谷酰多巴在清醒兔的交感神经抑制作用

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摘要 给清醒兔 iv 输注谷酰多巴（GD）25 和 100 $\mu g \cdot kg^{-1} \cdot min^{-1}$，MAP、HR 和动脉血浆多巴胺(DA)浓度无显著变化. 尿 DA 排泌率和肾 DA 增加. 动脉血浆 GD 和 *L*-多巴分别达到3.2±0.8 $\mu g \cdot ml^{-1}$和16.1±5.1 $ng \cdot ml^{-1}$. 肾脏和肾外去甲肾上腺素(NE)溢出率均降低，肾 NE 增加. 作为具有相对肾脏选择性的 DA 前体，GD 能抑制清醒家兔肾脏及肾外交感神经活性.

关键词 儿茶酚胺；多巴胺；多巴；肾；左旋多巴；去甲肾上腺素

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BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1994 Jan; 15 (1): 21—27

Presence of endothelium masks direct vasodilator effects of pyrogallol and methylthioninium chloride in perfused rat mesenteric artery[1]

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ABSTRACT In the perfused rat mesenteric artery vasoconstrictor responses to transmural nerve stimulation (TNS) were enhanced by pyrogallol (Pyr) 0.1 $mmol \cdot L^{-1}$ or methylthioninium chloride (Met) 0.01 $mmol \cdot L^{-1}$. But the duration of the effect of Pyr was brief, while the effect of Met remained stable. Met, but not Pyr, slightly increased the basal level of perfusion pressure. Contractile responses to the alpha adrenergic agonist methoxamine were also potentiated by both Pyr and Met, and in both cases their effects persisted as long as Pyr or Met was present. Superoxide dismutase (SOD) abolished or inhibited the potentiation produced by Pyr or Met. Both Pyr and Met inhibited the vasodilation produced by acetylcholine (ACh). However, after blockade of endothelial function both Pyr and Met inhibited vasoconstrictor responses to TNS in the presence of N^{ω}-nitro-*L*-arginine methyl ester (*L*-NAME) 0.1 $mmol \cdot L^{-1}$, an inhibitor of nitric oxide synthesis, or removal of endothelium. After removal of endothelium both Pyr and Met produced vasodilator responses in a concentration-dependent manner. These results suggest that the ability of both Pyr and Met to potentiate contractile responses and inhibit vasodilator responses to ACh is due to generation of superoxide anion, and that the actions of Met may also involve direct inactivation of guanylate cyclase. The present study also suggests that both Pyr and Met have direct relaxing effects on vascular smooth muscle, effects which are masked by enhancing actions in the presence of endothelium.

KEY WORDS mesenteric arteries; pyrogallol; methylthioninium chloride; N^{ω}-nitro-*L*-arginine methyl ester; superoxide dismutase

Methylthioninium chloride (methylene blue, Met), an inhibitor of guanylate cyclase, is widely used as a tool to evaluate the mechanism of action of vasodilators[1,2]. Besides inhibiting guanylate cyclase, Met has been shown to produce superoxide anion[3].

Pyrogallol (Pyr), a generator of superoxide anion, produces pharmacological responses similar to those of Met, including inhibition of relaxation to endothelium-dependent vasodilator and potentiation of contractile responses to vasoconstrictors, as well as inactivation of

Received 1993-06-12 Accepted 1993-11-15
[1] Supported by Grant #P01 DK36829 from the National Institutes of Health and by a postdoctoral fellowship from the California Affiliate of the American Heart Association.
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guanylate cyclase[1-3]. Endothelium-derived relaxing factor (EDRF), which has been postulated to be identical with nitric oxide (NO), activates guanylate cyclase, with a subsequent elevation of tissue levels of cGMP resulting in relaxation of vascular smooth muscle[4-6]. Thus inhibition of endothelial function produced by both Pyr and Met could possibly be due to inactivation of NO *via* generation of superoxide anion.

