原 著

Effects of Ketamine on Intracellular Free Calcium in Bovine Carotid Artery Endothelial Cells

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Abstract

We focused on endothelium-mediated vasodilation as a mechanism of the vasodilatory effects of ketamine. Cultured bovine carotid artery endothelial cells were loaded with the Ca^{2+} indicator dye: fluo3, and intracellular Ca^{2+} levels of the endothelial cells were monitored by a confocal laser-scanning microscope. After application of ketamine in final concentrations of 10^{-5}-10^{-3}M, intracellular Ca^{2+} levels were increased in a concentration-dependent manner. Our findings suggest that the Ca^{2+}-dependent production and/or release of vasodilator substances in the endothelium contributes, at least in part, to ketamine-induced vasodilation.

Key words : Ketamine, Endothelial cell, Intracellular free calcium, Fluo3, Confocal laser-scanning microscope

Introduction

Ketamine acts directly on vascular smooth muscle, causing relaxation. The mechanisms underlying this action have been shown to involve an interference with transmembrane Ca^{2+} influx and an inhibition of Ca^{2+} release from intracellular Ca^{2+} stores[1,2].

On the other hand, participation of endothelium-derived factors in ketamine-induced vasodilation has remained uncertain[3~5].

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Therefore, we examined the effect of ketamine on the intracellular Ca^{2+} level in the endothelium, since a transient increase in intracellular Ca^{2+} level stimulates production and/or release of vasodilator substances: prostacyclin (PGI_2), endothelium-derived relaxing factor (EDRF/ON), and endothelium-derived hyperpolarizing factor (EDHF) in the endothelium[6~8]. To our knowledge, this is the first report describing the direct effect of ketamine on intracellular Ca^{2+} level in the endothelium.

Materials and methods

(1) Cell culture

We obtained bovine carotid artery endothelial cells (BCAECs, original developers: Hagiwara, H and Inada, Y) from Japanese Cancer Research Resources Bank (JCRB). BCAECs were seeded on 35 mm-diameter coverglass dishes and grown to confluent monolayers in Eagle's minimum essential medium (MEM) containing 10 % fetal bovine serum (FBS) at 37 ℃ under humidified conditions of 95 % air-5 % CO_2. Subcultured BCAECs in the 20th passage were used in the experiment.

(2) Loading cells with the Ca^{2+} indicator dye: fluo3

For dye loading, endothelial cells were incubated with 2.5 μM fluo3 acetoxymethyl ester (fluo3-AM) in Hank's balanced salt solution (HBSS) at 37 ℃ under 5 % CO_2 in air for 1 hour.

(3) Calcium imaging

The fluorescence imaging was performed using a

confocal laser-scanning microscope (CLSM) : Meridian ACAS ultima (Okemos MI). Optical excitation of fluo3-Ca^{2+} complex was done by 488 nm line from an argon ion laser, and the emitted fluorescent light was monitored at 530 nm by a photomutiplier. For the time course measurement, fluorescence intensity data were collected through the kinetics image scan program in the computer system connected to the CLSM: a utility that displays serial two-dimensional (frame) images. Fluo3 is quite useful for detecting qualitative changes in calcium, however, since there is only one emission (and not a ratio of two separate emissions), quantitation into absolute calcium concentrations is complicated. Therefore, we measured relative changes in intracellular Ca^{2+} level.

(4) Experimental protocol

Dye-loaded confluent cells were washed three times with HBSS to remove unloaded dye; medium was ultimately replaced with 1 ml HBSS before the study. All experiments were performed at room temperature, because intracellular fluo3 would rapidly leak out at 37 ℃. Scan paramenters: laser spot intensity, laser pulse duration, and others were optimized for maximal sensitivity and minimal photobleaching.

To examine the effect of ketamine on the intracellular Ca^{2+} level, after the resting period in the time course measurement, ketamine (0 M, 2×10^{-6} M, 2×10^{-5} M, 2×10^{-4} M, and 2×10^{-3} M) diluted in 1 ml HBSS, was added to the dish with cells in 1 ml HBSS by a micropipette in final concentrations (0 M, 10^{-6} M, 10^{-5} M, 10^{-4} M, and 10^{-3} M). Changes in the mean fluorescence intensity for each individual cell was obtained and relative fluorescence intensity, normalized to the resting value, was plotted as a function of time.

