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Effects of TMB-8 on constriction of pial arteries in rats[1]

WANG Bin[2], XIAO Ji-Gao (*Department of Pharmacology, Nanjing Medical University, Nanjing 210029, China*)

KEY WORDS TMB-8; vasoconstriction; vasodilation; cerebral arteries; N^G-nitroarginine methyl ester; microcirculation; nimodipine; potassium chloride; serotonin

ABSTRACT

AIM: To study the effects of 8-(*N*, *N*-diethylamino)-*n*-octyl-3,4,5-trimethoxybenzoate (TMB-8) on constriction of pial arteries (PA). **METHODS**: The change of PA in rats was observed continuously and directly through cranial window using microcircular image-shearing system. **RESULTS**: Nimodipine (Nim) 1 μmol·L^{-1} produced dilatation of PA immediately and the maximal response occurred in 2 min. But the diameter was not changed by TMB-8 50 μmol·L^{-1}. PA diameter decreased immediately after the application of CSF containing KCl. TMB-8 25, 50 μmol·L^{-1} apparently inhibited KCl-induced PA constriction. When persistent constriction was evoked by 5-HT, diameter of PA were increased in a concentration-dependent manner after application of TMB-8, which was inhibited by N^{ω}-nitro-*L*-arginine methyl ester (*L*-NAME). **CONCLUSION**: TMB-8 inhibited the contraction induced by 5-HT or KCl in cerebral artery, probably via its calcium antagonization and nitric oxide release.

INTRODUCTION

8-(*N*, *N*-diethylamino)-*n*-octyl-3, 4, 5-trimethoxybenzoate (TMB-8) reduced the elevation of [Ca^{2+}] induced by His, 5-HT or Glu and inhibited the contraction of basilar artery evoked by NE or BHQ in rabbits, indicating that TMB-8 prevented Ca^{2+} release or increased Ca^{2+} uptake and might indirectly block Ca^{2+}-influx on plasma membrane[1-4]. Although TMB-8 showed protection and reduction of neural damage in experimental ischemic stokes[5], the direct effects of TMB-8 on cerebral artery have not been studied yet. By cranial window the dynamic effect of drug on cerebral microcirculation was observed continuously and directly, which has little harmful effects on cerebral physiological function. We made use of cranial window on the parietal cortex in rats and observed the direct effect of TMB-8 on cerebral vascular.

MATERIALS AND METHODS

Materials TMB-8, nimodipine (Nim), and serotonin (5-HT) were purchased from Sigma Chemical Co and were dissolved in artificial cerebral spinal fluid (CSF, NaCl 132, KCl 2.95, $CaCl_2$ 1.71, $MgCl_2$ 0.65, $NaHCO_3$ 24.6, *D*-glucose 3.69 mmol·L^{-1}). XY-3 microcircular microscope and MCIP microcircular image-shearing system were the products of Jiangnan Optical Instrument Factory. Sprague-Dawley rats were irrespective of sex (weighing 200 – 250 g, from Shanghai Experimental Animal Center, Chinese Academy of Sciences, Grade Ⅱ, Certificate № 005).

Preparation of rats Rats were anesthetized with sodium pentobarbital 40 mg·kg^{-1} ip. The depth of anesthesia was tested by applying pressure to paw or tail and by the response of heart rate and blood pressure. The additional sodium pentobarbital was administered when needed. After a tracheostomy was performed, each rat was mechanically ventilated with room air. A catheter was placed in the femoral artery to measure systemic arterial pressure and to draw arterial blood samples for gases and pH determination. Arterial blood gases were monitored within normal limits throughout the experiments, pH 7.40 ± 0.01, P_{CO_2} (4.60 ± 1.2) kPa, P_{O_2} (14 ± 3) kPa. Body temperature was maintained at 37 – 38 ℃ with a heating pad.