In contrast, Met, but not Pyr, decreased the basal levels of cGMP as well as increasing basal tone of blood vessels[1,5]. At higher concentrations Met produces constriction of isolated rabbit aorta due to release of endogenous norepinephrine from intramural adrenergic nerves *via* a mechanism independent of the lowering of cGMP[1,7]. These results suggest that the mechanism of pharmacological effect of Met is not totally similar to Pyr.

In view of the dissimilarities between Pyr and Met in the mode of inhibition of endothelial function, as well as effect on contractile responses to different vasoconstrictors, in the present study we compared the effects of Pyr and Met on contractile responses to the selective α adrenergic receptor agonist, methoxamine, and to endogenous norepinephrine released by TNS. Furthermore, we investigated the effect of inhibition of NO synthesis by the *L*-arginine antagonist, N^{ω}-nitro-*L*-arginine methyl ester (*L*-NAME), on contractile responses to TNS and the effects of Pyr and Met.

MATERIALS AND METHODS

Tissue preparation and perfusion Mesenteric vasculature of ♀ Sprague-Dawley rats (n=60, 275±s 25 g) was isolated for perfusion as described previously[8]. Rats were decapitated, and the mesenteric artery was quickly cannulated at its origin at the aorta with PE 50 tubing and perfused with warm Krebs' solution, saturated with 95 % O_2 + 5 % CO_2. Preparations were then placed in a water jacket (200 ml) maintained at 37 ℃. The system was perfused with Krebs' solution with peristaltic pump at a rate of 5 ±0.2 ml·min^{-1} and superfused by gravity feed at rate of 1±0.2 ml·min^{-1}. The Krebs' solution had the following composition: NaCl 118, KCl 4.8, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4$ 1.2, edetic acid 0.107, and dextrose 11.5 mmol·L^{-1}. The perfusion pressure was recorded by a pressure transducer. The resulting electric signals were digitized by Macintosh SE computer.

Two platinum electrodes, one placed around the superior mesenteric artery and the other resting on the vasculature in a lower part of the intestine, were used to create transmural field stimulation. TNS (amplitude of 60 V and pulse duration of 3 ms) was applied at 8 Hz for trains of 80 pulses using a Grass S48 stimulator. For vasoconstrictor responses to TNS, 2 min were allowed between each stimulation train.

Experimental procedures Tissues were equilibrated for 60 min before beginning each experiment. All drugs were administered by switching the perfusion solution to solution containing drug. For measurement of responses to Pyr, Met, or ACh, the tissues were pretreated with guanethidine 5 μmol·L^{-1} for 20 min and then contracted by methoxamine 5 μmol·L^{-1}. In the case of ACh, drugs were given into the perfusate in a cumulative fashion by changing to perfusion solution containing the indicated concentration of ACh. In the case of studies of the effect of Met on responses to Pyr, various concentrations of Pyr were tested in a non-cumulative fashion. To remove the endothelium, preparations were perfused with distilled water containing saponin 50 μg·ml^{-1} for 3 min. For all studies a paired design was used, that is the same tissue was studied both before and after treatment with Pyr or Met.

Drugs Pyr, *L*-NAME, SOD, saponin, ACh chloride, and methoxamine HCl (Sigma); Met (National Aniline) and guanethidine (Ciba Pharmaceutical Co). All drugs were dissolved and diluted in Krebs' solution. Pyr and Met were freshly prepared each day.

Statistics Results are expressed as $\bar{x}\pm s$. Contraction-response curves and EC_{50} of values (agonist concentration needed to produce 50 % of the maximal response) were derived from nonlinear, least-squares

sigmoid regression analysis of the concentration-response data. Statistical analyses were performed using paired *t* test and one way ANOVA. Tukey's test was used for multiple comparisons when ANOVA indicated statistically significant differences between groups.

