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Contractile Response of Methylene Blue on Vascular Smooth Muscles — Rabbit Thoracic Aorta and Porcine Mesenteric Artery —

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ABSTRACT

Methylene Blue (MeB) and gentian violet (10^{-6}~10^{-4} M) produced contractions in isolated thoracic aortic preparations of rabbits in a dose-dependent fashion, while other dyes, evans blue and eosine yellowish, did not affect the basal tension in the same range of doses. Porcine mesenteric arterial rings also responded to MeB with dose-dependent contractions. Single dose of 10^{-4} M MeB produced a biphasic response: contraction followed by relaxation. The contraction developed slowly within 2~4 min and peaked in about 20 minutes and then slowly relaxed to the basal level. Tyramine (10^{-4} M) also induced contraction but it developed faster and was more persistent than that of MeB. While the tyramine-induced tension was reproducible, the MeB-induced one was not reiterable until 3 to 5 hours after washing out the MeB. Adding 10^{-4} M MeB further potentiated the contraction induced by 10^{-4} M tyramine. However, the MeB contraction was not affected by further addition of tyramine. Both tyramine- and MeB-induced tensions were abolished or significantly inhibited by pretreatment with various drugs acting on the sympathetic nervous system. The tyramine-induced tension was more sensitive to guanethidine and 6-hydroxydopamine than the MeB-induced tension, while the latter was more sensitive to Ca^{2+}-free PSS and reserpine. But they have similar sensitivity to prazosin. The MeB-induced tension was significantly inhibited but not abolished by 6-hydroxydopamine pretreatment. However, either tyramine or 6-hydroxydopamine could not affect the basal tension of the ring that MeB once had been tested.

These results suggest that MeB-induced contractions of rabbit thoracic aorta and porcine mesenteric artery result from a release of endogenous norepinephrine from adrenergic nerve endings and are dependent in part on extracellular calcium, and that the potency of MeB to release or to deplete norepinephrine is greater than that of either tyramine or 6-hydroxydopamine.

Kew Words: Vascular Smooth muscle, Methylene blue, Norepinephrine-release

INTRODUCTION

Methylene blue (MeB) is one of the dyes most commonly employed in staining microorganisms and cells. It has two reciprocal functions in hemoglobin metabolism. Bowman and Rand (1980) reported that MeB with higher concentration led to methemoglobinemia by oxidizing Fe^{2+} of hemoglobin to Fe^{3+} and with lower concentration it promoted conversions of methemoglobin to normal one. It has been also known that MeB is effective in treating chronic urolithiasis and eczema herpeticum, and that it has some adverse effects such as nausea, abdominal and chest pain, sweating, hypotension, and tachycardia (Reynolds 1982). Sollman (1964) reported that MeB produced transient contractile response followed by relaxation in vessels when it was perfused.

It has been documented lately that some nitro compounds give rise to relaxation of vascular smooth muscles by activating guanylate cyclase and thus accumulating cyclic GMP, and that MeB blocks the

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relaxant response by inhibiting the guanylate cyclase activity (Gruetter *et al.*, 1979, 1981; Holzman 1982; Ignarro and Kadowitz 1985). Furchgott and Zawadzki (1980), observing that acetylcholine could relax the α-adrenoceptor agonist-induced contraction in intact endothelial arteries but not in deendothelial preparations, proposed that acetylcholine release a certain vaso-relaxing substance from endothelium, the so-called endothelium-derived relaxing factor (EDRF).

Recently, it has been established by many groups that MeB inhibits EDRF-dependent relaxation by inhibiting its release (Murakami *et al.*, 1987; Nagase *et al.*, 1987; Luckhoff, *et al.*, 1987; Gruetter *et al.*, 1988; Nishiye *et al.*, 1988).

Our study attempted to provide another evidence that MeB could release norepinephrine from adrenergic nerve endings in vascular smooth muscles.

MATERIALS AND METHODS

Rabbits of either sex weighing from 1.6 to 2.3 kg were used. On killing by a blow on the back of the head, the thoracic aorta was excised. Whole intestinal tracts were obtained from pigs of either sex as soon as slaughtered in local abattoir. The intestines were put into an icebox filled with ice flakes and rapidly transferred to the laboratory within 20 min. Small branches of mesenteric arteries were isolated from the small intestine. The thoracic aorta and mesenteric artery were soaked in cold (1~3°C) physiological salt solution (PSS) saturated with 95% O_2 and 5% CO_2. Both arteries were trimmed clean of connective and adipose tissues under a stereoscope. The cleaned arteries were then cut into rings of 5 mm in length.

In some experiments, the endothelium was removed by gently rubbing with a iron rod 2 to 3 times into the lumen of the arterial rings. Ring segments of arteries were mounted in a muscle bath by sliding the ring over two pararell stainless-steel hook (0.2 mm in diameter). The lower hook was fixed on the bottom of the bath and the upper was connected to an isometric transducer (Grass FT03) with a thread and the changes of the tension were recorded on a polygraph (Grass model 7D).

The volume of bath was 20 ml and the bath solution was saturated with mixed gas of 95% O_2 and 5% CO_2 at 37°C, to be maintained pH 7.4. The composition of physiological salt solution (PSS, mM) was: 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 18 $NaHCO_3$, 11 dextrose and 0.03 EDTA. Calcium free PSS (Ca^{2+}-free PSS), which was included 0.03 mM EGTA except $CaCl_2$ and EDTA, were used in some experiments. Reserpine treated preparations were obtained from rabbits which had been treated with 3 mg/kg intramuscular reserpine 24 hours before the experiment.