(5) Solutions and drugs

HBSS (138 mM NaCl, 5.0 mM KCl, 1.3 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.4mM $MaSO_4$, 4.0 mM $NaHCO_3$, 0.3 mM, Na_2HPO_4, 0.3mM KH_2PO_4, 5.6 mM D-glucose) was buffered to pH 7.4 at room temperature with 10 mM sodium Hepes. HBSS and FBS were purchased from Gibco Laboratories (Gland Island, NY). MEM was from Nissui Pharmaceutical (Tokyo, Japan). Fluo3-AM was from Dojindo (Kumamoto, Japan). Ketamine hydrochloride was from Sankyo Pharmaceutical (Tokyo, Japan). Sodium Hepes was from Sigma Chemical (St. Louis, MO).

(6) Data analysis

The measured values were expressed as means ± SD. One-way factorial analysis of variance with Scheffe's test was used to determine the concentration-dependent effects.

Results

Figure 1 shows the time course of changes in frame image of intracellular Ca^{2+} level ($[Ca^{2+}]i$) of BCAECs before and during exposure to 10^{-4} M ketamine. Fifteen serial images were stacked up, every 4.8 sec for 72 sec. After the 7-sec resting period, ketamine was added in final concentrations of 10^{-4} M. The matrix of images at fifteen points of time visualizes the prominent increase in $[Ca^{2+}]i$.

Figure 2 shows the time course of changes in relative fluorescence intensity, normalized to the resting value, before and during exposure to 10^{-4} M ketamine for ten individual cells. The relative fluorescence intensity reflects $[Ca^{2+}]i$. Addition of

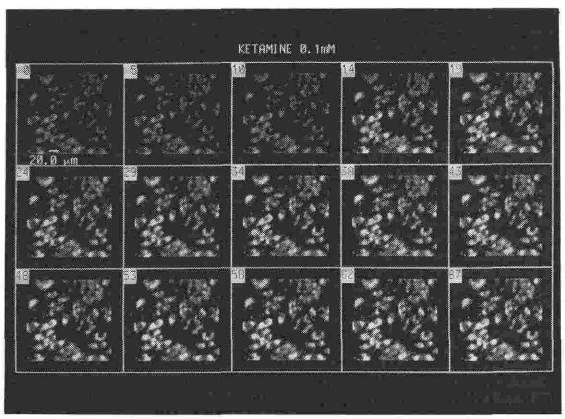

Figure 1 Two-dimensional images of the $[Ca^{2+}]i$ response to 10^{-4} M ketamine. Number of each stack indicates time from start point of scanning. Ketamine was applied at 7 sec from start point of scanning.

10^{-4} M ketamine induced a large and repid increase in $[Ca^{2+}]i$.

Figure 3 shows the time course of changes in relative fluorescence intensity before and during exposure to 0, 10^{-6} -10^{-3} M ketamine for ten individual cells in each of the five groups. Addition of 10^{-6} -10^{-3} M ketamine elevated $[Ca^{2+}]i$, compared with the controls. Relative fluorescence reduction observed in the control group could primarily result from photobleaching.

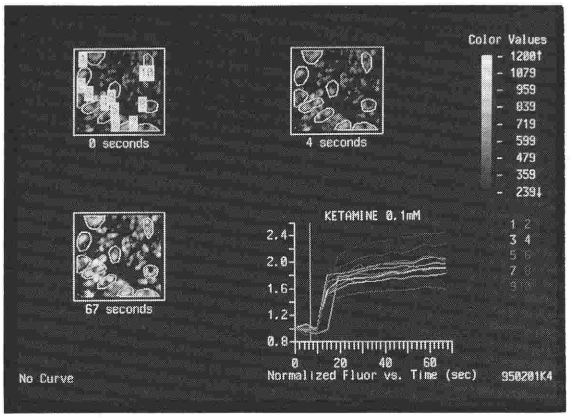

Figure 2 Time course of changes in relative fluorescence intensity, normalized to the resting value, before and during exposure to 10^{-4} M ketamine for each of ten individual cells. The time of addition (10^{-4} M ketamine) is marked by a vertical line. Relative fluorescence intensity of fluo3-Ca^{2+} complex for each individual cell was obtained by averaging the intensity of each pixel within a polygon enclosing each cell (lower right panel). Upper panels are the resting $[Ca^{2+}]i$ images. The lower left panel is the $[Ca^{2+}]i$ image at 1 min from addition of 10^{-4} M ketamine.