RESULTS

Vasoconstrictor responses to TNS or methoxamine TNS of the mesenteric vascular bed caused an increase in perfusion pressure, and this response was completely blocked by the adrenergic neuron blocker, guanethidine, confirming that vasoconstrictor responses were due to activation of adrenergic nerves (Fig 1). Pyr 0.1 mmol·L^{-1} potentiated vasoconstrictor responses to TNS, from 2.7±1.0 to 4.0±1.0 kPa, control and Pyr, respectively (n=4, P<0.01) (Fig 1). The potentiation by Pyr of vasoconstrictor responses to TNS was transient, and contractile responses quickly returned to control levels.

Vasoconstrictor responses to TNS were also potentiated by Met 0.01 mmol·L^{-1} (Fig 1). Increases in perfusion pressure with TNS were 2.9±0.8 and 5.4±1.5 kPa, control and Met, respectively (n=5, P<0.01). The response to Met was stable as long as Met remained in the bath. Met itself slightly increased the basal level of perfusion pressure from 2.9±0.3 to 3.3±0.3 kPa, control and Met, respectively (n=5, P<0.01).

Similarly, vasoconstrictor responses to methoxamine were also enhanced by Pyr (Fig 2). Increases in perfusion pressure produced by methoxamine were 6.1±0.9 and 8.2±1.5 kPa, control and in the presence of Pyr, respectively (n=5, P<0.01). Met also caused an augmentation of vasoconstrictor responses to methoxamine, from 5.8±1.5 to 19.7±3.7 kPa, control and in the presence of Met, respectively (n=5, P<0.01) (Fig 2). Potentiation of contractile responses to methoxamine

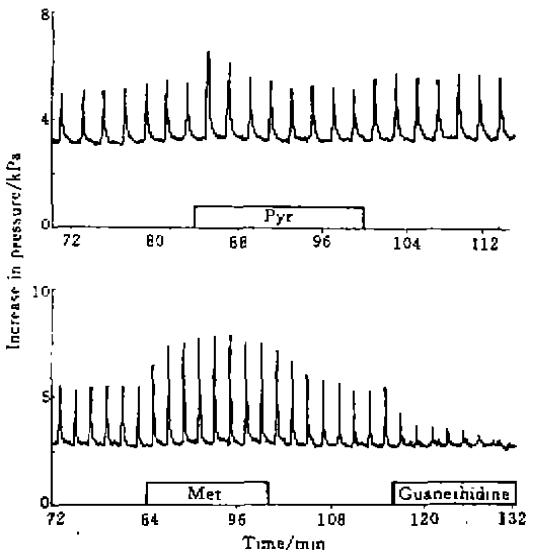

Fig 1. Effects of Pyr 0.1 mmol·L^{-1}, Met 0.01 mmol·L^{-1}, and guanethidine 5 μmol·L^{-1} on vasoconstrictor responses to TNS in perfused rat mesenteric artery. TNS was applied at 8 Hz for trains of 80 pulses.

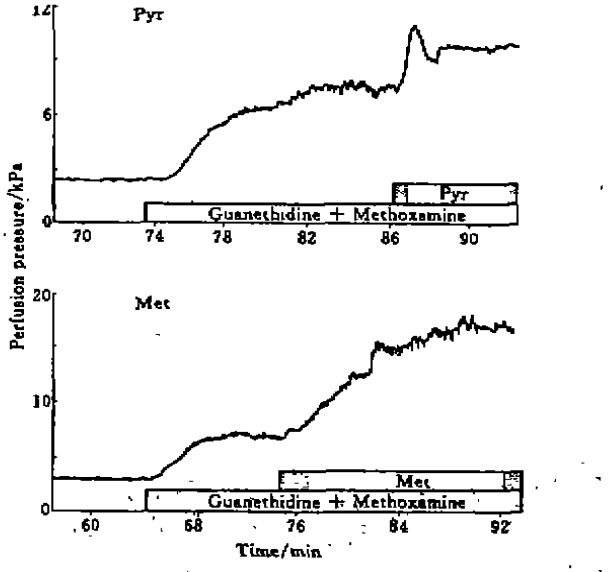

Fig 2. Effects of Pyr 0.1 mmol·L^{-1} or Met 0.01 mmol·L^{-1} on vasoconstrictor responses to methoxamine 5 μmol·L^{-1} in perfused rat mesenteric artery. Guanethidine 5 μmol·L^{-1} was used to block sympathetic nerves.

produced by Pyr or Met persisted as long as the drugs were present.