The arterial rings were equilibrated in normal PSS for 2 hours and brought up to a resting tension of 2 g in both animal preparations. After equilibration, the arterial ring was challenged with 35 mM KCl 2 to 3 times, and when their contractions became uniform, the proper experiments were started.

Drugs used in the present study were methylene blue (Difco Lab.), gentian violet (Hartman), evans blue (Hartman), eosin yellowish (Hartman), prazosin HCl (Pfizer), norepinephrine bitartrate (Sigma), 6-hydroxydopamine HCl (Sigma), tyramine HCl (Sigma), guanethidine sulfate (Ciba-Geigy), and reserpine (Ciba). The amines were dissolved to 10^{-1} M in acidic saline (pH = 4.0). And the others were dissolved in distilled water except for crystal violet, which was dissolved to 10^{-1} M in 95% ethanol. Subsequent dilutions were made with distilled water. Dose-response curves were obtained by a cumulative administration of the drugs. For comparing test values with the control, Student's t-test was employed. A p value<0.05 was taken as statistically significant.

RESULTS

I. Thoracic Aortae of Rabbits

Contractile response to MeB and gentian violet (GV)

MeB in concentrations lower than 10^{-6}M produced no or slight contractions, but with concentrations greater than 3.5×10^{-6}M dose-dependent contractions were elicited (Fig. 1). The maximally effective concentration of MeB when given in a single dose was 10^{-4} M. The contraction pattern, however, was different from those of norepinephrine (NE) and tyramine (Fig. 2). Contractile response to 10^{-6}M NE occurred immediately and peaked in 2 to 5 minutes. 10^{-4} M tyramine elicited contractile response more slowly than NE, but more rapidly than MeB (Fig. 2). NE- or tyramine-induced contraction was a persistent monophase. The MeB elicited a biphasic response: contraction followed by relaxation. The contraction slowly developed after 2 to 4 minutes latency and peaked in 12 to 20 minutes and then slowly relaxed to the baseline. The patterns of MeB-induced contractions were almost the same in both endothelium intact preparations (+Endo,

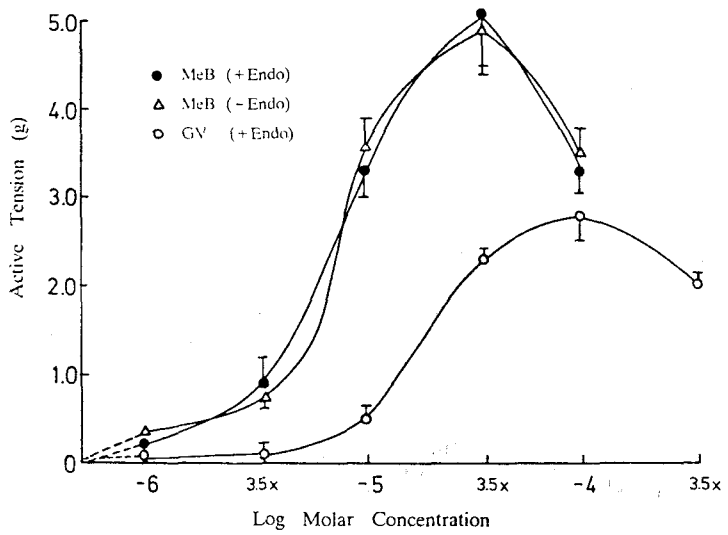

Fig. 1. Dose-contractile response curves to methylene blue (MeB) and gentian violet (GV) in isolated aortic rings of rabbits. +Endo and −Endo indicate intact endothelial rings and rings without endothelium, respectively. Each point shows the mean ± SEM from 7-12 aortae.

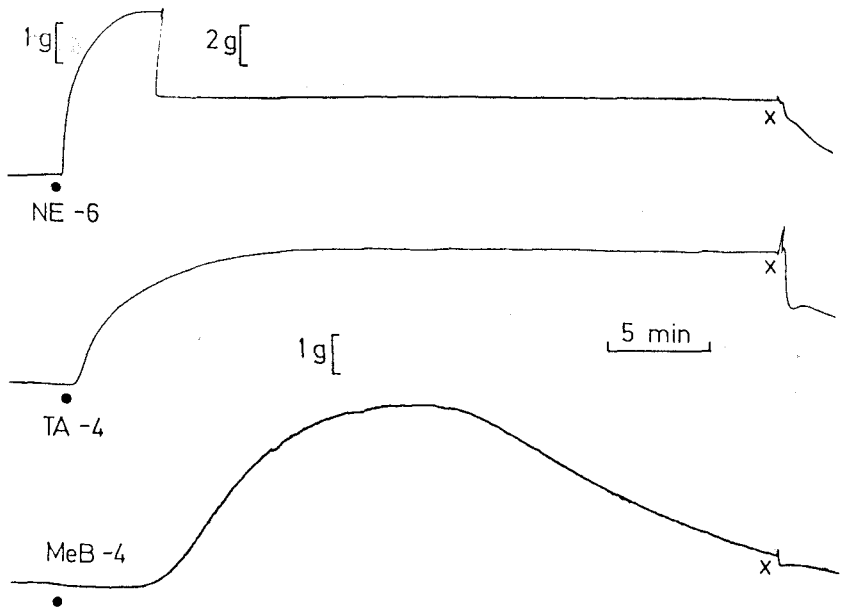

Fig. 2. Typical traces showing contractile responses of isolated aortic rings to norepinephrine (NE), tyramine (TA) and methylene blue (MeB). Three rings were obtained from a thoracic aorta. The indicated drug (10^{-4}M) was added to the bath at dots. All calibrations show 1 g, except the right part of the first trace. At x, bath fluid was changed.

