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Effects of berbamine on intracellular calcium concentration in cultured HeLa cells

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KEY WORD calcium; potassium; norepinephrine; caffeine; calcimycin; HeLa cells; fluorescent dyes; confocal microscopy

ABSTRACT

AIM: To study the involvement of Ca^{2+} signaling and the effects of berbamine (Ber) on intracellular calcium concentration ($[Ca^{2+}]_i$) elevated in cultured HeLa cells. **METHODS**: $[Ca^{2+}]_i$ was measured by confocal microscopy in single HeLa cell loaded with Fluo 3-AM. The change of $[Ca^{2+}]_i$ was represented by fluorescent intensity (FI). **RESULTS**: (1) In the presence of extracellular Ca^{2+} 1.3 mmol $\cdot L^{-1}$, the resting level of FI was 186 ± 44, $n = 49$ cells from all control experiments, and KCl, NE, caffeine, and calcimycin (Cal) all induced $[Ca^{2+}]_i$ elevations in cultured HeLa cells. (2) The resting level of FI was not affected by pretreatment with Ber. The FI increased by KCl 60 mmol$\cdot L^{-1}$, NE 100 μmol $\cdot L^{-1}$, and Cal 30 μmol $\cdot L^{-1}$ were attenuated ($P < 0.05$ or $P < 0.01$), the slope and the time to peak of FI increase were decreased and prolonged. (3) In the absence of extracellular Ca^{2+}, caffeine 80 mmol $\cdot L^{-1}$-induced $[Ca^{2+}]_i$ mobilization was not inhibited by Ber 100 μmol $\cdot L^{-1}$ pretreatment. (4) These effects of Ber were similar to those of verapamil (Ver) 10 μmol $\cdot L^{-1}$. **CONCLUSION**: Although it was derived from cervical cancer, the HeLa cells which were belong to the nonexcitable cell possessed the similar biological properties with excitable cells, and Ca^{2+} also played a crucial role in signal transduction processes.

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INTRODUCTION

As an important second messenger and mediator, calcium (Ca^{2+}) participated in signal transduction across the membrane and the modulations of biological functions, a variety of stimuli produced their cellular effects by changing the $[Ca^{2+}]_i$[1]. It is known that stimuli activate or modulate cellular Ca^{2+} signal by two general mechanisms: the influx of Ca^{2+} and the Ca^{2+} release from intracellular calcium store mediated by voltage-dependent Ca^{2+} channels[2] and stored depletion-dependent Ca^{2+} channels[3] in excitable cells. HeLa cells, derived from cervical carcinoma, are belong to nonexcitable cells[4], and it is not yet clear whether or not Ca^{2+} plays a role in signal transduction pathway like that in excitable cells. The aim of this experiment was to directly investigate agonist-induced $[Ca^{2+}]_i$ increase using confocal microscopy in cultured HeLa cells and the effect of Ber.

MATERIALS AND METHODS

Agents Fluo 3-AM (Sigma) was dissolved in Me_2SO 1 g $\cdot L^{-1}$ (Sigma) and stored at -20 ℃. Pluronic F-127 and HEPES were purchased from Sigma. KCl (Harbin Chemical Manufacturer), NE (Wuhan Pharmaceutical Co), and calcimycin (Cal) (Sigma) were all dissolved in standard buffer and kept at 4 ℃. Egtazic acid (EGTA, Sigma) was prepared according to previous method[5].

Preparation of cultured HeLa cells HeLa cells were cultured in cell culture flasks in RPMI-1640 medium (Gibco) with 10 % of fetal calf serum (FCS) at 37 ℃ in a cell incubator with 5 % CO_2 cell incubator and digested by using trypsin (Difco). The cell suspension was placed on the glass coverslips on the bottom of 6-well multidish and cultured under the same condition for another 20 h.