Effect of SOD Potentiation by Pyr of vasoconstrictor responses to methoxamine was abolished in the presence of SOD 100 U·ml^{-1} (Fig 3). The increase in pressure produced by methoxamine was 5.0±1.2 kPa in the control situation compared to 5.2±1.2 kPa in the presence of both Pyr and SOD (n=5). SOD 100 U·ml^{-1} also inhibited potentiation by Met of vasoconstrictor responses to methoxamine (Fig 3). Contractile responses to methoxamine were 6.1±1.2 and 9.2±2.1 kPa, control and Met in the presence of SOD, respectively (n=5). Although vasoconstrictor responses to methoxamine were still potentiated by Met in the presence of SOD, there was a significant difference in the vasoconstrictor response to methoxamine between Met in the absence and presence of SOD. The increase in the vasoconstrictor response to methoxamine produced by Met was 244±83 % compared to 53±24 % in the presence of SOD (n=5, P<0.01).

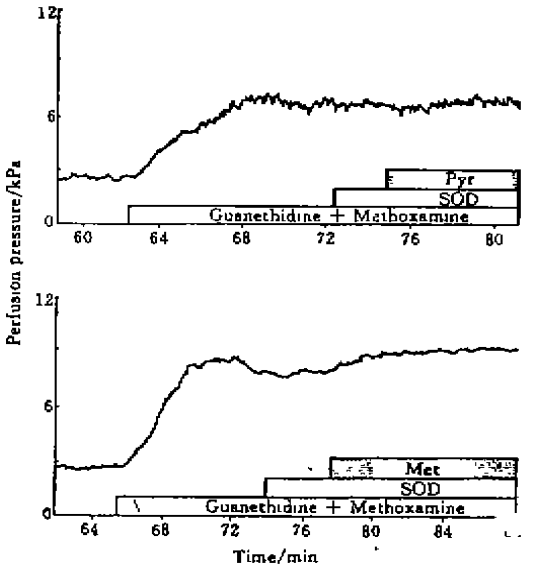

Fig 3. Effects of SOD 100 U·ml^{-1} on potentiation by Pyr 0.1 mmol·L^{-1} or Met 0.01 mmol·L^{-1} of vasoconstrictor responses to methoxamine 5 μmol·L^{-1} in perfused rat mesenteric artery.

Vasodilator responses to ACh In the presence of guanethidine 5 μmol·L^{-1} and methoxamine 5 — 10 μmol·L^{-1}, ACh caused relaxations in a concentration-dependent manner (Fig 4). This response was inhibited by both Pyr and Met (Fig 4). EC_{50} values for ACh were 0.012±0.006 μmol·L^{-1} in the absence of Pyr and 0.15±0.04 μmol·L^{-1} in the presence of Pyr. In the case of Met, ACh EC_{50} values were 0.045±0.002 and 0.56 ±0.04 μmol·L^{-1}, in the absence and presence of Met, respectively.