$ED_{50} = 7.4 \times 10^{-6}$M) and endothelium-removed ones (-Endo, $ED_{50} = 6.6 \times 10^{-6}$M) (Fig. 1). Therefore, the endothelium was left intact (+Endo) throughout the study unless specified otherwise and all data of MeB except dose-response curve were taken from single administration in a preparation. In cumulative dose-response curve, MeB produced maximum tension at 3.5×10^{-5} M and the higher dose rather elicited relaxant response. Even up to 3 to 5 hours after washing the ring with fresh PSS, the second dose of MeB

Table 1. Effects of various conditions on 10^{-4}M tyramine (TA)- and 10^{-4}M methylene blue (MeB)-induced tensions in isolated aortic rings of rabbits

Condition	NPSS	Ca^{2+}-free PSS	6-OHDA	Prazosin	GUAN	Reserpine
Conc.	—	— (%)	10^{-4}M (%)	10^{-4}M (%)	10^{-4}M (%)	3 mg/kg
TA	3.3±0.3	1.9±0.3 (42.4)	0.1±0.3 (97.0)**	0.1±0.3 (97.0)	0.0±0.1 (100)**	1.3±0.3 (60.6)
MeB	4.2±0.5	1.1±0.2 (73.8)*	1.3±0.2 (69.1)	0.1±0.2 (97.6)	4.1±0.4 (2.4)	0.2±0.2 (95.3)**

Numerals represent the mean active tension ±SEM (g) of 10-18 rings from 4-7 rabbits. %: Inhibitory rate to each control response in NPSS. Asterisks indicate significantly greater inhibition between both inhibitory rates of TA vs MeB (*: $P<0.05$, **: $P<0.01$). NPSS: normal physiological salt solution, 6-OHDA: 6-hydroxydopamine, GUAN: guanethidine, Conc: concentration. Other details are in text.

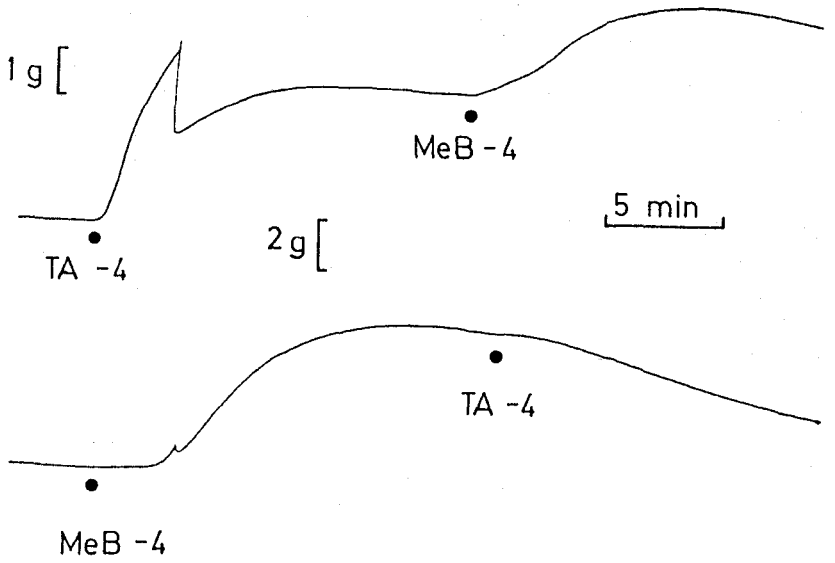

Fig. 3. Effect of an additive dose of methylene blue (MeB) or tyramine (TA) on tyramine (upper)- or methylene blue (lower)-induced tension in an isolated aortic ring, respectivley. The calibration of the left part of upper trace is 1 g and other parts are 2g. Upper and lower traces were obtained from two different rings of an aorta. The indicated drug (10^{-4}M) was added to the bath at each dot.

could not produce any contraction.

GV also produced dose-dependent contractile responses (6 experiments, $ED_{50} = 1.9 \times 10^{-5}$M), but the magnitude was less than that of MeB. The maximal contraction at 10^{-4}M GV was about 50% of the MeB and higher dose rather produced relaxation (Fig. 1).

To see whether these vascular responses are specific to only these blue dyes, effects of another blue dye, evans blue, and a yellow dye, eosine yellowish, were examined. But up to 10^{-4}M concentrations, they did not produced any contractile responses at all in six rings.

