ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Aug; 20 (8): 705 – 708
http://www.simm.ac.cn; www.chinainfo.gov.cn periodical

Effects of berbamine on ATP-induced $[Ca^{2+}]_i$ mobilization in cultured vascular smooth muscle cells and cardiomyocytes[1]

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KEY WORDS vascular smooth muscle; myocardium; cultured cells; berbamine; verapamil; adenosine triphosphate; calcium; Fura 3-AM; confocal microscopy

ABSTRACT

AIM: To study the effects of berbamine (Ber) on $[Ca^{2+}]_i$ homeostasis induced by adenosine triphosphate (ATP) in vascular smooth muscle cells (VSMC) of rabbits and cardiomyocytes of rats. **METHODS**: Both cell types were cultured and loaded with Fura 3-AM. $[Ca^{2+}]_i$ was measured by fluorescent intensity (FI) in each cell with confocal microscopy. **RESULTS**: (1) ATP 30 $\mu mol \cdot L^{-1}$ elevated $[Ca^{2+}]_i$ in VSMC and cardiomyocytes, FI values reached 660 ± 258 and 1058 ± 252 from 250 ± 84 and 218 ± 76 at 19 s ± 5 s and 11.8 s ± 2.4 s, but FI in nucleus was not changed in VSMC. (2) Ber 30 $\mu mol \cdot L^{-1}$ did not affect the resting FI in both cell types, but prolonged the time to peak ($P < 0.01$) and reduced the FI elevated by ATP ($P < 0.01$), but not completely inhibited even at 100 $\mu mol \cdot L^{-1}$. (3) In D-Hanks' solution or in the presence of egtazic acid (EGTA) 3 $mmol \cdot L^{-1}$, the inhibitory effect of Ber was not seen ($P > 0.05$). (4) All effects of Ber on ATP-induced $[Ca^{2+}]_i$ mobilization were similar to those of Ver 10 $\mu mol \cdot L^{-1}$. **CONCLUSION**: In VSMC and cardiomyocytes, ATP-induced Ca^{2+} influx was inhibited by Ber and Ver, while the Ca^{2+} release was not.

[1] Project supported by Key Projects for the Eighth Five-year Plan of China (№ 859150308) and research fund (1997 – 1999) from Education Committee of Heilongjiang Province.
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Received 1998-08-10 Accepted 1998-12-23

INTRODUCTION

ATP exerted its effects either directly via P_2 receptor or through a metabolite and adenosine A receptor[1,2], and ATP could stimulate the formation of inositol phosphates and raise $[Ca^{2+}]_i$ which might be involved in P_2Y_2 receptor[3,4]. The elevation of $[Ca^{2+}]_i$ in vascular smooth muscle is an important early event in normal vascular functions. The overload of $[Ca^{2+}]_i$ would result in the pathogenesis of vascular functions. Berbamine (Ber) possessed important pharmacological effects through noncompetitive calcium antagonism[5-11]. The aim of the present study was to study the effects of Ber on ATP-induced $[Ca^{2+}]_i$ raise in vascular smooth muscle cells (VSMC) and cardiomyocytes.

MATERIALS AND METHODS

Agents Berbamine (Ber) crystals provided by the Institute of Applied Ecology of Chinese Academy of Sciences were dissolved in distilled water (pH 5.3 – 5.4 at 22 ℃) as stock solution and diluted with Hanks'solution before use. Verapamil (Ver, Orion Pharm, Helsinki) was dissolved in PBS. Fura 3-AM (Molecular Probes, Eugene OR, USA) was dissolved in Me_2SO_4 1 $g \cdot L^{-1}$ (Sigma Co) and stored at – 20 ℃. Pluronic F-127, HEPES, ATP, and egtazic acid (EGTA) were purchased from Sigma Co.

Preparation of VSMC and cardiomyocytes VSMC were isolated from thoracic aorta of New Zealand rabbits (3 – 4 wk) provided by the Experimental Animal Center of Harbin Medical University (Certificate № 0921). The cell suspension was adjusted into $5 \times 10^8 \cdot L^{-1}$, plated on 25 mm round coverslip on the bottom of 6-well multidish, and cultured in DMEM medium containing 20 % fetal bovine serum (FBS) in CO_2 incubator for 48 h, and

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Aug; 20 (8)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

then in DMEM without FBS for 24 – 48 h. VSMC was identified by "valley and hill appearance". The experiments were performed with monolayer of cells from passage 4 – 7. Cultured cardiomyocytes were prepared according to the previous method [12].

Measurement of $[Ca^{2+}]_i$ [12] VSMC and cardiomyocytes were loaded in Fura 3-AM 10 and 2 μmol · L^{-1}, respectively, for the measurement of $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ was measured by confocal microscopy and represented by fluorescent intensity (FI) [13].

