Involvement of Endogenous Noradrenaline Release in Methylene Blue-Induced Contraction of Isolated Rabbit Aorta

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Abstract—The vasocontractile response to methylene blue (Meb) was investigated in isolated rabbit aorta. Meb (1–100 μM) induced a slowly developing contraction after a long latency in rabbit aortic strip. The maximal contraction was obtained by 50 μM Meb, which corresponded to 1 μM noradrenaline (NA)-induced contraction. Once the maximal contraction was induced by Meb, the strip completely lost the contractile response to a further application of Meb. The usual NA-induced contraction, however, could be observed in such a Meb-insensitive aortic strip. Meb-induced contractions were not affected by atropine, diphenhydramine, methysergide, indomethacin, nordihydroguaiaretic acid and removal of endothelial cells from the aortic strip, but they were abolished by prazosin. In aortic strips from rabbits pretreated with reserpine (3.0 mg/kg, i.m.) for a day, Meb failed to induce contraction. Meb evoked the $[^3$H] release from $[^3$H]NA-preloaded aortic strips. In high performance liquid chromatographic analysis, a considerable amount of NA was found in the bathing fluid of the aortic strip in the presence of Meb. In addition, Meb pretreatment inhibited $[^3$H]NA uptake by the aortic strip and abolished the contractile response to an electrical stimulation of adrenergic nerve terminals. Although Meb decreased the basal level of cyclic GMP in the aortic strip, Meb-induced $[^3$H]NA release from the aortic strip was not affected by 8-bromo cyclic GMP. These results suggest that Meb-induced contraction of rabbit aorta is due to the release of endogenous NA from its storage pools of intramural adrenergic nerves through an independent mechanism of its cyclic GMP lowering effect. In addition, incubation of aortic strips with Meb resulted in depleting the storage NA and blocking the nerve function, suggesting that Meb might be useful for a pharmacological tool as an adrenergic neuron blocking agent in vitro.

Recent studies have proposed that cyclic GMP is a mediator of vascular smooth muscle relaxation induced by nitric oxide-yielding (1–5) and endothelium-dependent (5–8) vasodilators. In these observations, methylene blue (Meb) was often used as an inhibitor of the activation of guanylate cyclase. It has been demonstrated that Meb inhibits the vascular relaxation as well as the increase in tissue cyclic GMP levels induced by several vasodilators (2–4, 6). We also reported that Meb inhibited isosorbide 5-mononitrate-induced relaxation and cyclic GMP accumulation in isolated rabbit aorta and vena cava (9). In the preliminary experiment of the previous study, we observed that Meb itself had a contractile effect on rabbit aorta and vena cava (9). Although Martin et al. (10) have recently reported that Meb augments the tone in rabbit aortic rings as the result of interfering with the effect of spontaneous released endothelium-derived relaxing factor, little information is available on the direct effect of Meb on vascular tissue. Therefore, the purpose of the study reported here was to analyze the mechanism of Meb-induced contraction in isolated rabbit aorta and to clarify the direct action of Meb on vascular tissue. Part of this study has been reported previously in abstract form (11).
Materials and Methods

Rabbit aortic strips were prepared as described previously (9). Helical strips (3–5 mm wide, 20–25 mm long) were mounted in an organ bath containing 20 ml of physiological salt solution (PSS). The PSS had the following composition: 130 mM NaCl, 4.7 mM KCl, 1.6 mM CaCl₂, 1.17 mM MgSO₄, 14.9 mM NaHCO₃, 1.18 mM KH₂PO₄, 27 μM EDTA and 5.5 mM dextrose. The solution was continuously bubbled with a mixture of 95% O₂ and 5% CO₂ and maintained at 37°C. Under an optimal resting tension of 2.0 g, the strips were equilibrated for 90 min, during which the bathing fluids were replaced with fresh PSS every 20 min. All experiments were performed under these conditions unless otherwise indicated.

Mechanical measurement: The contractile responses were measured isometrically through an FD-pick-up (Nihon Kohden, TB-611T) and carrier amplifier (Nihon Kohden, AP-600G). In early experiments, strips were exposed to cumulative concentrations of noradrenaline (NA). After determination of concentration-response relationships for NA, the strips were rinsed with PSS every 15 min for 45 min. The strips were then contracted with 0.5 μM NA (the contraction was in a range between 60 and 75% of the maximal contraction). After repeated rinses with PSS and 40–60 min equilibration, the strips were exposed to Meb. The extent of Meb-induced contraction was expressed as a percentage of NA (0.5 μM)-induced tone which was observed just before the application of Meb. To study responses induced by Meb in the presence of several inhibitors, the strips were preincubated with the inhibitors for 10 min.