A cranial window was prepared over the right parietal cortex[6]. Briefly the head was fixed in prone position with a stereotaxic apparatus, the dura was opened without touching the brain. A glass cranial window was placed in the hole and secured with dental cement. The window field was superfused at 0.3 mL·min^{-1} with CSF at the temperature of 37 ℃. Pial arteries, with diameter

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[2] Correspondence to Dr WANG Bin. Phn 86-25-666-2884. Fax 86-25-650-8960. E-mail BinWang@njmu.edu.cn
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ranging from 35 to 45 μm, were visualized through the cranial window. The diameters of PA were measured using a microscope equipped with a television camera coupled to a video monitor and width analyzer. The images were recorded on videotape for later analysis. In each rat, one arteriole was observed under the window.

Cerebral microvessels were equilibrated for 40 min after installation of the cranial window. After application of drug arteries was suffused with CSF to allow diameters to return to stable baseline before the next drug was applied. The order of drugs application in different terms was randomized. 36 rats were used.

Statistical analysis Data were analyzed with *t* test.

RESULTS

The effect of TMB-8 on diameter of PA PA diameters were kept constant for 3 h when cranial window was perfused by CSF, which suggested that there was a relatively stable milieu in cranial window. CSF containing Nim 1 μmol·L^{-1} produced dilatation of PA immediately and the maximum response occurred at 2 min then remained unchanged. CSF containing TMB-8 50 μmol·L^{-1} did not change the diameter of PA, indicating TMB-8 was different from Nim in effects on PA (Fig 1).