Influences of various drugs on the contraction induced by tyramine or MeB

Tyramine. The contractile response to 10^{-4}M tyramine was reproducible, when it was repeatedly added to the bath at intervals of 30 to 60 minutes. An addition of 10^{-4}M tyramine, when 10^{-4}M MeB-induced contraction was at the peak, did not further increase the contraction at all. On the contrary, an addition of the MeB during the steady-state contrac-

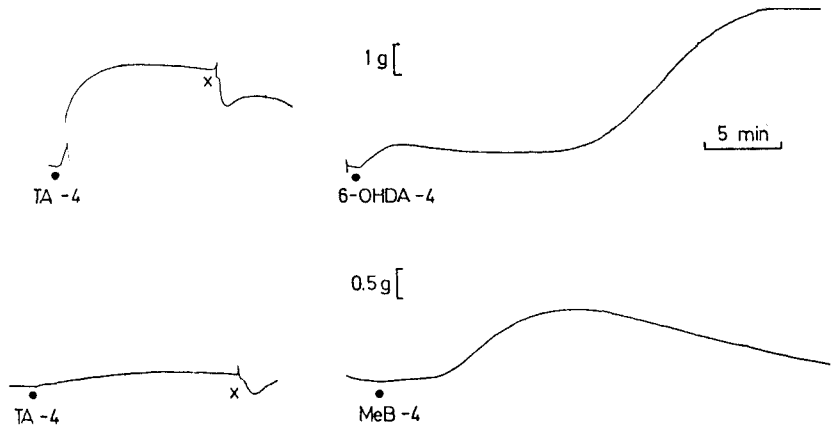

Fig. 4. Effect of 6-hydroxydopamine (6-OHDA) treatment on tyramine (TA)- and methylene blue (MeB)-induced tension in an isolated aortic ring. Lower traces were obtained 30 min after washout of 6-hydroxydopamine. All calibrations are 1 g, except 0.5 g of lower right trace. The indicated drug (10^{-4}M) was added to the bath at dots and bath fluid was changed at x.

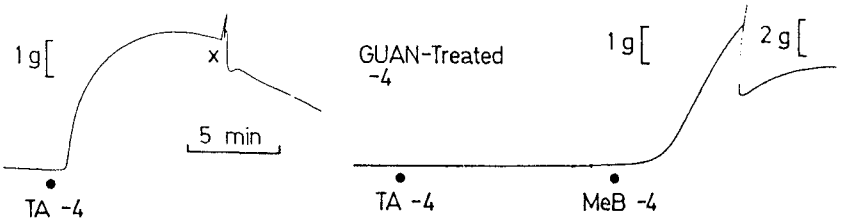

Fig. 5. Effect of guanethidine (GUAN) treatment on tyramine (TA)- and methylene blue (MeB)-induced tension in an isolated aortic ring. Right trace was obtained from the same ring treated with 10^{-4}M guanethidine for 20 min. The indicated drug (10^{-4}M) was added to the bath at dots and bath fluid was changed at x.

tion induced by tyramine markedly augmented the contraction (Fig. 3).

Ca^{2+}-free PSS. The contractile response to 10^{-4}M MeB was 4.2 ± 0.5 g (18 rings, mean $\pm$ SEM) and that to 10^{-4}M tyramine was 3.3 ± 0.3 g (10 rings) in normal PSS. In Ca^{2+}-free PSS which is completely devoid of $CaCl_2$, the tyramine-induced contraction was partially inhibited to 42.4% of the tension in normal PSS, and the MeB response was more markedly attenuated to 73.8% (Table 1).

6-Hydroxydopamine. 10^{-4}M 6-hydroxydopamine (6-OHDA) produced slight contraction at first, and then the ring was markedly contracted about 5 to 8 minutes later (Fig. 4). After the preparations were treated with 6-OHDA for 20 minutes, bath fluid was exchanged with fresh PSS. When the 6-OHDA-induced tension returned to the basal level, either tyramine or MeB was added to the bath. Tyramine could induce no contraction, while the MeB-induced contraction was markedly attenuated but not completely abolished (Fig. 4) (Table 1). Also the 6-OHDA-induced contraction was not reproducible.

Guanethidine. Guanethidine (10^{-4}M) itself has no effect on the basal tension. Tyramine-induced contraction was completely abolished by pretreatment with guanethidine for 30 min, but MeB-induced one was not affected (Fig. 5) (Table 1).

Prazosin. Prazosin (10^{-6}M) produced a slight relaxation. Both tyramine- and MeB-induced contractions were almost abolished 30 minutes after prazosin treatment (Table 1).

Reserpine. In rings obtained from reserpine-pretreated rabbits, the tyramine-induced contraction was markedly attenuated and MeB-induced one was

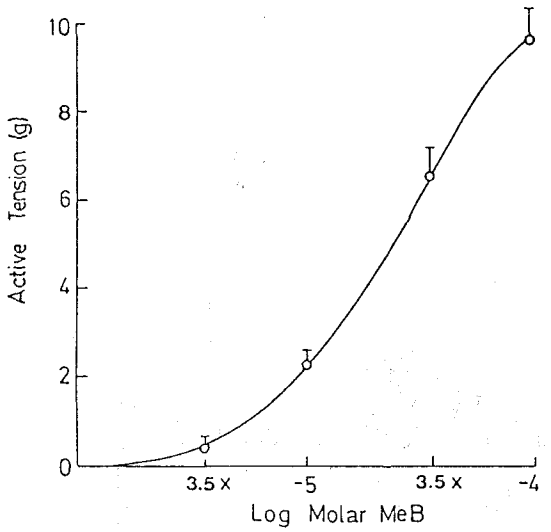

Fig. 6. Dose-contractile response curve to methylene blue (MeB) in isolated mesenteric rings of pigs. Each point shows the mean ± SEM from 16 rings.

almost abolished (Table 1).