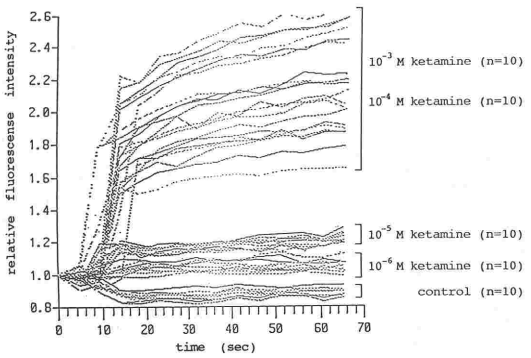

Figure 3 Time coures of changes in relative fluorescence intensity before and during exposure to 0, 10^{-6}-10^{-3} M ketamine for ten individual cells in each of the five groups.

Figure 4 shows the relative fluorescence intensity in each of the five groups at 1 min from addition of ketamine. Ketamine $> 10^{-5}$ M elevated $[Ca^{2+}]i$ in a concentration-dependent manner.

Discussion

Ketamine has been used with widespread success in the anesthetic induction on certain patients with hemodynamic compromise because of its cardiovascular stimulation[9]. Ketamine increases blood pressure, heart rate, and cardiac output; yet its use has also resulted in cardiovascular decompensation in critically ill patients[10]. The negative effect on cardiovasculature has been suggested to be due to the direct vasodilatory and myocadial depressant actions of ketamine[10,11].

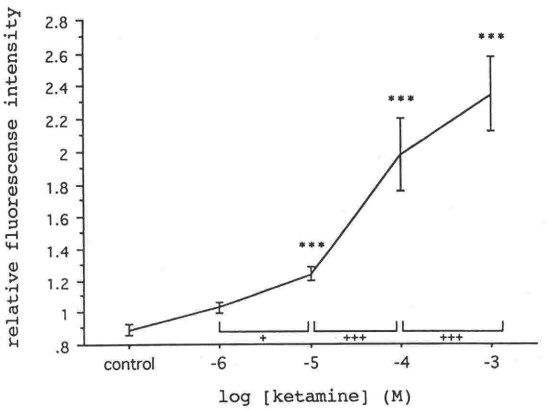

Figure 4 Dose-dependent effects of ketamine on $[Ca^{2+}]i$. Values, expressed as means ± SD, are relative fluorescence intensity in each five group at 1 min from addition of ketamine. ***Statistically significant compared with controls (***$p < 0.001$). $^{+}$, $^{+++}$Statistically significant compared with group next to each other ($^{+}p < 0.05$, $^{+++}p < 0.001$).

While the role of the endothelium in modulating the constrictive or dilatory effects of many anesthetic agents has been demonstrated, little is known about its role in modifying the respones to ketamine[3~5].

Endothelial cells are located on the luminal surface of blood vessels and play an important role in the regulation of vascular tone. At least three major dilator substances are released, PGI_2, EDRF/NO, and EDHF[12,13]. Real time measurements of these substances have been required for their physiological and pathological importance, but to date it has been almost impossible because of their short biological half life. The biosynthetic pathways of these substances are activated in parallel by rise in intracellular Ca^{2+} level of endothelial cells[6~8]. Therefore, we focused on the changes in intracellular Ca^{2+} level as an alternative.

The ketamine concentrations in human plasma amounted to as large as 1.1×10^{-4} and 6.0×10^{-5} M, 1 and 5 min after administration of 2.0 and 2.2 mg/kg, intravenously, respectively[14,15]. Because ketamine's plasma protein binding is only 12%, our findings demonstrate that a clinically relevant concentration of ketamine elevates the intracellular Ca^{2+} level of cultured endothelial cells[16].

