Environmental Toxicology and Pharmacology 29 (2010) 320–322

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

Short communication

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

Xiaoping Zhang[a], Junyu Xiong[a,*], Yuxia Jiao[a], Guan Wang[a], Zhiyi Zuo[b,**]

[a] Department of Anesthesiology, the Second Affiliated Hospital, Dalian Medical University, 467 Zhongshan Road, Dalian, Liaoning Province 116027, China
[b] Department of Anesthesiology, University of Virginia Health System, 1 Hospital Drive, PO Box 800710, Charlottesville, VA 22908-0710, USA

ARTICLE INFO

Article history:
Received 2 December 2009
Received in revised form 12 February 2010
Accepted 16 February 2010
Available online 23 February 2010

Keywords:
Etomidate
Mitochondrial K_{ATP} channel
Mitochondrial permeability transition pore
Preconditioning

ABSTRACT

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 (mitoK$_{ATP}$ channel) inhibitor 5-hydroxydecanoic acid reduced the etomidate preconditioning effects. The mitoK$_{ATP}$ 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 mitoK$_{ATP}$ channel activation.

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 immunocyte injury.

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 (mitoK$_{ATP}$) 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. (Kumanmoto, Japan) and the nitric oxide Assay Kit was procured from the Beyotime Institute of Biotechnology (Shanghai, China). Diazoxide (a mitoK$_{ATP}$ channel activator), 5-hydroxydecanoic acid sodium salt (5-HD, a specific mitoK$_{ATP}$ 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 °C in an incubator gassed with 95% air and 5% CO_2. Cell densities were maintained at 1.0×10^5 to 1.0×10^6 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 1.5×10^5/ml. They were then used in the following study groups.

* Corresponding author. Tel.: +86 411 84671291.
** Corresponding author. Tel.: +1 434 924 2283; fax: +1 434 924 2105.
E-mail addresses: jyxiong0639@sina.com (J. Xiong), zz3c@virginia.edu (Z. Zuo).

1382-6689/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.etap.2010.02.003

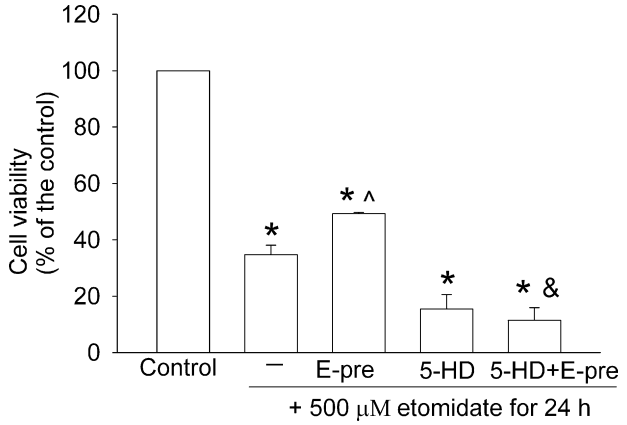

Fig. 1. Cell viability under various experimental conditions. Results are normalized by the data from control group with the control group data set at 100%. The final results are presented as mean ± SD ($n = 3$). E-pre: etomidate preconditioning with 1 μM etomidate for 1 h; 5-HD: 5-hydroxydecanoic acid. $^{*}P < 0.01$ compared with control group; $^{\wedge}P < 0.01$ compared with cells exposed to 500 μM etomidate for 24 h only; $^{\&}P < 0.01$ compared with the group that had etomidate preconditioning and then exposure to 500 μM etomidate for 24 h.

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 1 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.
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 + 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 etomidate 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 kit is reduced by dehydrogenases in cells to produce a yellow product (formazan) that is soluble in the tissue culture medium. The amount of the formazan dye generated by cells is proportional to the number of living cells. Absorbance of the samples was measured at 450 nm using a microplate reader. The cell viability (%) = (the optical density value of experimental group − the optical density value of blank group)/(the optical density value of control group − the optical density value of blank group) × 100%.