II. Porcine Mesenteric Arteries

Contractile response to MeB

Cumulative additions of MeB between 3.5×10^{-6} M and 10^{-4}M produced concentration-dependent increases in tension in porcine mesenteric artieral rings, with the ED_{50} being 2.6×10^{-5} M (Fig. 6). The maximally effective concentration when given in single dose was 10^{-4} M and the dose elicited a biphasic response like in the rabbit aorta (Fig. 7). The maximal active tension induced by MeB was 9.6 ± 0.8g (28 rings), about twice tension produced in the rabbit ring (Table 2). No contraction was observed, when MeB was repeatedly added.

Influences of various drugs on the contraction induced by tyramine or MeB

Tyramine. The mesenteric ring was persistently

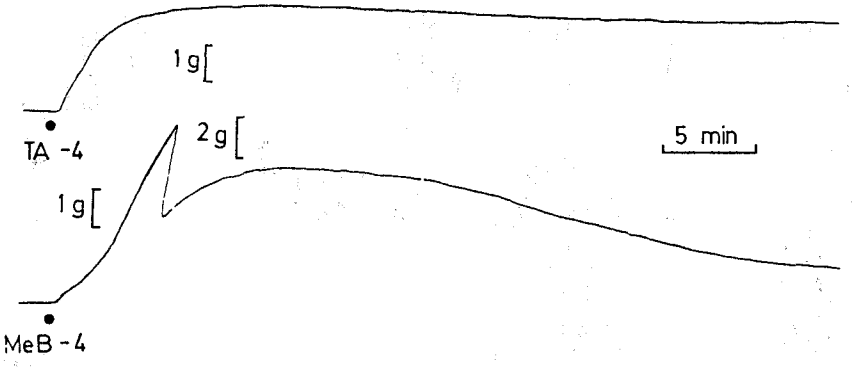

Fig. 7. Typical traces showing contractile responses of isolated mesenteric rings to tyramine (TA) and methylene blue (MeB). Two rings were obtained from a porcine mesenteric branch. The indicated drug (10^{-4}M) was added to the bath at dots. Calibration marks are drawn at each part of traces.

Table 2. Effects of various conditions on 10^{-4}M tyramine (TA)- and 10^{-4}M methylene blue (MeB)-induced tensions in isolated mesenteric rings of pigs

Condition	NPSS	Ca^{2+}-free PSS	6-OHDA	6-OHDA	Prazosin
Conc.	—	— (%)	10^{-4}M (%)	2×10^{-4}M (%)	10^{-6}M (%)
TA	4.7 ± 0.5	2.2 ± 0.3 (53.2)	4.8 ± 0.6 (−2.1)	2.0 ± 0.4 (57.5)*	0.8 ± 0.2 (82.9)
MeB	9.6 ± 0.8	2.7 ± 0.4 (71.9)*	10.5 ± 1.8 (−9.4)	6.7 ± 0.6 (30.2)	2.1 ± 0.4 (78.1)

Numerals show the mean active tension ± SEM (g) of 12-28 rings from 6-12 pigs. (− %) shows a potentiation. Other details are in text and table 1.

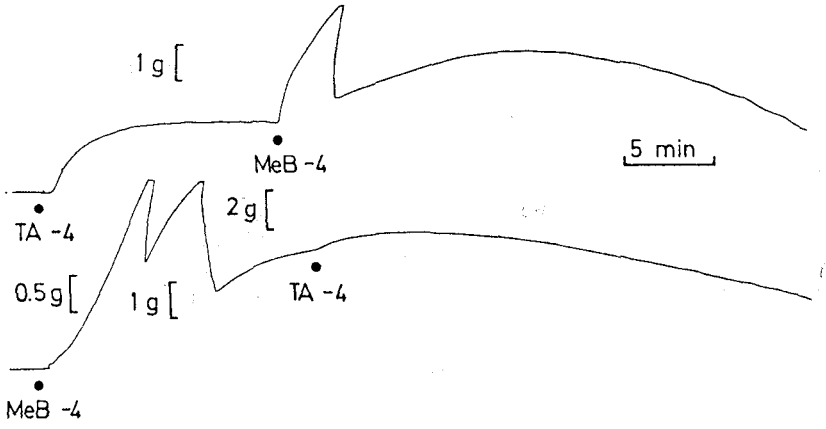

Fig. 8. Effect of an additive dose of methylene blue (MeB) or tyramine (TA) on tyramine (upper)- or methylene blue (lower)-induced tension in an isolated mesenteric ring, respectively: Upper and lower traces are obtained from two different rings of a mesenteric branch. The indicated drug (10^{-4}M) was added to the bath at each dot. Calibration marks are drawn at each part of traces.

