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8-(*N*, *N*-Diethylamino)-*n*-octyl-3,4,5-trimethoxybenzoate reduced $[Ca^{2+}]_i$ elevation induced by histamine, serotonin, and glutamate in cultured calf basilar artery smooth muscle cells

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KEY WORDS TMB-8; calcium; basilar artery; vascular smooth muscle; cultured cells; histamine; serotonin; sodium glutamate

AIM: To study the effects of 8-(*N*, *N*-diethylamino)-*n*-octyl-3,4,5-trimethoxybenzoate (TMB-8) on intracellular free calcium ($[Ca^{2+}]_i$) in cultured calf basilar artery smooth muscle cells. **METHODS:** $[Ca^{2+}]_i$ was examined by a system of measurement of AR-CM-MIC, using Fura 2-AM as a fluorescent indicator. **RESULTS:** In the presence of extracellular Ca^{2+} 1.3 mmol $\cdot L^{-1}$, histamine (His), serotonin (5-HT), and sodium glutamate (Glu) markedly increased the $[Ca^{2+}]_i$ which was attenuated by TMB-8. In Ca^{2+} free Hanks' solution containing egtazic acid 0.1 mmol $\cdot L^{-1}$, TMB-8 not only reduced the resting $[Ca^{2+}]_i$, but also inhibited the elevation of $[Ca^{2+}]_i$ evoked by His and 5-HT. **CONCLUSION:** TMB-8 reduced the resting $[Ca^{2+}]_i$ and attenuated His-, 5-HT-, and Glu-induced increases of $[Ca^{2+}]_i$ in basilar artery smooth muscle cells.

Histamine (His), serotonin (5-HT), and sodium glutamate (Glu) were found in mammalian central nervous system[1]. They increased $[Ca^{2+}]_i$ of brain neurons, deteriorated brain ischemia, produced contractions or dilations in the cerebral arteries in various physiologic or pathologic conditions, and affected some pathologic processes[2,3]. 8-(*N*, *N*-diethylamino)-*n*-octyl-3, 4, 5-trimethoxybenzoate (TMB-8) was regarded as intracellular calcium antagonist[4,5] and showed protection and reduction of neural damage in experimental ischemic strokes[6]. TMB-8 was presumed to reduce $[Ca^{2+}]_i$ of cerebral vascular smooth muscle cells. The present work was undertaken to investigate the effects of TMB-8 and these transmitters on $[Ca^{2+}]_i$ in cultured calf basilar artery smooth muscle cells.

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MATERIALS AND METHODS

Materials TMB-8, Fura 2-AM, Me_2SO, histamine, serotonin, and *L*-glutamic acid were purchased from Sigma Chemical Co. TMB-8 was dissolved freshly by distilled water. *L*-Glutamic acid was dissolved by Hanks' solution, regulating pH to 7.0 with NaOH. Fura 2-AM was dissolved in Me_2SO. Dulbecco's modified Eagle's medium (DMEM) was obtained from Gibco. The system of measurement of AR-CM-MIC was the product of Spex Co, USA.

Cell culture The smooth muscle cells of calf basilar artery were cultured according to the method[7] with some modifications. The basilar artery of newborn calf (Nanjiang Weigang Calf Serum Factory) was isolated rapidly. The endothelium was removed first by erasing when the blood vessels were cut longitudinally, the pieces (1 - 2 mm^2) of the blood vessels were placed endosurface downright in culture dishes and were incubated at 37 ℃ in a humidified incubator with 95 % air and 5 % CO_2 in DMEM with 10 % (vol/vol) fetal calf serum (FCS). Cells migrated from the blood vessels about in 7 d. When cells covered the culture dish, small pieces were removed and cells were washed with D-Hanks' solution twice, then incubated at 37 ℃ for 10 min in Ca^{2+} and Mg^{2+}-free Hanks' solution with 0.125 % trypsin. The trypsinization was discontinued by adding ice-cold DMEM 10 mL containing 10 % (vol/vol) FCS. The cell pellets were resuspended in fresh medium with FCS to 1×10^8 cells $\cdot L^{-1}$ and cultured. The medium was changed every 2 - 3 d. Cells reached confluence in about 5 d and then passaged every 5 - 6 d. $[Ca^{2+}]_i$ was measured in passages 4 and 5.

$[Ca^{2+}]_i$ measurement Cells were

incubated in DMEM with Fura 2-AM 3 $\mu mol \cdot L^{-1}$ for 40 min. The loaded cells were washed twice with Hanks' solution or D-Hanks' solution. The fluorescence was recorded in a system of measurement of AR-CM-MIC at λ_{ex} = 340 nm/380 nm, λ_{em} = 505 nm, directly after the addition of an agonist. $[Ca^{2+}]_i$ of single cell was calculated according to the formula[8]:

$$[Ca^{2+}]_i = K_d (sb_1/sb_2) [(R - R_{min})/(R_{max} - R)] \ nmol \cdot L^{-1}$$

K_d = 224 $nmol \cdot L^{-1}$ at 37 ℃. R was fluorescence intensity, R_{max} and R_{min} were obtained by the addition of ionomycin and egtazic acid respectively.