2.5. Mitochondrial permeability transition pore (mPTP) opening assay

mPTP opening assay was performed by using calcein acetoxymethyl ester that is fluorescent, is accumulated in the mitochondria and will be quenched $CoCl_2$ after it has gone through the mPTP to be in the cytosol (Petronilli et al., 1998). Cells were first loaded with calcein acetoxymethyl 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 (NO^{3-}) and nitrite (NO^{2-}) (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 Greiss reaction to measure the nitrite concentrations. Absorbance of the samples was measured at 540 nm using a microplate reader.

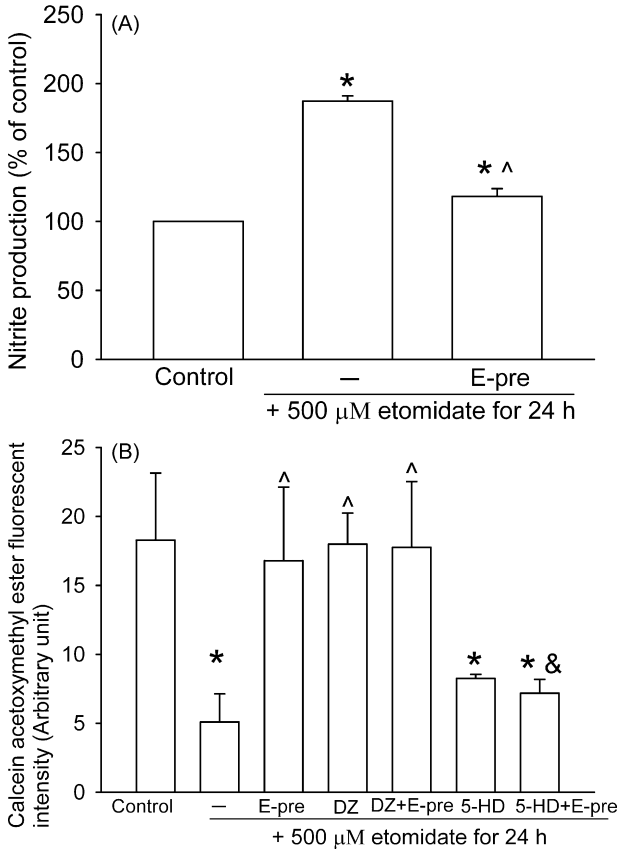

Fig. 2. Nitrite production (panel A) and mitochondrial permeability transition pore (mPTP) opening (panel B) under various conditions. Cell culture medium was collected at the end of experiments for nitrite concentration quantification. The mPTP opening was measured by decreased calcein acetoxymethyl ester fluorescent intensity. The final results are presented as mean ± SD ($n = 3$). E-pre: etomidate preconditioning with 1 μM etomidate for 1 h; DZ: diazoxide; 5-HD: 5-hydroxydecanoic acid. $^{*}P < 0.05$ compared with control group; $^{\wedge}P < 0.05$ compared with cells exposed to 500 μM etomidate for 24 h only; and $^{\&}P < 0.05$ compared with the group that had etomidate preconditioning and then exposure 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 mitoK$_{ATP}$ channel inhibitor 5-hydroxydecanoic acid reduced the etomidate preconditioning-induced attenuation of decreased cell viability and increased mPTP opening (Figs. 1 and 2). The mitoK$_{ATP}$ 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 mitoK_{ATP} channels in the etomidate preconditioning effect because 5-HD, a specific mitoK_{ATP} channel inhibitor, inhibited the etomidate preconditioning effect and the mitoK_{ATP} 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 K_{ATP} 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. MitoK_{ATP} 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.

Acknowledgement

This study was supported by internal source of the Department of Anesthesiology, the Second Affiliated Hospital, Dalian Medical University.

References

Badhwar, A., Bihari, A., Dungey, A.A., Scott, J.R., Albion, C.D., Forbes, T.L., Harris, K.A., Potter, R.F., 2004. Protective mechanisms during ischemic tolerance in skeletal muscle. Free Radic. Biol. Med. 36, 371–379.