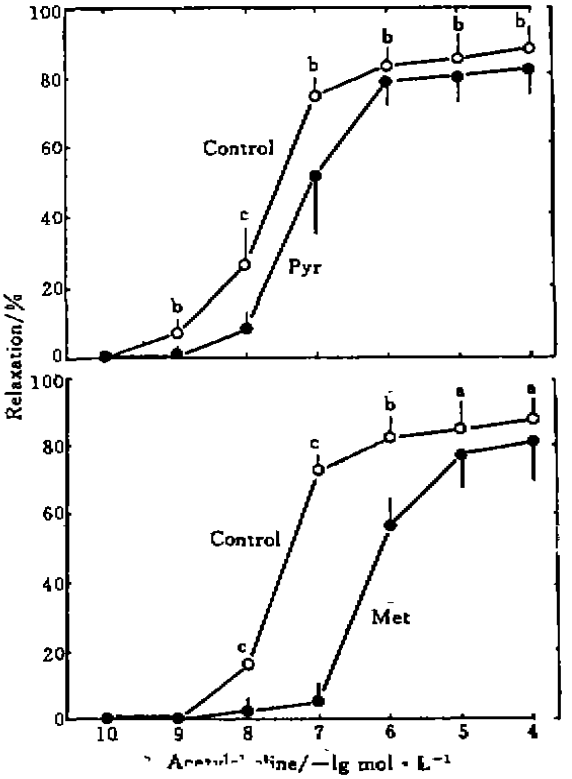

Fig 4. Effects of Pyr 0.1 mmol·L^{-1} or Met 0.01 mmol·L^{-1} on relaxation to ACh which was measured in preparations treated with guanethidine 5 μmol·L^{-1} and in the presence of methoxamine 5—10 μmol·L^{-1}. Vasodilation is expressed as % of contraction induced by methoxamine. n=5, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ vs control.

Effect of *L*-NAME on vasoconstrictor responses *L*-NAME, an inhibitor of NO synthesis, augmented vasoconstrictor responses to TNS. In the presence of *L*-NAME, Pyr reduced vasoconstrictor responses to TNS. In contrast, in the presence of *L*-NAME, Met had no effect on vasoconstrictor responses to TNS (Fig 5).

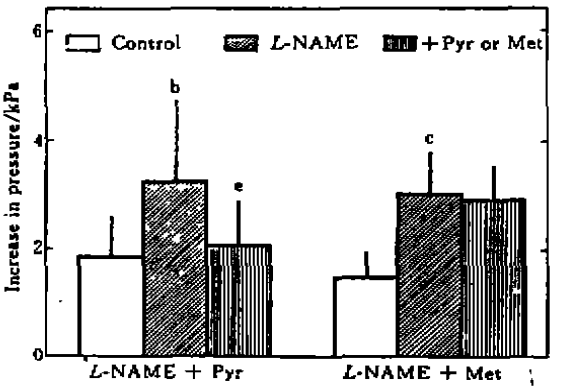

Fig 5. Effects of Pyr 0.1 mmol·L^{-1} (n=5) or Met 0.01 mmol·L^{-1} (n=4) on vasoconstrictor responses to TNS in the presence of *L*-NAME 0.1 mmol·L^{-1}. Contractile responses to TNS at 8 Hz for trains of 80 pulses. $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$ vs control; $^eP<0.05$ vs *L*-NAME.

Effect of endothelial damage on responses to Met After the mesenteric artery was exposed to saponin 50 μg·ml^{-1} for 3 min, vasoconstrictor responses to TNS were enhanced. Contractile responses to TNS were 1.5±0.5 and 2.8±0.5 kPa, control and saponin, respectively (n=5, $P<0.01$). After this treatment with saponin to damage the endothelium, instead of potentiating vasoconstrictor responses to TNS as seen in Fig 1, Met inhibited vasoconstrictor responses to TNS, which were 2.8±0.5 and 2.0±0.4 kPa for saponin and saponin in the presence of Met, respectively (n=5, $P<0.01$).

Vasodilator responses to Pyr or Met Perfused mesenteric arteries were pretreated with saponin to remove endothelium and exposed to guanethidine 5 μmol·L^{-1} to block sympathetic nerves. Methoxamine 5 μmol·L^{-1} was added to increase smooth muscle tone. Both Pyr and Met caused vasodilator responses in a concentration-dependent manner (Fig 6).