Statistics Data were read from the computer and analyzed using worksheet program, Excel. The values were compared by t-test.

RESULTS

Effect of Ber on $[Ca^{2+}]_i$ elevated by ATP in VSMC ATP 30 μmol · L^{-1} induced a transient increase in cytosolic $[Ca^{2+}]_i$ and the FI value reached 660 ± 258 from the resting level (250 ± 84) at 19 s ± 5 s after the addition of ATP. The FI values in nucleus area showed no difference before and after ATP (Fig 1).

Pretreatment of VSMC with Ber 30 μmol · L^{-1} for 10 min decreased the FI value elevated by ATP without effect on the resting level. The similar result was also observed with Ver 10 μmol · L^{-1} (Tab 1).

Effect of Ber on $[Ca^{2+}]_i$ elevated by ATP in cardiomyocytes $[Ca^{2+}]_i$ was increased by ATP 30 μmol · L^{-1} in cultured cardiomyocytes (Fig 1). After the addition of ATP, the peak value of FI was 1058 ± 252 from resting at 11.8 s ± 2.4 s. When the cells were pretreated with Ber 30 μmol · L^{-1} or Ver 10 μmol · L^{-1} for 10 min, ATP-induced $[Ca^{2+}]_i$ elevation was reduced ($P < 0.01$) and the time to peak was delayed ($P < 0.01$) (Tab 2).

Tab 2. Effects of Ber and Ver 10 μmol · L^{-1} on $[Ca^{2+}]_i$ mobilized by ATP 30 μmol · L^{-1} in Hanks' or D-Hanks' solution in single cultured cardiomyocytes of rats. $[Ca^{2+}]_i$ change was represented by fluorescent intensity (FI) in each cell. $x \pm s$. $^cP < 0.01$ *vs* resting. $^dP > 0.05$, $^fP < 0.01$ *vs* ATP. $^gP > 0.05$ *vs* Ber.

Group	n	$[Ca^{2+}]_i$ FI/Cell		
		Resting	Peak	Time to Peak
ATP	30	218 ± 76	1058 ± 252^c	11.8 ± 2.4
Ber 30 μmol · L^{-1}	16	243 ± 48^d	367 ± 68^f	17 ± 5^f
Ber 100 μmol · L^{-1}	9	225 ± 41^d	289 ± 55^f	21 ± 7^f
in D-Hank	8	231 ± 53^g	349 ± 85^g	18 ± 6^g
in EGTA	7	235 ± 47^g	343 ± 56^g	19 ± 7^g
Verapamil	6	229 ± 60^d	334 ± 85^f	16 ± 5^f

Effect of Ber on $[Ca^{2+}]_i$ elevation in the absence of extracellular Ca^{2+} In the presence of EGTA 3 mmol · L^{-1} or in D-Hanks' solution, the inhibitory effects of Ber on $[Ca^{2+}]_i$ mobilized by ATP were not observed ($P > 0.05$, Tab 1,2).

DISCUSSION

The regulatory effects of ATP on cardiovascular system were closely associated with vascular functions and recently concentrated. ATP is mainly released from the blood platelets during their activation and plays an important role on cardiovascular system directly or indirectly through its metabolites, also this regulatory

Tab 1. Effects of Ber and Ver 10 μmol · L^{-1} on $[Ca^{2+}]_i$ mobilized by ATP 30 μmol · L^{-1} in Hanks' or D-Hanks' solution in single cultured VSMC of rabbits. $[Ca^{2+}]_i$ change was represented by fluorescent intensity (FI) in each cell. $x \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* resting. $^dP > 0.05$, $^fP < 0.01$ *vs* ATP. $^gP > 0.05$ *vs* Ber.

Group	n	FI/Cell			FI/Nucleus	
		Resting	Peak	Time to Peak	Resting	Peak
ATP	14	250 ± 84	660 ± 258^c	19 ± 5	96 ± 28	103 ± 30^a
Ber 30 μmol · L^{-1}	8	211 ± 28^d	359 ± 103^f	27 ± 6^f	107 ± 33^d	112 ± 24^d
Ber 100 μmol · L^{-1}	6	229 ± 46^d	293 ± 66^f	30 ± 8^f	122 ± 28^d	117 ± 31^d
in D-Hank	7	236 ± 67^g	384 ± 72^g	26 ± 6^g	112 ± 31^g	119 ± 35^g
in EGTA	7	244 ± 45^g	343 ± 56^g	30 ± 7^g	98 ± 37^g	113 ± 27^g
Verapamil	6	229 ± 23^d	321 ± 27^f	32 ± 5^f	102 ± 28^d	114 ± 30^d