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Fluo 3-AM loading[6] The coverslips with cultured HeLa cells were washed once with standard buffer (NaCl 138, KCl 5.0, KH_2PO_4 0.3, $MgSO_4$ 0.4, Na_2HPO_4 0.3, $CaCl_2$ 1.3, HEPES 20 mmol · L^{-1}, glucose 0.1 %, and BSA (bovine serum albumin, 0.01 %, pH 7.2) at 22 ℃. This preparation was then incubated with Fluo 3-AM working solution (Fluo 3-AM 4.4 μmol · L^{-1} and Pluronic F-127 0.03 % dissolved in standard buffer) at 37 ℃ for 60 min. After incubation it was washed with standard buffer twice immediately at 22 ℃.

Measurement of $[Ca^{2+}]_i$[6] The coverslip was mounted in the Auttoflour® cell chamber (Molecular Probe) with 200 μL PBS (phosphate buffer saline). The FI was detected by confocal microscope (InSight Plus-IQ, Meridian) with 40 × objective at 488 nm for excitation and 530/30 for emission at room temperature. 20 μL of stimulant was added to the preparation between the second and third scan. Ber or Ver was pretreated for 10 min before addition of agonists.

Statistic analysis All data were expressed as $\bar{x} \pm s$ and evaluated by t-test. P values less than 0.05 were considered to be significant.

RESULTS

Effects of Ber on $[Ca^{2+}]_i$ induced by KCl KCl 60 mmol · L^{-1} elevated $[Ca^{2+}]_i$, and FI was increased from 183 ± 56 to 674 ± 173 and FI reached the peak at (48 ± 15) s after addition of KCl in the presence of extracellular Ca^{2+}. Pretreatment of Ber 3, 10, 30, and 100 μmol · L^{-1} inhibited KCl-induced FI elevation in a concentration-dependent manner, but the resting level of FI was not affected. The time to peak of FI increase was prolonged by the same treatment. The similar effect of Ber was also seen with Ver 10 μmol · L^{-1}. The increase in FI by KCl was inhibited completely in the presence of egtazic acid 3 mmol · L^{-1} (Tab 1).

Tab 1. Effects of pretreatment with Ber on $[Ca^{2+}]_i$ mobilization and the time to peak of fluorescent intensity increase induced by KCl 60 mmol · L^{-1} in cultured HeLa cells. The change of $[Ca^{2+}]_i$ was represented by fluorescent intensity (FI). $\bar{x} \pm s$. ${}^cP<0.01$ vs resting, ${}^eP<0.05$, ${}^fP<0.01$ vs KCl.

Groups	n	$[Ca^{2+}]_i$/FI Resting	Peak	Time to peak/s
KCl	12	183 ± 56	674 ± 173^c	48 ± 15
Ber 3 μmol · L^{-1}	9	177 ± 43	678 ± 185^c	55 ± 8
Ber 10 μmol · L^{-1}	8	186 ± 50	592 ± 149^c	59 ± 8
Ber 30 μmol · L^{-1}	11	181 ± 49	473 ± 85^{ce}	69 ± 7^f
Ber 100 μmol · L^{-1}	14	179 ± 61	438 ± 77^{cf}	67 ± 13^f
Ver 10 μmol · L^{-1}	10	175 ± 47	526 ± 95^{ce}	71 ± 10^f
Egtazic acid 3 mmol · L^{-1}	10	181 ± 46	194 ± 61^f	

Effects of Ber on $[Ca^{2+}]_i$ induced by NE NE 100 μmol · L^{-1}-induced FI elevation was partially reduced ($P<0.05$, Tab 2) by pretreatment of Ber 30

Tab 2. Effects of pretreatment with Ber on $[Ca^{2+}]_i$ elevation, the slope and the time to peak of fluorescent intensity increase induced by NE 100 μmol · L^{-1} and Cal 30 μmol · L^{-} in cultured HeLa cells. The change of $[Ca^{2+}]_i$ was represented by fluorescent intensity (FI). $\bar{x} \pm s$. ${}^cP<0.01$ vs resting, ${}^eP<0.05$, ${}^fP<0.01$ vs NE or Cal.