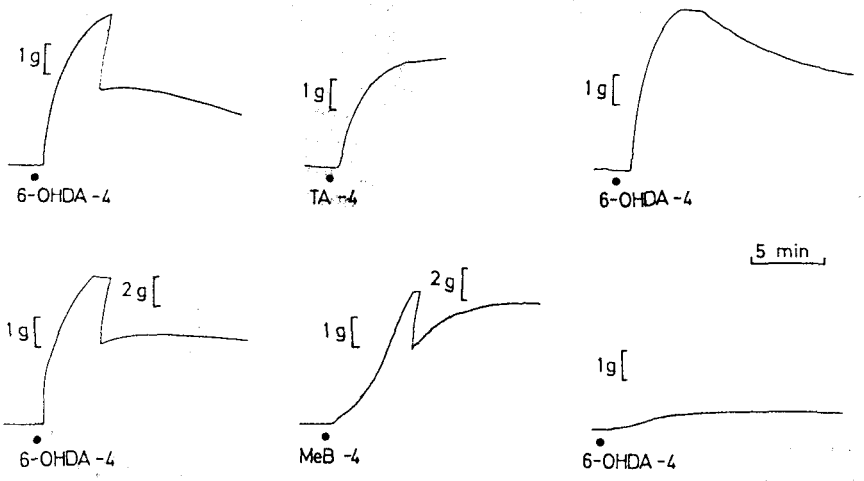

Fig. 9. Effect of 6-hydroxydopamine (6-OHDA) treatment on tyramine (TA)- and methylene blue (MeB)-induced tension in an isolated mesenteric ring. Second and third panels were obtained at 30 and 60 min after washout of the previous drugs, respectively. Upper and lower rings are two different ones obtained from a mesenteric branch. The indicated drug (10^{-4}M) was added to the bath at each dot. Calibration marks are drawn at each part of traces.

contracted by 10^{-4} M tyramine, with the maximum tension of 4.7 ± 0.5g (28 rings). When the MeB-induced tension had maximally developed, further addition of the tyramine did not significantly augment the contraction (Fig. 8). To the contrary, when the tyramine-induced tension had reached steady-state, the addition of MeB markedly potentiated the contraction (Fig. 8).

Ca^{2+}-free PSS. In Ca^{2+}-free media both contractions induced by MeB and tyramine were inhibited to 71.9% and 53.2% of the tension produced in normal PSS, respectively. And the inhibitory rate to MeB was significantly greater than to tyramine (Table 2).

6-Hydroxydopamine. The 10^{-4} M 6-OHDA produced an abrupt and persistent contraction and the 6-OHDA-induced tension was reproducible when

repeatedly given at intervals of 1 to 2 hours (Fig. 9). Both contractions induced by tyramine and MeB were not affected by pretreatment with 10^{-4}M 6-OHDA for 20 minutes. On the contrary, the 6-OHDA did not produce any contraction in the preparation that MeB once had been tested (Fig. 9). After the rings were treated with 2×10^{-4} M 6-OHDA for 60 minutes, however, both the tyramine- and MeB-induced tensions were inhibited to 57.5% and 30.2% of the control, respectively (Table 2).

Prazosin. Pretreatment with 10^{-6}M prazosin for 30 minutes markedly inhibited both tyramine- and MeB-induced tensions (Table 2).

DISCUSSION

Among the four dyes tested in the present study, MeB and gentian violet produced contractions of the rabbit thoracic aorta in a dose-dependent fashion, whereas evans blue and eosin yellowish, up to 10^{-4}M, had no effects. It is mostly likely that the difference results from the specificity of the molecular structures and being the only common structure found in those which elicit contractile response, the dimethylamino radical is suggested to the structure involved in the specificity.

MeB is able to pass through cell membranes easily, thus having inherent ability to modulate the physiological process in cells. Indeed, a wide variety of pharmacological action of MeB is being reported recently. It has been reported that MeB as well as hemoglobin or myoglobin inhibits the nitric oxide-induced relaxation of the vascular smooth muscle, but not catecholamine-induced one (Gruetter *et al.*, 1979, 1980; Lippton *et al.*, 1982). However, recent studies have shown that hemoglobin does not inhibit relaxations induced by sodium nitroprusside, organic nitrate and nitrosoguanidine but MeB is capable of effectively inhibiting them. The reason suggested is that lipid-soluble nitro compounds easily pass through cell membrane and produce relaxant response by releasing or synthesizing nitric oxide in the cell, and that MeB also can easily enter into the interior of the cell, while hemoprotein can not. As expected from their permeabilities, MeB inhibits the action of the nitric oxide released in the cells, but hemoprotein does not (Grutter *et al.*, 1981; Ignarro *et al.*, 1981).

It has been shown by several research groups that nitrogen oxide-containing vasodilators increase the guanylate cyclase activity of vascular smooth muscles, leading to increased synthesis of cyclic GMP which initiates the relaxation process. Thus, guanylate cyclase may be the intracellular receptor for the nitro compounds (Ignarro *et al.*, 1982; Wolin *et al.*, 1982; Ignarro and Kadowitz 1985). MeB selectively inhibits vascular smooth muscle relaxation induced by the nitro compounds and this is shown to be due to inhibition of both guanylate cyclase activity and tissue accumulation of cyclic GMP (Ignarro *et al.*, 1981; Edwards *et al.*, 1984). MeB is also able to increase the "resting tone" of vascular smooth muscle by decreasing cyclic GMP level. These facts indicate that the cyclic GMP level and vascular smooth muscle relaxation are closely related biological process (Kukovetz *et al.*, 1981; Ignarro and Kadowitz 1985). Furthermore, MeB inhibits EDRF-dependent relaxant response to acetylcholine and enhances contractile response to acetylcholine itself, owing to its inhibitory action of EDRF release (Martin *et al.*, 1985; Luckhoff *et al.*, 1987; Nagase *et al.*, 1987). As reported above, MeB is capable of blocking the various processes involving the relaxant response of vascular smooth muscles, so that the MeB-induced contraction observed in the present study could also be accounted for by its effects of decreasing some factors involved in the relaxant response, such as cyclic EMP and/or EDRF.