Statistical analysis Data were analyzed with t test.

RESULTS

In normal Hanks' solution, $[Ca^{2+}]_i$ was increased by His (1, 10, and 100 $\mu mol \cdot L^{-1}$) which was markedly inhibited by TMB-8 (30 $\mu mol \cdot L^{-1}$) (Tab 1).

Tab 1. The effect of TMB-8 30 $\mu mol \cdot L^{-1}$ on the rises of $[Ca^{2+}]_i$ induced by His and Glu in Hanks' solution containing Ca^{2+} 1.3 $mmol \cdot L^{-1}$. $n = 6$, $\bar{x} \pm s$. $^cP < 0.01$ vs before His or Glu. $^dP > 0.05$, $^eP < 0.05$, $^fP < 0.01$ vs control.

	$\mu mol \cdot L^{-1}$	$[Ca^{2+}]_i$/$nmol \cdot L^{-1}$ Control	TMB-8
His	0	98 ± 5	97 ± 9^d
	1	120 ± 6^c	109 ± 8^e
	10	145 ± 8^c	132 ± 5^e
	100	171 ± 10^c	149 ± 7^f
Glu	0	96 ± 4	95 ± 6^d
	1	118 ± 9^c	106 ± 9^e
	3	139 ± 8^c	119 ± 9^f
	10	146 ± 8^c	132 ± 4^f

In Ca^{2+}-free Hanks' solution containing egtazic acid 0.1 $mmol \cdot L^{-1}$, TMB-8 30 $\mu mol \cdot L^{-1}$ reduced the resting $[Ca^{2+}]_i$, TMB-8 3, 10 $\mu mol \cdot L^{-1}$ decreased the elevation of $[Ca^{2+}]_i$ induced by His (1, 10, and 100 $\mu mol \cdot L^{-1}$) and TMB-8 30 $\mu mol \cdot L^{-1}$ almost completely blocked this elevation (Fig 1).

5-HT (1, 10, and 100 $\mu mol \cdot L^{-1}$) increased the $[Ca^{2+}]_i$ efficiently in normal Hanks' solution, these effects were partially

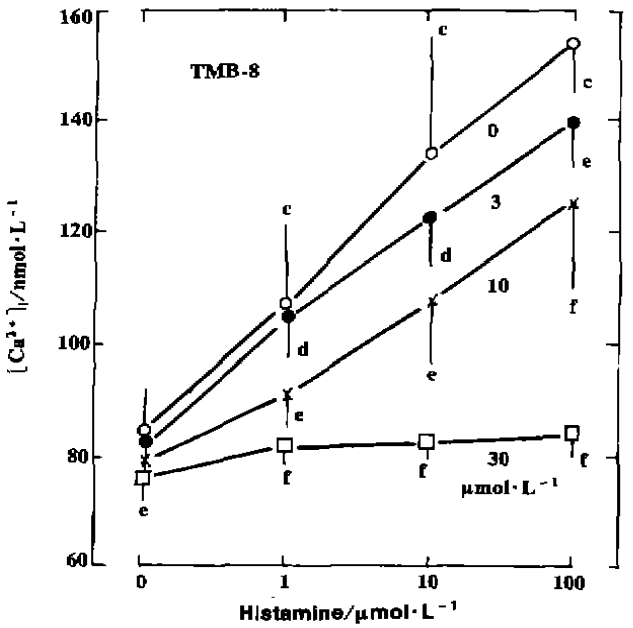

Fig 1. Effect of TMB-8 on the rises of $[Ca^{2+}]_i$ induced by His in Ca^{2+}-free Hanks' solution containing egtazic acid 0.1 $mmol \cdot L^{-1}$. $n = 6$, $\bar{x} \pm s$. $^cP < 0.01$ vs before His. $^dP > 0.05$, $^eP < 0.05$, $^fP < 0.01$ vs control.

blocked by TMB-8 30 $\mu mol \cdot L^{-1}$. The elevation of $[Ca^{2+}]_i$ induced by 5-HT (1, 10, and 100 $\mu mol \cdot L^{-1}$) in Ca^{2+} free Hanks' solution containing egtazic acid 0.1 $mmol \cdot L^{-1}$ was blocked almost completely by TMB-8 30 $\mu mol \cdot L^{-1}$ (Fig 2).

