), 5-hydroxydecanoic acid sodium salt (5-HD, a specific mitoKATP channel inhibitor) and a MitoProbe™ transition pore assay kit were purchased from Sigma Co. (St. Louis, MO, USA).

2.2. HL-60 cell culture

HL-60 cells (Cell Bank of Chinese Academy of Sciences, Shanghai, China) were cultured in suspension in RPMI 1640 medium containing 10% fetal bovine serum, antibiotics and 2 mM l-glutamine at 37 ºC in an incubator gassed with 95% air and 5% CO₂. Cell densities were maintained at 1.0 × 10⁵ to 1.0 × 10⁶ cells/ml. Cell cultures were split every 2–3 days using the following method. Cells in culture medium were centrifuged for 5 min at 1000 rpm. The supernatant was removed and cell pellet was resuspended in 3 ml medium. About 1 ml cell suspension with 9 ml fresh medium was placed into a culture flask that was maintained in an incubator. Cells at exponential growth phase with viability ≥95% as assessed by trypan blue exclusion assay were used in the study.

2.3. Study groups

HL-60 cells were plated at a density of 1.5 × 10⁵/ml. They were then used in the following study groups.

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5-HD + preconditioning + high-dose etomidate group: cells were treated with 100 µM etomidate for 1 h, washed with medium to remove etomidate, allowed to have an etomidate-free period of 4 h, and then exposed to 500 µM etomidate for 24 h.

Diazoxide + high-dose etomidate group: cells were preconditioned with 100 µM etomidate for 1 h, washed with medium to remove etomidate, allowed to have a 5-HD-free period of 4 h, and then exposed to 500 µM etomidate for 24 h.

5-HD + preconditioning + high-dose etomidate group: cells were treated with 100 µM 5-HD for 30 min, washed with medium to remove 5-HD, preconditioned with 100 µM diazoxide for 1 h, washed with medium to remove etomidate, allowed to have a diazoxide-free period of 4 h, and then exposed to 500 µM etomidate for 24 h.

Diazoxide + high-dose etomidate group: cells were treated with 100 µM diazoxide for 30 min, washed with medium to remove diazoxide, allowed to have a diazoxide-free period of 4 h, and then exposed to 500 µM etomidate for 24 h.

Diazoxide + preconditioning + high-dose etomidate group: cells were preconditioned with 100 µM diazoxide for 30 min, washed with medium to remove diazoxide, preconditioned with 100 µM 5-HD for 1 h, washed with medium to remove etomidate, allowed to have a diazoxide- and etomidate-free period of 4 h, and then exposed to 500 µM etomidate for 24 h.

2.4. Cell viability assay

CCK-8 assay is a nonradioactive method to allow sensitive colorimetric determination of the number of viable cells. [2-(2-Methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] (WST-8) in the medium is reduced by dehydrogenases in cells to produce a yellow product (3-(2-Methoxy-4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) (WST-8) in the medium. The absorbance of the samples was measured at 450 nm using a microplate reader. The cell viability is fluorescent, is accumulated in the mitochondria and will be quenched CoCl2 after it has gone through the mPTP to be in the cytosol (Petronilli et al., 1998). Cells were first loaded with calcine acetoxyethyl ester and the fluorescent intensity in cells was monitored by flow cytometry at the excitation wavelength of 488 nm.

2.5. Mitochondrial permeability transition pore (mPTP) opening assay

mPTP opening assay was performed by using calcine acetoxyethyl ester that is fluorescent, is accumulated in the mitochondria and will be quenched CoCl2 after it has gone through the mPTP to be in the cytosol (Petronilli et al., 1998). Cells were first loaded with calcine acetoxyethyl ester and the fluorescent intensity in cells was monitored by flow cytometry at the excitation wavelength of 488 nm.

2.6. Nitric oxide (NO) production quantification

Because NO has an extremely short half-life, we quantified NO production by measuring the concentrations of the two stable NO products nitrate (NO3−) and nitrite (NO2−) (Ko et al., 2008). The culture medium was collected and analyzed by using the Nitric Oxide Assay Kit. The assay included a process to convert nitrate to nitrite and then to use a Griess reaction to measure the nitrite concentrations. Absorbance of the samples was measured at 540 nm using a microplate reader.