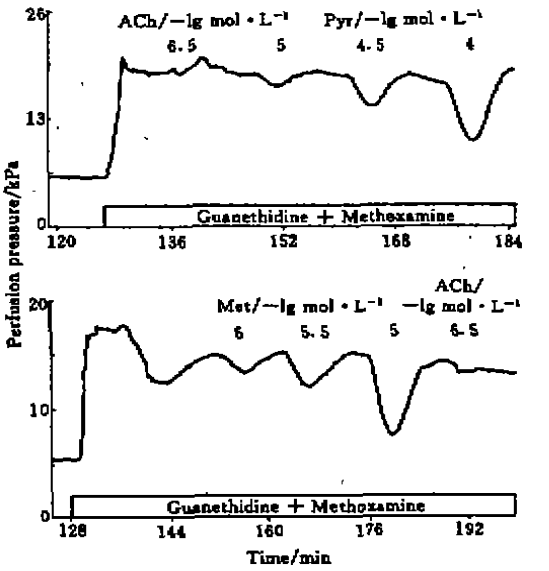

Fig 6. Vasodilator responses to Pyr or Met. Preparations were pretreated with saponin to remove endothelium and were then exposed to guanethidine 5 μmol·L^{-1} and methoxamine 5 μmol·L^{-1}.

Effect of Met on responses to Pyr Met enhanced methoxamine-induced vasoconstriction, but had no effect by itself (Fig 2). In contrast, when Met was present Pyr 0.01—0.1 mmol·L^{-1} caused vasodilator responses in a concentration-dependent manner.

DISCUSSION

The present studies demonstrate that, in the perfused rat mesenteric artery, Pyr or Met augmented vasoconstrictor responses to both endogenous norepinephrine and the selective α_1 adrenergic receptor agonist, methoxamine, an augmentation which resembles the effect of removal of the endothelium or treatment with an

L-arginine antagonist to inhibit NO synthesis. Similar results have been seen in various tissues[7,9]. In the present study potentiation by Pyr or Met of methoxamine-induced vasoconstriction was abolished or inhibited by SOD. This suggests that the effects of both Pyr and Met depend on the generation of superoxide anion.

Pyr or Met also suppressed relaxation to ACh in the perfused rat mesenteric vasculature. This suppression was also abolished by SOD, suggesting that Pyr and Met may inhibit ACh-induced relaxation by generation of oxygen radicals.

NO causes relaxation of smooth muscle *via* activation of guanylate cyclase and increased production of cGMP, and EDRF was spontaneously released from vascular endothelium[1,9]. Both Pyr and Met decrease cGMP production[4-6,10]. Therefore, potentiation of vasoconstrictor responses by Pyr or Met may be due to inactivation of NO by generation of superoxide anion leading to enhanced contractile responses.

Superoxide anion, besides its ability to oxidize NO, may work through a catalase pathway. Superoxide anion inhibits activity of catalase, and catalase activates guanylate cyclase[11]. Therefore, our results suggest that the potentiation by Pyr or Met of contractile responses to vasoconstrictors may be a consequence of decreased guanylate cyclase activity due to inactivation of both NO and catalase through generation of superoxide anion.

Met, but not Pyr, increased the basal perfusion pressure in the perfused rat mesenteric artery[1,10]. In our studies done in the presence of *L*-NAME to inhibit NO synthase, Pyr caused vasodilation, but Met had no effect under these conditions. Even in the presence of Met to inactivate guanylate cyclase Pyr caused a concentration-dependent relaxation of methoxamine-induced vasoconstriction. Thus it is probable that the effects of Pyr may be entirely due to generation of superoxide anion, while the effects of Met depend on both an oxygen-free radical pathway and direct inhibition of guanylate cyclase.

Both Pyr and Met caused an inhibition of vasoconstrictor responses to TNS when endothelial function was blocked, in the presence of *L*-NAME and after endothelial damage. It has been reported that reactive oxygen metabolites such as superoxide anion, hydrogen peroxide and hydroxyl radical relax vascular anion, hydrogen peroxide and hydroxyl radical relax vascular smooth muscle through direct or indirect means[1,12]. On the one hand, oxyradicals may potentiate contractile responses to vasoconstrictors through inactivation of NO, an action which is dependent on an intact endothelium; on the other hand oxyradicals may act directly on vascular smooth muscle to cause relaxation. In tissues with intact endothelium, the enhancing effect of Pyr or Met may conceal a vasodilator response. This hypothesis was documented by our findings that both Pyr and Met produced vasodilator responses after blockade of endothelial function. Therefore, influence of endothelium on responses to Pyr and Met should be considered when they are used as tools to investigate the mechanism of action of vasodilators.