In some experiments, the endothelium of the strip was removed by careful abrasion of the intimal surface with filter paper. The presence or absence of the endothelium was assessed by measuring the response to 1 μM acetylcholine on the NA-precontracted strips. In the endothelium-intact strips, acetylcholine produced a relaxation ranging from 30–60% of the NA-induced tone, but no significant relaxation was obtained in the endothelium-removed strips.

In electrical stimulation experiments, all conditions were identical to the above except that the strips were suspended between two platinum rings in an organ bath. Electrical stimulation was applied with rectangular pulses of 1 msec duration, supramaximal voltage, 30 Hz frequency for 10 sec using an electrical stimulator (Nihon Kohden, MSE-3R). This electrical stimulation was selected to cause optimal activation of adrenergic nerve terminals in the aortic strips.

[^3H]NA release: In[^3H]NA release experiments, the aortic strips were mounted in a small organ bath containing 3 ml of PSS. After 90 min equilibration under a resting tension of 2.0 g, the strips were incubated with[^3H]NA (22.7 nM, 44 Ci/mmol) for 90 min and were then rinsed with PSS every 15 min for 60 min. Thereafter, the incubation fluids were collected and replaced with a fresh one every 10 min. In order to determine the basal[^3H]NA levels, the strips were incubated with normal PSS for 20–40 min before changing the incubation fluid to Meb (50 μM)-containing PSS. The radioactivity in 0.5 ml of each incubation fluid was measured with a liquid scintillation counter.

[^3H]NA uptake: The aortic strips were mounted in a 3 ml-organ bath, equilibrated for 90 min under a resting tension of 2.0 g. The strips were then incubated for 60 min in the presence or absence of 50 μM Meb. After repeated rinses with PSS, the strips were exposed to[^3H]NA (22.7 nM, 44 Ci/mmol) for 90 min. To determine the tissue content of radioactivity, each strip was rinsed with PSS, removed from the bath, weighed and solubilized in a NCS tissue solubilizer (Amersham). The radioactivity was measured with a liquid scintillation counter.

Measurement of NA by high performance liquid chromatography (HPLC): Aortic strips were mounted in a 3 ml-organ bath, equilibrated for 90 min and then contracted with 0.5 μM NA. After repeated rinses with PSS, the strips were incubated for 45 min in the presence or absence of 50 μM Meb. The incubation fluid (3.0 ml) was collected and mixed with 1 ml of 0.1 M Tris-HCl (pH 8.6), 0.1 ml of 0.1 mM EDTA, 0.1 ml of 0.1 M Na₂S₂O₅ and 2 ng of 3,4-dihydroxybenzylamine (DHBA, a chromatographic internal standard). The mixtures were shaken for 20
Methylene Blue on Rabbit Aorta

min in a shaker (Ikemoto Rika, MW-1) following the addition of 30 mg alumina (Aluminum oxide activated, Wako). The alumina was collected by centrifuging at 1,700×g for 5 min and washed three times with cooled purified water. The catecholamines adsorbed in alumina were extracted with 0.1 ml of 0.1 N HCl and separated by HPLC (12). Reversed phase HPLC was performed on a Yanapak ODS-A (4.6×250 mm, 7 μm particles, Yanaco) at 30°C using a model L-2000 pump (Yanaco) and an electronchemical detector (VMD-101, Yanaco). Methanol/water (containing 0.1 M sodium acetate, 0.02 M citric acid, 150 mg/l 1-octan sulfonate and 50 mg/l EDTA) 1:9 (v/v) at a flow rate of 0.5 ml/min was used as the mobile phase for the column. Recoveries of authentic standards (NA, adrenaline and dopamine) were reproducibly greater than 75%.

Measurement of cyclic GMP: Aortic strips were prepared and equilibrated in a manner identical to the mechanical experiments. The strips were removed from the bath at various intervals following the addition of Meb, frozen in liquid N2 and stored at -80°C until use. The frozen strips were lyophilized and weighed on a microbalance. The lyophilized strips were homogenized in ice-cold 6% trichloroacetic acid with a glass-glass homogenizer in an ice bath. The homogenates were centrifuged at 1,700×g for 15 min at 4°C, and the supernatants were extracted with H2O-saturated ethylether. Aliquots of the aqueous phase were lyophilized and the residues were dissolved in 0.1 M sodium acetate buffer (pH 6.2). Cyclic GMP was measured by radioimmunoassay (13) as described previously (9).