1. Control group: cells were cultured under normal conditions for 24 h.
2. High-dose etomidate group: cells were exposed to 500 µM etomidate for 24 h.
3. Preconditioning group: cells were preconditioned with 100 µM etomidate for 1 h, allowed to have an etomidate-free period of 4 h, and then exposed to 500 µM etomidate for 24 h.
4. 5-HD + high-dose etomidate group: cells were treated with 100 µM 5-HD for 30 min, washed with medium to remove 5-HD, allowed to have a 5-HD-free period of 4 h, and then exposed to 500 µM etomidate for 24 h.
5. 5-HD + preconditioning + high-dose etomidate group: cells were treated with 100 µM 5-HD for 30 min, washed with medium to remove 5-HD, preconditioned with 1 µM etomidate for 1 h, washed with medium to remove etomidate, allowed to have a 5-HD- and etomidate-free period of 4 h, and then exposed to 500 µM etomidate for 24 h.
6. Diazoxide + preconditioning + high-dose etomidate group: cells were treated with 100 µM diazoxide for 30 min, washed with medium to remove diazoxide, allowed to have a diazoxide-free period of 4 h, and then exposed to 500 µM etomidate for 24 h.
7. Diazoxide + preconditioning + high-dose etomidate group: cells were treated with 100 µM diazoxide for 30 min, washed with medium to remove diazoxide, preconditioned with 1 µM 5-HD for 1 h, washed with medium to remove etomidate, allowed to have a diazoxide- and etomidate-free period of 4 h, and then exposed to 500 µM etomidate for 24 h.

2.7. Statistics

Data are presented as means ± S.D. All experiments were repeated for three times with different sets of cells. One-way analysis of variance followed by Tukey test was used for statistical analysis. All data were analyzed by SPSS (version 13.0). A P < 0.05 was considered significant.

3. Results

Compared with control, exposure to 500 µM etomidate for 24 h reduced cell viability (Fig. 1) and increased NO production and mPTP opening (Fig. 2). Preconditioning with a small dose of etomidate attenuated those detrimental effects (Figs. 1 and 2). The mitoKATP channel inhibitor 5-hydroxydecanoic acid reduced the etomidate preconditioning-induced attenuation of decreased cell viability and increased mPTP opening (Figs. 1 and 2). The mitoKATP channel opener diazoxide also attenuated the increased mPTP opening caused by 500 µM etomidate (Fig. 2).

4. Discussion

We showed in this study that 500 µM etomidate reduced the viability of HL-60 cells. We selected this dose on the basis of our previous work which showed that middle or high micromolar concentrations of etomidate reduced HL-60 viability (Jiao et al., 2007). However, the clinical importance of the effects of high concentration etomidate on HL-60 cells as found in our studies is not...
clear because the blood concentrations of etomidate during anaesthesia or sedation are at low micromolar concentrations (Hebron, 1983).

Application of 1 μM etomidate before the exposure to 500 μM etomidate reduced the decreased cell viability caused by the large dose of etomidate, suggesting that the low concentration of etomidate can induce a preconditioning effect. This is a situation similar to ischemic preconditioning where a short episode of ischemia induces protection against subsequent severe ischemia. Our study suggests that NO may be involved in the detrimental effect of etomidate at high doses because 500 μM etomidate induced a large amount of NO production and this increase was attenuated by etomidate preconditioning. NO is involved in signal transduction of preconditioning–induced protection in various organs including heart and brain (Guo et al., 1999; Zhao and Zuo, 2004). However, NO, as a reactive free radical, at high concentrations can cause cell injury (Xu et al., 2008a,b).

mPTP is located at the junction of inner and outer mitochondrial membrane. mPTP opening increases the inner membrane permeability to solutes smaller than 1.5 kDa, which results in mitochondrial swelling, collapse of mitochondrial membrane potential, uncoupling of oxidative phosphorylation and release of cytochrome c from the mitochondria (Petronilli et al., 2001). These effects ultimately result in cell injury and death.

Our report strongly suggests the involvement of mitoKATP channels in the etomidate preconditioning effect because 5-HD, a specific mitoKATP channel inhibitor, inhibited the etomidate preconditioning effect and the mitoKATP channel opener diazoxide mimicked the etomidate preconditioning effect in attenuating the mPTP opening caused by the large dose of etomidate. Consistent with our findings, the opening of KATP channels is considered as an important effector for the protection induced by various preconditioning stimuli in the heart and brain (Tomai et al., 1994; Watanabe et al., 2008).

In summary, we have shown that etomidate at a high concentration reduces HL-60 cell viability. This effect may be caused by an increase of NO production and the opening of mPTP. Preconditioning with a small dose of etomidate attenuates these detrimental effects of the large dose of etomidate. MitoKATP channels may well be responsible for mediating the etomidate preconditioning effect.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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References


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