REFERENCES

1 Martin W, Villani GM, Jothianandan D, Furchgott RF. Selective blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta.
J Pharmacol Exp Ther 1985; **232**: 708—16.

2 Wolin MS, Cherry PD, Rodenburg JM, Messina EJ, Kaley G. Methylene blue inhibits vasodilation of skeletal muscle arterioles to acetylcholine and nitric oxide *via* the extracellular generation of superoxide anion.
J Pharmacol Exp Ther 1990; **254**: 872—6.

3 Beauchamp C, Fridovich I. Superoxide dismutase: Im-

proved assays and an assay applicable to acrylamide gels. Anal Biochem 1971; 44: 276—87.

4 Ignarro LJ, Byrns RE, Buga GM, Wood KS. Endothelium-derived relaxing factor from pulmonary artery and vein possesses pharmacologic and chemical properties identical to those of nitric oxide radical. Circ Res 1987; 61: 866—79.

5 Ignarro LJ, Byrns RE, Buga GM, Wood KS, Chaudhuri G. Pharmacological evidence that endothelium-derived relaxing factor is nitric oxide: Use of pyrogallol and superoxide dismutase to study endothelium-dependent and nitric oxide-elicited vascular smooth muscle relaxation. J Pharmacol Exp Ther 1988; 244: 181—9.

6 Gruetter CA, Gruetter DY, Lyon JE, Kadowitz PJ, Ignarro LJ. Relationship between cyclic guanosine 3':5'-monophosphate formation and relaxation of coronary arterial smooth muscle by glyceryl trinitrate, nitroprusside, nitrite and nitric oxide: Effects of methylene blue and methemoglobin. J Pharmacol Exp Ther 1981; 219: 181—6.

7 Matsuoka I, Sakurai K, Ono T, Nakanishi H. Involvement of endogenous noradrenaline release in methylene blue-induced contraction of isolated rabbit aorta. Jpn J Pharmacol 1987; 44: 23—33.

8 Li YJ, Duckles SP. Effect of endothelium on the actions of sympathetic and sensory nerves in the perfused rat mesentery. Eur J Pharmacol 1992; 210: 23—30.

9 Tesfamariam B, Weisbrod RM, Cohen RA. Endothelium inhibits responses of rabbit carotid artery to adrenergic nerve stimulation. Am J Physiol 1987; 253: H792—8.

10 Iguarro LJ, Harbison RG, Wood KS, Kadowitz PJ. Dissimilarities between methylene blue and cyanide on relaxation and cyclic GMP formation in endothelium-intact intrapulmonary artery caused by nitrogen oxide-containing vasodilators and acetylcholine. J Pharmacol Exp Ther 1986; 236: 30—6.

11 Kono Y, Fridovich I. Superoxide radical inhibits catalase. J Biol Chem 1982; 257: 5751—4.

12 Rubanyi GM. Vascular effects of oxygen-derived free radicals. Free Rad Biol Med 1988; 4: 107—20.

21-27

内皮的存在掩盖了邻苯三酚与亚甲蓝直接舒张大鼠肠系膜动脉的效应

R965.2

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摘要 在灌注的大鼠肠系膜血管，邻苯三酚与亚甲蓝能增强甲氧胺或电刺激释放去甲肾上腺素的缩血管效应. 然而，当亚硝基精氨酸甲酯存在或去内皮时，两者可抑制 TNS 所致的缩血管效应. 去除内皮后，邻苯三酚与亚甲蓝产生浓度依赖性舒血管效应. 结果提示，血管内皮存在可能掩盖邻苯三酚与亚甲蓝两者的直接舒张血管平滑肌作用.

关键词 肠系膜动脉；邻苯三酚；亚甲蓝；亚硝基精氨酸甲酯；超氧化物歧化酶