However, it is doubtful whether decrease of the relaxant factor itself will bring about such a marked contraction. For the contractions induced by either MeB or gentian violet were dose-dependent and the MeB contraction was inhibited by the drugs which are not directly related to cyclic GMP or EDRF. Such results strongly suggest that some mechanisms, beside the process concerned with cyclic GMP and/or EDRF, participate in the MeB contraction. The MeB contraction was completely abolished by prazosin, α_1-adrenoceptor antagonist, and was abolished or significantly attenuated either by reserpine, NE depletor in the sympathetic nerve endings, or by 6-OHDA, sympathetic nerve ending degenerator. These results may be interpreted as indicating that MeB releases NE from sympathetic nerve endings and produces the contractile response of vascular smooth muscle through α_1-adrenoceptors. In support of this postulation Matsuoka *et al.*, (1985, 1987) claimed that cyclic GMP-decrease itself could not play a "trigger" role for the contraction, even though the decrease of cyclic GMP level by MeB could augment other drugs-induced contractions. Thus it seems reasonable to assume that the MeB-induced contraction in vascular smooth muscle is not caused by decreasing of cyclic GMP level, but induced by releasing of NE from nerve endings.

Tyramine is one of the drugs which release NE from nerve endings and elicits contraction in vascular smooth muscle. Therefore, in the present study the contractile response to MeB was compared with that to tyramine. In the rabbit rings, guanethidine, which blocks release and uptake of NE from sympathetic nerve endings, completely abolished the tyramine-induced tension but did not affect the MeB-induced tensions. The 10^{-4} M 6-OHDA-pretreament completely abolished the tyramine-induced tension while markedly inhibited but did not abolish the MeB-induced one. Both tyramine and MeB contractions in the porcine rings were resistant to the 10^{-4}M 6-OHDA. However, both MeB- and tyramine-induced tensions were significantly inhibited when 6-OHDA concentration is double to 2×10^{-4}M, with the former being more sensitive to 6-OHDA than the latter. Both tensions were also significantly inhibited in Ca^{2+}-free PSS, with the tyramine being more resistant than the MeB. Summing up, the tyramine-induced tension was more sensitive to guanethidine and 6-OHDA than the MeB-induced one, while the latter was more sensitive to Ca^{2+}-free PSS and reserpine than the former. And both tensions showed similar sensitivity to prazosin ihese, results strongly suggest that both tyramine and MeB elicit contractile response through the α_1-adrenoceptors in vascular smooth muscle by releasing NE from adrenergic nerve endings, even though mechanisms involved in NE release by the two drugs may not be identical. Moreover, further addition of 10^{-4}M MeB, when 10^{-4}M tyramine-induced tension was at the maximum, produced markedly additive contraction. And the tyamine-induced contraction itself was persistent and reproducible, whereas the MeB-induced one was neither persistent nor reproducible. Once MeB (10^{-4}M) contraction had been tested, either tyramine or MeB could not elicit any responses until 3 to 5 hours after washing the preparations with fresh PSS. In addition, in the porcine mesenteric rings 6-OHDA can produce contraction repeatedly. However, it could not elicit any changes in basal level of the ring, once the MeB had been applied before. These suggest that the NE-releasing action of MeB is irreversible and more powerful than that of tyramine or 6-OHDA, and that MeB may also inhibit reuptake of released NE. Indeed, Matsuoka *et al.* (1987) have reported that MeB inhibited [^{3}H]-norepinephrine uptake.

Conclusively, our present data suggest that MeB-induced contractions of rabbit thoracic aorta and porcine mesenteric artery result from a release of endogenous NE from adrenergic nerve endings, and are dependent in part on extracellular calcium, and that MeB depletes NE in nerve endings by releasing NE and inhibiting reuptake of NE.

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REFERENCES

Bowman WC and Rand MI: *Textbook of pharmacology. 2nd ed. Blackwell Scientific Publications, Oxford, pp 21.46-39.4, 1980*

Edward JC, Ignarro LJ, Hyman AL and Kadowitz PJ: *Relaxation of intrapulmonary artery and vein by nitrogen oxide-containing vasodilators and cyclic GMP. J Pharmacol Exp Ther 228:33-42, 1984*

Furchgott RF and Zawadzki JV: *The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288: 373-647, 1980*

Gruetters CA, Barry BK, McNamara DB, Gruetter DY, Kadowitz PJ and Ignarro LJ: *Relaxation of bovine coronary artery and activation of coronary arterial guanylate cyclase by nitric oxide, nitroprusside and a carcinogenic nitrosoamine. J Cyclic Nucl Res 5:211-224, 1979*

Gruetter CA, Kadowitz PJ and Ignarro LJ: *Methlene blue ihibits coronary arterial relaxation and guanylate cyclase activation by nitroglycerin, sodium nitrite and amyl nitric. Can J Physiol Pharmacol 59: 150-156, 1980*

Gruetter CA, Gruetter DY, Lyon JE, Kodowitz PJ and 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 219:181-186, 1981*

Gruetter CA, Ryan ET, Lemke SM, Bailly DA, Fox MK and Schoepp DD: *Endothelium-dependent modulation of angiotensin II-induced contraction in blood vessels. Eur J Pharmacol 146:85-95, 1988*

Holzmann S: *Endothelium-induced relaxation by acetylcholine associated with larger use in cyclic GMP in coronary alterial strips. J Cyclic Nucl Res 8:409-419, 1982*

Ignarro LJ, Lippton H, Edwards JC, Baricos WH, Hyman AL, Kadowitz PJ and Gruetter CA: *Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide: Evidence for the involvement of S-nitrosothiols as active intermediates. J Pharmacol Exp Ther 218:739-749, 1981*