Groups	n (cells)	$[Ca^{2+}]_i$/FI Resting	Peak	Slope/FI · s^{-1}	Time to peak/s
NE (control)	13	193 ± 49	812 ± 157^c	89 ± 22	43 ± 12
Ber 30 μmol · L^{-1}	9	187 ± 43	536 ± 85^{cf}	47 ± 12^f	49 ± 11
Ber 100 μmol · L^{-1}	10	181 ± 40	411 ± 88^{cf}	34 ± 9^f	62 ± 15^f
Ver 10 μmol · L^{-1}	12	189 ± 51	446 ± 75^{ce}	48 ± 13^f	60 ± 9^f
Egtazic acid 3 mmol · L^{-1}	12	173 ± 47	399 ± 104^{cf}	9 ± 6^f	82 ± 13^f
Cal (control)	8	184 ± 61	1024 ± 217^c	129 ± 33	21 ± 7
Ber 30 μmol · L^{-1}	14	188 ± 67	731 ± 175^{ce}	92 ± 18^e	36 ± 9^f
Ber 100 μmol · L^{-1}	11	174 ± 51	594 ± 163^{cf}	64 ± 20^f	47 ± 14^f
Ver 10 μmol · L^{-1}	9	190 ± 55	636 ± 125^{cf}	87 ± 17^e	74 ± 10^f
Egtazic acid 3 mmol · L^{-1}	8	177 ± 62	446 ± 151^{cf}	73 ± 15^f	68 ± 11^f

and 100 μmol·L^{-1} and Ver 10 μmol·L^{-1}. The slope and the time to peak of FI elevation were decreased and prolonged, respectively ($P<0.05$ or $P<0.01$, Tab 2). In the presence of egtazic acid 3 mmol·L^{-1}, FI elevation by NE was inhibited, but not completely.

Effects of Ber on $[Ca^{2+}]_i$ induced by Cal The preincubation of HeLa cells with Cal 30 μmol·L^{-1} increased FI to 1024 ± 217 from resting level with the slope of (89 ± 22) FI·s^{-1} and the FI reached the peak at (25 ± 8) s after addition of Cal. After pretreatment with Ber 30 and 100 μmol·L^{-1}, Cal-induced FI increase was decreased by 38 % and 49 % ($P<0.01$). The slope and the time to peak of FI change were also reduced and prolonged, respectively ($P<0.05$ or $P<0.01$, Tab 2).

Effects of Ber on $[Ca^{2+}]_i$ mobilized by caffeine In D-Hanks' solution or in the presence of egtazic acid 3 mmol·L^{-1}, the $[Ca^{2+}]_i$ mobilization by caffeine 80 mmol·L^{-1} was not inhibited ($P>0.05$) by pretreatment of Ber 30 and 100 μmol·L^{-1} and Ver 10 μmol·L^{-1}, and both the slope and the time to peak of FI increase were not affected ($P>0.05$, Tab 3).

Tab 3. Effects of pretreatment with Ber on $[Ca^{2+}]_i$ mobilization and the time to peak of fluorescent intensity increase induced by caffeine 80 mmol·L^{-1} in cultured HeLa cells in D-Hanks' solution or in the presence of egtazic acid. The change of $[Ca^{2+}]_i$ was represented by fluorescent intensity (FI). $\bar{x} \pm s$. $^cP<0.01$ vs resting.

Groups	n (cells)	$[Ca^{2+}]_i$/FI Resting	Peak	Time to peak/s
Caffeine (control)	16	173 ± 56	488 ± 143^c	38 ± 15
Ber 30 μmol·L^{-1}	14	177 ± 45	470 ± 125^c	41 ± 10
Ber 100 μmol·L^{-1}	15	180 ± 62	445 ± 147^c	36 ± 13
Ver 10 μmol·L^{-1}	12	193 ± 44	479 ± 111^c	42 ± 12
Egtazic acid 3 mmol·L^{-1}	11	187 ± 49	501 ± 138^c	35 ± 16

DISCUSSION

More recently, confocal microscopy has become a useful technique in biomedical analysis, especially in the measurement of intracellular ions. Compared with the method[5] in which the ultraviolet (UV) light was used as light resource, the laser is employed as light resource in confocal microscopy, therefore, it is possible to detect a lower fluorescence with combination use of Fluo 3-AM, a new generation of calcium indicator. In addition, the fluorescent bleaching and the noxious effect on living cells resulted from the laser are much less than those induced by UV light. The most important thing is that the background noise is easily avoided by using confocal microscopy because of its property of cell tomography, so the data and images are more reliable and clear.