Cochrane, J., Williams, B.T., Banerjee, A., Harken, A.H., Burke, T.J., Cairns, C.B., Shapiro, J.I., 1999. Ischemic preconditioning attenuates functional, metabolic, and morphologic injury from ischemic acute renal failure in the rat. Ren. Fail. 21, 135–145.

Ferrari, R., Ceconi, C., Curello, S., Percoco, G., Toselli, T., Antonioli, G., 1999. Ischemic preconditioning, myocardial stunning, and hibernation: basic aspects. Am. Heart J. 138, S61–S68.

Guo, Y., Jones, W.K., Xuan, Y.-T., Tang, X.-L., Bao, W., Wu, W.-J., Han, H., Laubach, V.E., Ping, P., Yang, Z., et al., 1999. The late phase of ischemic preconditioning is abrogated by targeted disruption of the inducible NO synthase gene. Proc. Natl. Acad. Sci. U.S.A. 96, 11507–11512.

Hebron, B.S., 1983. Plasma concentrations of etomidate during an intravenous infusion over 48 hours. Anaesthesia 38 (Suppl.), 39–43.

Jiao, Y., Xiong, J., Sun, Y., Liu, M., Lu, S., 2007. Toxic effect of etomidate on HL-60 cells and influence of etomidate preconditioning. Chin. J. Anesthesiol. 27, 236–239.

Ko, S.H., Ryu, G.R., Kim, S., Ahn, Y.B., Yoon, K.H., Kaneto, H., Ha, H., Kim, Y.S., Song, K.H., 2008. Inducible nitric oxide synthase-nitric oxide plays an important role in acute and severe hypoxic injury to pancreatic beta cells. Transplantation 85, 323–330.

Patel, S., 2002. Cardiovascular effects of intravenous anaesthetics. Int. Anesthesiol. Clin. 40, 15–33.

Peralta, C., Serafin, A., Fernandez-Zabalegui, L., Wu, Z.Y., Rosello-Catafau, J., 2003. Liver ischemic preconditioning: a new strategy for the prevention of ischemia-reperfusion injury. Transplant. Proc. 35, 1800–1802.

Petronilli, V., Miotto, G., Canton, M., Colonna, R., Bernardi, P., Di Lisa, F., 1998. Imaging the mitochondrial permeability transition pore in intact cells. Biofactors 8, 263–272.

Petronilli, V., Penzo, D., Scorrano, L., Bernardi, P., Di Lisa, F., 2001. The mitochondrial permeability transition, release of cytochrome c and cell death. Correlation with the duration of pore openings in situ. J. Biol. Chem. 276, 12030–12034.

Tomai, F., Crea, F., Gaspardone, A., Versaci, F., De Paulis, R., Penta de Peppo, A., Chiariello, L., Gioffre, P.A., 1994. Ischemic preconditioning during coronary angioplasty is prevented by glibenclamide, a selective ATP-sensitive K^+ channel blocker. Circulation 90, 700–705.

Watanabe, M., Katsura, K., Ohsawa, I., Mizukoshi, G., Takahashi, K., Asoh, S., Ohta, S., Katayama, Y., 2008. Involvement of mitoKATP channel in protective mechanisms of cerebral ischemic tolerance. Brain Res. 1238, 199–207.

Xu, X., Feng, J., Zuo, Z., 2008a. Isoflurane preconditioning reduces the rat NR8383 macrophage injury induced by lipopolysaccharide and interferon gamma. Anesthesiology 108, 643–650.

Xu, X., Kim, J.A., Zuo, Z., 2008b. Isoflurane preconditioning reduces mouse microglial activation and injury induced by lipopolysaccharide and interferon-gamma. Neuroscience 154, 1002–1008.

Zhao, P., Zuo, Z., 2004. Isoflurane preconditioning induces neuroprotection that is inducible nitric oxide synthase-dependent in the neonatal rats. Anesthesiology 101, 695–702.