Ignarro LJ, Degnan JN, Baricos WH, Kadowitz PJ and

Wolin MS: *Activation of purified guanylate cyclase by nitric oxide rquires heme: Comparison of heme-deficient, heme-reconstituted and heme-containing forms of soluble enzyme from bovine lung. Biochim Biophys Acta 718:45-59, 1982*

Ignarro LJ and Kadowitz PJ: *The pharmacological and physiological role of cyclic GMP in vascular smooth muscle relaxation. Ann Rev Pharmacol Toxicol 25:171-191, 1985*

Kukovetz WR, Poch G and Holzmann S: *Cyclic nucleotides and relaxation of vascular smooth muscle. In Vasodilatation, ed. P.M. Vanhoutte, I. Leusen, New York: Raven, pp 339-353, 1981*

Lippton HL, Gruetter CA, Ignarro LJ, Meyer RL and Kadowitz, PJ: *Vasodilator actions of several N-nitroso compounds. Can J Physiol Pharmacol 60:68-75, 1982*

Luckoff A, Busse R, Winter I and Bassenge E: *Characterization of vascular relaxant factor released from cultured endothealial cells. Hypertension 9:295-302, 1987*

Martin W, Villani GM, Jothianandan D and Furchgott RF: *Selective blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation by hemoglobin and methylene blue in rabbit aorta. J Pharmacol Exp Ther 232:708-712, 1985*

Matsuoka I, Sakurai K and Nakanishi H: *Isosorbide 5-mononitrate effects on isolated rabbit aorta and vena cava: Relationship between cyclic GMP and relaxation of vascular smooth muscle. Eur J Pharmacol 118:155-162, 1985*

Matsuoka I, Sakurai K, Ono T and Kakanishi H: *Involvement of endogenous noradrenaline release in methylene blue-induced contraction of isolated rabbit aorta. Japan J Pharmacol 44:23-33, 1987*

Murakami K, Karaki H and Urakawa N: *Comparison of the inhibitory effects of nicorandil, nitroglycerin and isosorbide dinitrate on vascular smooth muscle of rabbit aorta. Eur J Pharmacol 141:195-202, 1987*

Nagase H, Karaki H and Urakawa N: *Palytoxin-induced endothelium-dependent relaxation in the isolated rat aorta. Naunyn-Schmiedeberg's Arch Pharmacol 335:575-579, 1987*

Nishiye E, Itoh T and Kuriyama H: *Some effects of leukotriene D_4 on the mechanical properties of the guinea-pig basiler artery. Br J Pharmacol 93:591-600, 1988*

Reynolds EF: *Martindale, the extrapharmacopoeia. 28th ed. The Pharmacentical Press (London), pp 384-385, 1982*

Sollmann T: *Methylene blue oxidation-reduction reaction. In a mannual of pharmacology and its applications to therapeutics and toxicology (eight edition). W.B. Saunders Company, Philadelphia and London, pp 829-831, 1964*

Wolin MS, Wood KS and Ignarro LJ: *Guanylate cyclase from bovine lung: A kinetic analysis of the regulation of the purified soluble enzyme by protoporphyrin IX, heme and nitrosyl-heme. J Biol Chem 257:13312-13320, 1982*

= 국문초록 =

혈관평활근에 대한 Methylene Blue의 수축작용
— 가토흉부대동맥근과 돼지장간막동맥근 —

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가토흉부대동맥근에서 MeB와 gentian violet는 용량-의존성 수축반응을 일으켰으나 evans blue와 eosine yellowish는 전혀 수축반응을 일으키지 못하였다. MeB는 돼지 장간막 동맥에서도 용량-의존성 수축반응을 일으켰다. 양표본에서 MeB 10^{-4} M의 단회투여는 수축반응에 이어 이완반응이 나타나는 양상성 반응을 일으켰으나, tyramine은 지속적인 수축반응을 일으켰다. Tyramine의 수축반응은 반복적이었으나 MeB의 그것은 일차 수축반응후 3~5시간후까지도 반복되지 않았다. Tyramine 10^{-4} M의 최대 수축반응 상태에서 MeB 10^{-4} M의 추가투여는 현저한 추가수축반응을 일으켰으나 반대로 MeB의 최대수축반응 상태에서 tyramine의 추가투여는 그이상의 수축반응을 일으키지 못하였다.

Tyramine과 MeB 수축반응은 교감신경계 약물로 소실 또는 유의하게 억제되었다. Tyramine 수축반응은 MeB 수축보다 guanethidine과 6-hydroxydopamine에 더 예민한 반면, Ca^{2+}-free PSS와 reserpine에 대하여는 MeB 수축반응이 tyramine 수축보다 더 예민하였고 prazosin하에서는 두 수축반응이 비슷하게 억제되었다. MeB 수축반응은 6-hydroxydopamine으로 유의하게 억제는 되었으나 소실되지 않았고, MeB 수축반응 관찰후에는 tyramine 뿐만아니라 6-hydroxydopamine의 수축반응도 소실되었다.

이상의 성적은 가토흉부대동맥과 돼지 장간막동맥에서 MeB 수축반응은 부분적으로 세포외 calcium 의존성이고 adrenaline성 신경말단으로부터의 norepinephrine유리에 기인하며, MeB의 norepinephrine유리 및 고갈작용이 tyramine 또는 6-hydroxydopamine의 작용보다 더 강력함을 시사하고 있다.