Materials and data analysis: Drugs used were methylene blue (Wako), noradrenaline hydrochloride (Aldrich), atropine sulfate (Sanko), methysergide hydrogenmaleinate (Sandoz), prazosin hydrochloride (Eisai), guanethidine (Ciba-Geigy), reserpine (Daiichi Seiyaku), acetylcholine chloride (Daiichi Pure Chemicals), 8-bromo cyclic GMP sodium salt, nordihydroguaiaretic acid, diphenhydramine and tetrodotoxin (Sigma). 1-[7,8-3H] noradrenaline (44 Ci/mmol) was from Amersham. The radioimmunoassay kit for cyclic GMP was obtained from Yamasa Shoyu Co. The statistical difference of the values obtained was tested with the paired Student’s t-test.

Results

Contractile response to Meb: Meb induced a slowly developing contraction of rabbit aortic strip, in a concentration-dependent manner (Fig. 1). When the aortic strip was exposed to 50 μM Meb, the tension was slowly increased after a latency about 3 min and reached to the maximum within 30 min, followed by a slow relaxation. The threshold concentration for Meb in eliciting contraction was around 1 μM; and the maximal effect, which was equipotent to the effect of 1 μM NA, was obtained at the concentration over 50 μM. When a lower concentration of Meb was used, there was a longer period of latency before the onset of contraction, and the contraction developed more slowly. Unlike the response to NA, Meb-induced contraction was not reproducible in the same strip. When the aortic strips were exposed again to the half-effective concentration of Meb (10 μM), the second response was significantly smaller (23%±7%, n=5, P<0.01) than the first one (100%). Furthermore, once the maximal response had been induced by Meb (50 μM), the strips completely lost the contractile response to Meb (Fig. 2). The normal contraction induced by NA, however, could be observed in such a Meb-insensitive strip (Fig. 2). The extents of Meb-induced contraction in the following results are therefore represented as a percentage of the 0.5 μM NA-induced tone which were observed just before applying Meb.

Effects of several inhibitors: The contractions induced by 50 μM Meb were not inhibited by a serotonin antagonist, methysergide (1 μM), and a histamine antagonist diphenhydramine (1 μM), but they could be completely abolished by an a1-adrenoceptor antagonist, prazosin (1 μM) (Fig. 3). Meb-induced contractions were also unaffected by an acetylcholine antagonist, atropine (1 μM); a cyclooxygenase inhibitor, indomethacin (2.5 μM); and a lipoxygenase inhibitor, nordihydroguaiaretic acid (50 μM) (data are not shown). These results indicate
Fig. 1. Meb-induced contractions of rabbit aortic strips. Left: Time course of Meb (50 μM)-induced contractions. Values represent the mean±S.E.M. from 6 observations. Right: Concentration-response curve of Meb-induced contractions. Open circle shows NA (1 μM)-induced contraction. Values represent the mean±S.E.M. from 4 to 7 observations.

an involvement of α₁-adrenergic action in the response to Meb.

Effects of drugs acting on adrenergic nerve terminals: Tetrodotoxin (1 μM) completely inhibited the response to electrical stimulation of adrenergic nerve terminals in aortic strips (Fig. 7), but it had no effect on Meb-induced contraction (Fig. 4). Guanethidine (50 μM) sometimes produced a slight contraction and then blocked the response to electrical stimulation of adrenergic nerve terminals (data are not shown). In addition, guanethidine augmented the response to exogenously applied NA probably as the result of inhibition of NA uptake (Fig. 4). However, guanethidine had little effect on Meb-induced contractions (Fig. 4). Meb (50 μM)-induced contractions (128% ±7% of 0.5 μM NA-induced tone, n=5) were also little affected by pretreatment with the combination of 50 μM tyramine and 10 μM guanethidine (106%±9% of 0.5 μM NA-induced tone, n=5), under which the pool of NA available for displacement with tyramine had been depleted (data are not shown). When rabbits were pretreated with reserpine (3.0 mg/kg, i.m.) for a day, a supersensitivity to NA was observed in the isolated aortic strips (Fig. 4). In these reserpine-pretreated aortic strips, Meb failed to induce

Fig. 2. Contractile responses of one strip to repeated application of NA (0.5 μM) and Meb (50 μM).
Methylene Blue on Rabbit Aorta

Fig. 3. Effect of methysergide (Met, 1 μM), diphenhydramine (Dih, 1 μM) and prazosin (Pra, 1 μM) on Meb (50 μM)-induced contractions. All drugs were preincubated with aortic strips for 10 min. Contractions are shown as a percentage of 0.5 μM NA-induced tone in the absence of drugs. Values represent the mean±S.E.M. from 5 observations. *Significantly different from the control (None) (P<0.05).

contraction (Fig. 4). Both Meb- and NA-induced contraction could be observed in calcium-free and 1 mM EGTA containing PSS (Fig. 4).