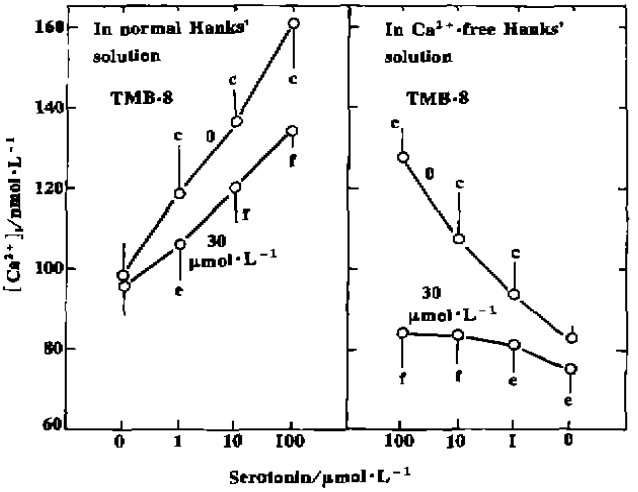

Fig 2. Effect of TMB-8 on the rises of $[Ca^{2+}]_i$ induced by 5-HT in Hanks' solution containing Ca^{2+} 1.3 $mmol \cdot L^{-1}$ (left) and in Ca^{2+}-free Hanks' solution containing egtazic acid 0.1 $mmol \cdot L^{-1}$ (right). $n = 6$, $\bar{x} \pm s$. $^cP < 0.01$ vs before 5-HT. $^eP < 0.05$, $^fP < 0.01$ vs control.

Glu (1, 3, and 10 $\mu mol \cdot L^{-1}$) increased $[Ca^{2+}]_i$ in normal Hanks' solution, TMB-8 30 $\mu mol \cdot L^{-1}$ inhibited Glu-induced increase of $[Ca^{2+}]_i$, but it did not affect the resting $[Ca^{2+}]_i$ ($P > 0.05$) (Tab 1).

DISCUSSION

In the present of Ca^{2+}-free extracellular medium, resting $[Ca^{2+}]_i$ is dependent on intracellular Ca^{2+} mobilization and the elevation of $[Ca^{2+}]_i$ induced by agonists relyed on intracellular Ca^{2+} release. Our studies in cultured calf basilar artery smooth muscle cells found that in Ca^{2+}-free extracellular medium, TMB-8 30 $\mu mol \cdot L^{-1}$ reduced resting $[Ca^{2+}]_i$; TMB-8 3, 10 $\mu mol \cdot L^{-1}$ decreased the elevation of $[Ca^{2+}]_i$ induced by His or 5-HT and TMB-8 30 $\mu mol \cdot L^{-1}$ almost completely blocked this elevation, indicating that TMB-8 may increase intracellular Ca^{2+} uptaking and inhibit the release of intracellular Ca^{2+} pools.

In the present of extracellular Ca^{2+}, TMB-8 30 $\mu mol \cdot L^{-1}$ inhibited the increase of $[Ca^{2+}]_i$ evoked by His, 5-HT or Glu. It was reported that TMB-8 could block *N*-receptor[9], but action of TMB-8 on His, 5-HT or Glu receptor needed forward study. The saturation of the sarcoplasmic reticulum (SR) with calcium can *inhibit Ca^{2+}-influx from extracellular site*[10]. The action of TMB-8 blocking Ca^{2+}-influx is highly possible that this mechanism may be an indirect action.

Neural tissues are highly dependent on glucose and O_2 supplies. An adequate continuous blood supply to the brain is essential to ensure the normal function of the brain. The cerebro-vasodilator and agents increasing cerebral blood flow and improving cerebral circulation are important for protection/treatment of brain ischemic. TMB-8, regarded as intracellular calcium antagonist, may be useful in protection/treatment of these diseases.

It is concluded that TMB-8 significantly reduced the elevation of $[Ca^{2+}]_i$ induced by His, 5-HT or Glu. The results indicated that TMB-8 prevented Ca^{2+} release or increased Ca^{2+} uptaking and might indirectly block Ca^{2+}-influx on plasma membrane. Anticerebral ischemic effect of TMB-8 may be related to these actions.

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TMB-8抑制组胺、5-羟色胺和谷氨酸引起的培养乳牛基底动脉平滑肌细胞内游离钙的升高

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关键词 TMB-8; 钙; 基底动脉; 血管平滑肌; 培养的细胞; 组胺; 5-羟色胺; 谷氨酸钠

目的: 研究 8-(*N*, *N*-二乙胺)-*n*-辛基-3, 4, 5-三甲氧基苯甲酸酯对培养乳牛基底动脉平滑肌$[Ca^{2+}]_i$的作用. **方法**: 采用 AR-CM-MIC 阳离子测定系统, 测量细胞内游离钙浓度($[Ca^{2+}]_i$). **结果**: 在细胞外钙浓度为 1.3 $mmol \cdot L^{-1}$时, TMB-8 30 $\mu mol \cdot L^{-1}$可明显抑制组胺, 5-羟色胺和谷氨酸引起的$[Ca^{2+}]_i$的升高. 在外钙为零 + 依他酸 0.1 $mmol \cdot L^{-1}$ 时, TMB-8 30 $\mu mol \cdot L^{-1}$可明显降低静息$[Ca^{2+}]_i$, TMB-8 30 $\mu mol \cdot L^{-1}$可几乎完全阻断组胺和 5-羟色胺增加$[Ca^{2+}]_i$的作用. **结论**: TMB-8 降低培养乳牛基底动脉平滑肌静息$[Ca^{2+}]_i$, 抑制 His, 5-HT 和 Glu 引起的$[Ca^{2+}]_i$的增加.