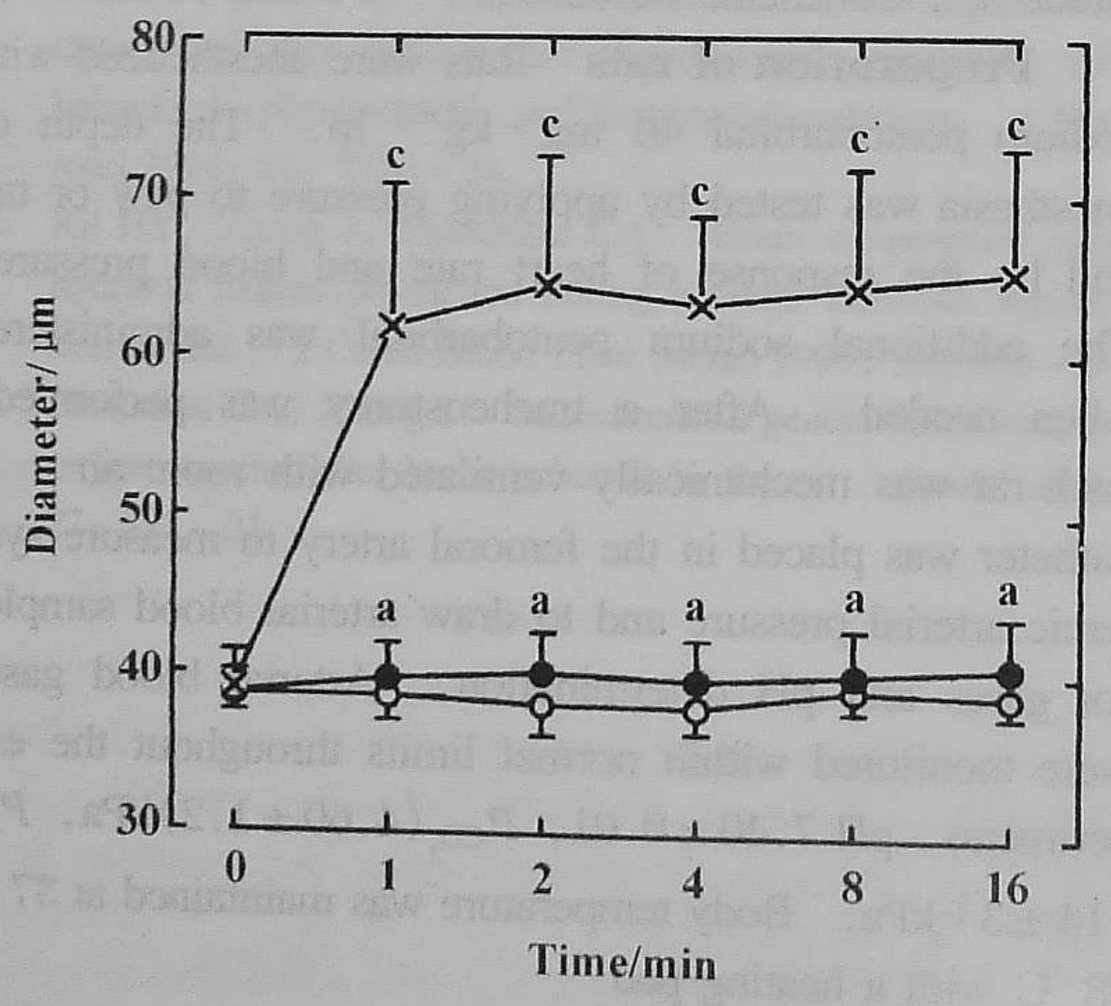

Fig 1. Effects of TMB-8 on the diameter of pial artery in rats. ○: Control; ●: TMB-8 50 μmol·L^{-1}; ×: Nim 1 μmol·L^{-1}. $n = 6$ rats, $\bar{x} \pm s$. $^{a}P > 0.05$, $^{c}P < 0.01$ *vs* Control.

The effect of TMB-8 on KCl-induced PA constriction PA constricted immediately after the application of CSF containing KCl. PA diameter was decreased from 39.4 ± 2.5 μm to 33.1 ± 3.3 ($P < 0.01$) and 25.5 ± 3.4 μm ($P < 0.01$) by KCl 20 and 40 mmol·L^{-1} respectively. The vasoconstriction induced by KCl was inhibited by pre-suffusion with TMB-8 25 or 50 μmol·L^{-1} for 20 min (Tab 1).

Tab 1. Effects of TMB-8 on KCl-induced decrease in diameter in pial artery of rats. $n = 6$ rats. $\bar{x} \pm s$. $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* TMB-8 0 μmol·L^{-1}.

TMB-8/ μmol·L^{-1}	Diameter/μm KCl 20 mmol·L^{-1}	KCl 40 mmol·L^{-1}
0	33 ± 3	26 ± 3
25	36.8 ± 1.7^{b}	29.8 ± 3.1^{b}
50	38.8 ± 1.5^{c}	33 ± 3^{b}

The effect of TMB-8 on 5-HT-induced PA constriction PA almost constricted immediately and then its diameter was kept constant during 120 min by continuous perfusion of CSF containing 5-HT 1 μmol·L^{-1}. TMB-8 12.5, 25, 50 μmol·L^{-1} were given respectively 30 min after application of 5-HT, diameter of PA were increased in a concentration-dependent manner (Tab 2).

Tab 2. Effects of TMB-8 on 5-HT 1 μmol·L^{-1} induced decrease in diameter of pial artery. $n = 5$. $\bar{x} \pm s$. $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* TMB-8 0 μmol·L^{-1}.

TMB-8/μmol·L^{-1}	Diameter/μm
0	16.4 ± 1.9
12.5	19 ± 3^{b}
25	22 ± 3^{c}
50	28 ± 6^{c}

The effect of *L*-NAME on PA dilation induced by TMB-8 The cranial window was superfused with CSF containing 5-HT 1 μmol·L^{-1} for 30 min in each group, diameter of PA decreased immediately and then kept constant. Group 1 served as control. *L*-NAME 10 μmol·L^{-1} was applied in group 2 and TMB-8 50 μmol·L^{-1} was added in group 3 respectively. In group 4, 10 min after application of *L*-NAME, TMB-8 50 μmol·L^{-1} was applied. It was *L*-NAME that did not produce change of PA diameter in group 2 and TMB-8 produced dilation of PA in group 3. However, in group 4, the diameter was significantly greater than that in

group 2 and smaller than that in group 3, suggesting that *L*-NAME partly inhibited TMB-8-induced vasodilation (Fig 2).