Unlikely the excitable cells, the understanding of properties of nonexcitable cells are much concerned in recently, especially the role of intracellular Ca^{2+} signal transmembrane process. In this experiment, KCl induced $[Ca^{2+}]_i$ elevation. This result suggested that there was a concurrent voltage-dependent process in HeLa cells, and this process is related to extracellular Ca^{2+} and may be involved in KCl-induced Ca^{2+} influx. The present result also demonstrated that Ber possessed the inhibitory effect on this concurrent voltage-dependent process, and it was consistent with our previous data[7]. The intracellular Ca^{2+} release was not observed after addition of KCl in this preparation. To determine whether or not receptor-operated Ca^{2+} channel is involved in the regulation of Ca^{2+}, NE was selected for this aim. The data suggested that NE induced both Ca^{2+} influx and Ca^{2+} release because NE-induced $[Ca^{2+}]_i$ elevation was only inhibited partially by egtazic acid 3 mmol·L^{-1}. The Ca^{2+} influx by NE was reduced by pretreatment with Ber or Ver, without the effect on Ca^{2+} release. In addition, Cal-induced $[Ca^{2+}]_i$ increase was related to extracellular Ca^{2+} although it was not inhibited completely in the presence of edetic acid. The inhibitory effect of Ber on Cal-induced $[Ca^{2+}]_i$ elevation might be associated with the decrease of the permeability of Ca^{2+} across membrane. In Ca^{2+}-free solution, caffeine-induced $[Ca^{2+}]_i$ was not affected by pretreatment with Ber, Ver, and edetic acid, suggesting that caffeine-induced $[Ca^{2+}]_i$ mobilization was mediated by intracellular Ca^{2+} release probably due to activation of ryanodine receptor[8], and there was no inhibitory effect of Ber on Ca^{2+} release.

In conclusion, the HeLa cells, derived from cervical cancer, had similar biological properties in part with excitable cells[7,9,10], and $[Ca^{2+}]_i$ also played an important role in transduction system mediated by different mechanisms. Meanwhile, Ber showed an

antagonizing effect on Ca^{2+} influx through voltage-dependent or receptor-operated mechanisms, but without obvious effect on Ca^{2+} release in cultured HeLa cells.

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小檗胺对培养的HeLa细胞内游离钙浓度的作用

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关键词 钙; 钾; 去甲肾上腺素; 咖啡因; 卡西霉素; HeLa细胞; 荧光染料; 共聚焦显微镜检查

目的: 研究Ca^{2+}信号传导是否参与Hela细胞的信号传导过程以及小檗胺(Ber)对HeLa细胞内钙浓度($[Ca^{2+}]_i$)变化的影响. **方法**: Fluo 3-AM负载HeLa细胞, 共聚焦法测定$[Ca^{2+}]_i$, 结果以荧光强度(FI)表示. **结果**: (1) 有外钙时, HeLa细胞静息FI为186±44, KCl、NE、Cal、及咖啡因均升高HeLa细胞的$[Ca^{2+}]_i$. (2) Ber处理后,静息FI无影响, 但抑制KCl、NE和Cal引起的$[Ca^{2+}]_i$升高($P<0.01$), FI变化的速率减慢, 达峰值的时间延长. (3) 无外钙时, 咖啡因诱导的$[Ca^{2+}]_i$升高不被Ber抑制. (4) Ber的上述作用与Ver的作用相似. **结论**: HeLa细胞属于非兴奋性细胞,但部分生物学特征与兴奋性细胞相似, Ca^{2+}同样在其信息转导中发挥重要作用.

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