In the present experiment, we used bovine carotid artery endothelial cells. There are species specificity and heterogeneity between different vascular beds in the endothelium-dependent responses of mammalian blood vessels[17~19]. It is, therefore, difficult to discuss the effect of ketamine on blood pressure in man, on the basis of our findings. More definitive experiments are needed to determine the role of endothelium-derived factors in ketamine-induced hypotension. Furthermore, studies in vivo are required to determine if the endothelium contributes to the overall hemodynamic action of ketamine.

In this preliminary study, we found that the Ca^{2+}-dependent production and/or release of vasodilator substances in the endothelium could participate, at least in part, in ketamine-induced vasodilation.

References

1) Yamazaki M, Ito Y, Kuze S, et al : Effects of ketamine on voltage-dependent Ca^{2+} currents in single smooth muscle cells from rabbit portal vein. Pharmacology 45 : 162-169, 1992
2) Kanmura Y, Kajikuri J, Ito T, et al : Effects of ketamine on contraction and synthesis of inositol 1,4,5-trisphosphate in smooth muscle of the rabbit mesenteric artery. Anesthesiology 79 : 571-579, 1993
3) Yamazaki M, Momose Y, Shakunaga K, et al : The vasodilatory effects of ketamine on isolated rabbit portal veins. Pharmacol Toxicol 75 : 3-8, 1994
4) Xuan Y, Galss PSA : Ketamine regulation of endothelial cell nitric oxide pathway. Anesth Analg 78 : S491, 1994
5) Chung HC, Ho ST, Ho W, et al : Partially endothelium-dependent relaxing effect of ketamine on the canine basilar artery in vitro. Acta Anaesthesiol Sin 30 : 1-6, 1992
6) Parsaee H, McEwan JR, Joseph S, et al : Differential sensitivities of the prostacyclin and nitric oxide biosynthetic pathways to cytosolic calcium in bovine aortic endothelial cells. Br J Pharmacol 107 : 1013-1019, 1992
7) Korenaga R, Ando J, Ohtsuka A, Sakuma I, et al : Close correlation between cytoplasmic Ca^{++} levels and release of an endothelium-derived relaxing factor from cultured endothelial cells. Cell Struct Funct 18 : 95-104, 1993
8) Nagano T, Vanhoutte PM : Endothelium-derived hyperpolarizing factor and endothelium-dependent relaxations. Am J Respir Cell Mol Biol 8 : 1-6, 1993
9) White PF, Way WL, Trevor AJ: Ketamine: Its pharmacology and therapeutic uses. Anesthesiology 56 : 119-136, 1982
10) Waxman K, Shoemaker WC, Lippman M : Cardiovascular effects of anethetic induction with ketamine. Anesth Analg 59 : 355-358, 1980
11) Schwartz DA, Horrwitz LD : Effects of ketamine on left ventricular performance. J Pharmacol Exp Ther 194 : 410-414, 1975
12) Moncada S, Gryglewski R, Bunting S, et al : An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature 263 : 663-665, 1976
13) Furchgott RF, Zawadzki JV : The obligatory role of endothelial cells in the relaxation of smooth muscle by acetylcholine. Nature 288 : 373-376, 1980
14) Domino EF, Zsigmond EK, Domino LE, et al : Plasma levels of ketamine and two of its metabolites in surgical patients using a gas chromatographic mass flagmentographic assay. Anesth Analg 61 : 87-92, 1982
15) Idvall J, Ahlgren I, Aronsen KF, et al : Ketamine infusions: Pharmacokinetics and clinical effects. Brit J Anaesth 51 : 1167-1173, 1979
16) Wieber J, Gugler R, Hengstmann JH, et al : Pharmacokinetics of ketamine in man. Anaesthesist 24 : 260-263, 1975
17) Toda N : Mechanisms of histamine-induced relaxation in isolated monkey and dog coronary arteries. J Pharmacol

Exp Ther 239 : 529-535, 1986
18) Toda N : Mechanism of histamine actions in human coronary arteries. Circ Res 61 : 280-286, 1987
19) Vanhoutte PM, Miller VM : Heterogeneity of endothelium-dependent responses in mammalian blood vessels. J Cardiovasc Pharmacol 7 : S12-S23, 1985

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