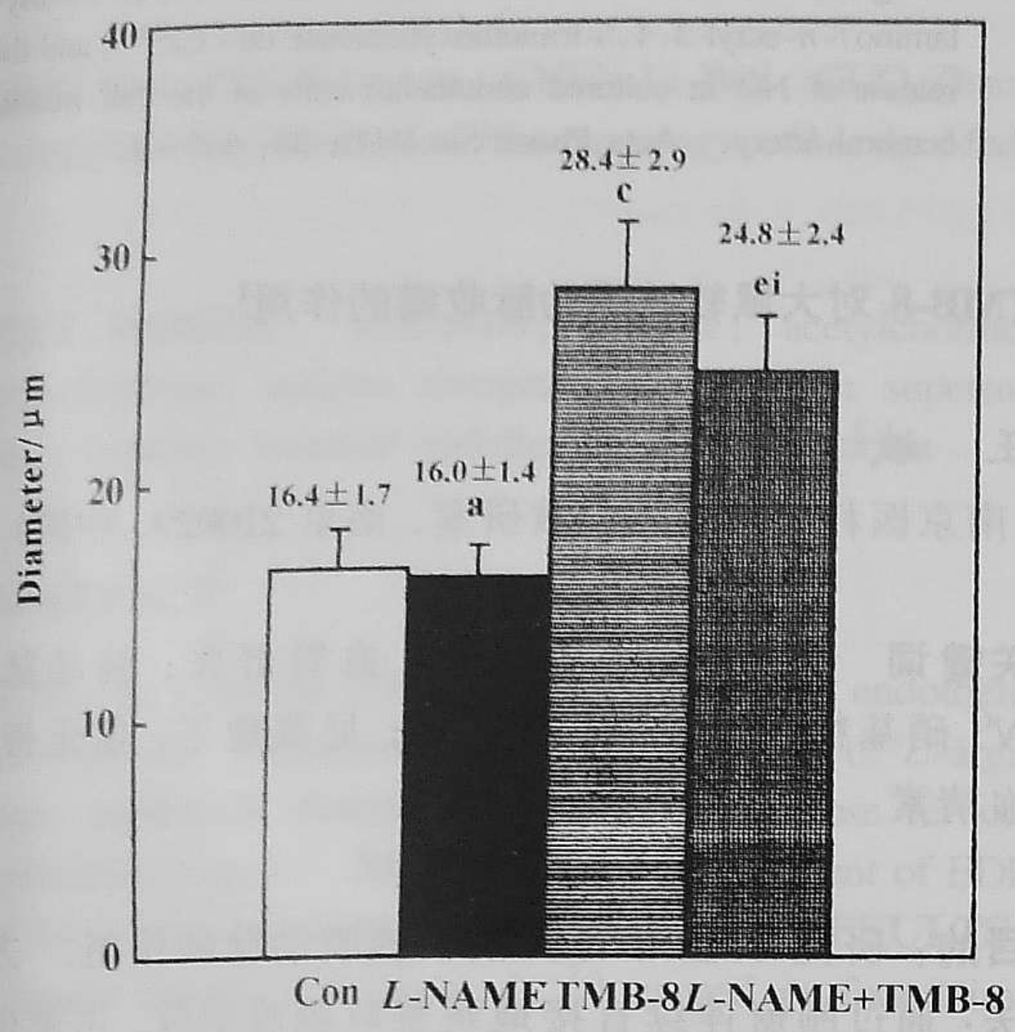

Fig 2. Effects of *L*-NAME on PA dilation induced by TMB-8. TMB-8 or *L*-NAME was perfused 30 min after 5-HT 1 μmol·L^{-1}. $n=6$, $\bar{x}\pm s$. $^{a}P>0.05$, $^{c}P<0.01$ *vs* Control. $^{e}P<0.05$ *vs* TMB-8 50 μmol·L^{-1}. $^{i}P<0.01$ *vs* *L*-NAME 10 μmol·L^{-1}.

DISCUSSION

Dynamic change of pial microcirculation could be observed continuously and directly by means of cranial window, the condition of cerebral vascular bed could be analyzed in time. Cerebral physiological function was not less influenced by superfusing CSF with constant speed. If the drug was applied by dropping CSF containing drugs on pial surface without using suffusion system, there were some problems difficult to solve. Firstly, the gradient of drug concentration was formed in different area, due to uneven dropping. Secondly, the diameter of PA decreased after the medium on the pial surface vaporized, in that case, application of CSF containing no drugs would produce increase of diameter. As a result, it would be hard to distinguish the effect of drug from that of experimental condition. By setting a suffusion system, we could maintain a stable condition in cranial window and solved the problems. In control, no distinct change of PA diameter was observed within 3 h of CSF suffusing.

TMB-8 did not have an apparent direct effect on resting arterial diameter, which was consistent with our previous studies, TMB-8 did not have influence on the rest tones of basilar artery in rabbits and on the rest $[Ca^{2+}]_i$ in cultured calf basilar artery smooth muscle cells in normal Hanks' solution respectively[1,4]. The result distinguish it from other Ca^{2+} antagonists, such as Nim[7]. Suffusion of CSF containing KCl induced influx of calcium in vascular smooth muscle cell, which increased cytosolic calcium and produced contraction of vessel[2]. KCl-induced vasoconstriction was inhibited by previously application of TMB-8, suggesting that TMB-8 played a role in inhibition of the influx of calcium. 5-HT increase influx of Ca^{2+} and trigger release of Ca^{2+} from sarcoplasmic reticulum (SR) and increase cytosolic Ca^{2+}[1]. 5-HT-induced persistent contraction of PA was reduced by application of TMB-8 in a concentration-dependent manner. The result suggested that TMB-8 influenced metabolism of Ca^{2+} storage, activated pump of Ca^{2+} and decrease cytosolic Ca^{2+}, thus induced dilation of PA[8,9].

Nitric oxide (NO) participate regulation of physiological function of cardiovascular, nervous and immunological system. Central nerve, heart and vascular endothelium can release NO[10]. NO released by vascular endothelium diffuses from where it is synthesized to vascular smooth muscle, to which smooth muscle cell relaxes by increasing guanosine 3′, 5′-cyclic monophosphate (cGMP) through activation of cylase guanylate[11].

NO synthase may be divided into constitutive, inducible and nervous forms and so on. NO produced by the constitutive NO synthase (cNOS) was released by vascular endothelial cell, and dilates vessel without neurotoxicity[12]. It was reported that some calcium antagonist may induce NO-release from coronary vascular endothelium presumably by activating endothelial NO-synthase, this effect may be mediated by an influence on the intracellular calcium in endothelial cells[13]. Single application of cNOS inhibitor *L*-NAME induced no diameter change while *L*-NAME inhibited TMB-8-induced vasodilation described above. In our previous studies[14], we found TMB-8 50 μmol·L^{-1} increased release of NO in cultured endothelial cells of the calf middle cerebral artery. These results imply that the effect of PA dilation induced by TMB-8 may be related to NO release of endothelial cell of PA. Underlying mechanisms need further research.

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TMB-8 对大鼠软脑膜动脉收缩的作用[1]

王 斌[2], 肖继皋
(南京医科大学药理学教研室, 南京 210029, 中国)

关键词 TMB-8; 血管收缩; 血管舒张; 脑动脉; N^G-硝基精氨酸甲酯; 微循环; 尼莫地平; 氯化钾; 血清素

目的: 研究 TMB-8 对大鼠软脑膜动脉的作用. **方法**: 通过颅窗连续直接地观察软脑膜动脉, 用微循环系统记录和分析动脉直径的变化. **结果**: Nim l $\mu mol \cdot L^{-1}$使 PA 直径立即增加, 在给药后 2 min 时达到最大, 而 TMB-8 50 $\mu mol \cdot L^{-1}$对 PA 无明显影响. 预先给予 TMB-8 25, 50 $\mu mol \cdot L^{-1}$则明显抑制 KCl 引起的 PA 收缩幅度. 在 CSF 中存在 5-HT 使 PA 持续收缩的情况下, TMB-8 浓度依赖性地扩张 PA, 在 TMB-8 50 $\mu mol \cdot L^{-1}$加入前, 灌流一氧化氮(NO)合酶抑制剂 *L*-NAME 10 $\mu mol \cdot L^{-1}$则 TMB-8 扩张 PA 的作用减弱. **结论**: TMB-8 能扩张痉挛的脑血管, 可能与其钙拮抗和一氧化氮释放作用有关.

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