Effects of Meb on [3H]NA distribution in aortic strips: Meb (50 μM) evoked [3H]-release from aortic strips which were preloaded with [3H]NA (Fig. 5A). The maximal release was obtained 30 min after addition of Meb, which was consistent with the time when the maximal contraction was caused by Meb (Fig. 1). No [3H]release was observed in the absence of Meb. When aortic strips were incubated with [3H]NA for 90 min, the tissue content of radioactivity was accumulated to 9.5×10^5 dpm/g wet weight (Fig. 5B). The [3H]NA uptake was markedly inhibited by pretreatment with Meb (Fig. 5B).

Analysis of catecholamines with HPLC: We next examined whether Meb actually caused a release of endogenous NA from aortic strips. The aortic strips were incubated for 45 min in the presence or absence of 50 μM Meb. Catecholamines in the incubation fluids were analyzed with a reversed phase HPLC. Results are shown in Fig. 6. The chromatogram of the incubation fluid in the absence of Meb (control) had no detectable peak except for the internal standard DHBA. In contrast, there were three prominent peaks in the chromatogram of Meb-containing incubation fluid. Although peaks 2 and 3 were unidentified substances (peak 2 was close to that of adrenaline, but could be separated from it in a cochromatography), peak 1 was NA. The concentration of NA released by Meb from the aortic strip into the incubation fluid amounted to about 10 nM.

Effect of Meb on the response to electrical stimulation: The aortic strips were contracted in response to an electrical stimulation at 30 Hz, 1 msec, 50 V/cm for 10 sec. These contractions were completely blocked by tetrodotoxin (1 μM) and prazosin (1 μM), indicating that NA is released from adrenergic nerve terminals by the electrical stimulation (Fig. 7A). When aortic strip was electrically stimulated at 10 min intervals for 90 min in the presence of 10 μM Meb, the contractile response to the electrical stimulation was augmented time-dependently.

Fig. 4. Effect of tetrodotoxin (TTX, 1 μM), guanethidine (Gua, 50 μM), reserpine (Res, 3.0 mg/kg) and EGTA (1 mM) on Meb (50 μM)-induced contractions. TTX and Gua: Drugs were pretreated with aortic strips for 10 min (n=5). Res: Aortic strips from rabbits pretreated with reserpine (3.0 mg/kg, i.m.) for a day were used (n=3). EGTA: Calcium in PSS was exchanged with 1 mM EGTA (n=4). Contractions are shown as a percentage of 0.5 μM NA-induced tone without any treatment. Values represent the mean±S.E.M. *Significantly different from the control (None) (P<0.05).
Fig. 5. Effects of Meb on $[^3]$H release from preparations preloaded with $[^3]$HNA (A) and on $[^3]$HNA uptake by aortic strips (B). A: $[^3]$HNA-preloaded aortic strips were prepared as described in "Materials and Methods". The strips were incubated with PSS (○) for 40 min. Thereafter, the strips were incubated with PSS (○) or Meb-containing PSS (●) for 80 min. The incubation fluids were collected and replaced with fresh one every 10 min for measuring radioactivity. Values represent the mean±S.E.M. from 5 observations. B: Aortic strips were incubated for 60 min in the presence or absence of 50 μM Meb. After repeated rinses with PSS, aortic strips were treated with $[^3]$HNA for 90 min. Values represent the mean from triplicate observations.

until the first 40 min and then gradually decreased. After 80 min incubation with Meb, the response to electrical stimulation was decreased to 10–20% of the control response. This inhibition persisted for 150 min after Meb was washed out, and there was no recovery even after incubation with 1 μM NA for 30 min (Fig. 7B). When the concentration of Meb was increased to 50 μM, the contractile response to electrical stimulation was completely abolished after the treatment with Meb for 45 min (Fig. 7A).

Effect of removal of endothelium on Meb-induced contraction: It has been shown that Meb is a potent inhibitor of vascular relaxation induced by endothelium-dependent vasodilators such as acetylcholine (6, 13). Therefore, the role of endothelium in Meb-induced aortic contraction was examined (Fig. 8). In the presence of endothelium, acetylcholine (1 μM) induced a relaxation in NA (0.5 μM)-precontracted aortic strips. The acetylcholine induced relaxation was abolished by removal of endothelium from the intimal surface of aortic strips. Meb-induced contraction could be observed equally whether the endothelium was present or absent in the aortic strip.

Relationship between Meb-induced NA release and cyclic GMP: It is well recognized that Meb inhibits the activation of guanylate cyclase and prevents cyclic GMP accumulation induced by some vasodilators in several vascular tissues (2–4). Meb itself slightly, but significantly decreased cyclic GMP content of aortic strips (Fig. 9). The contraction induced by 50 μM Meb as well as 0.5 μM NA were attenuated by 100 μM 8-bromo cyclic GMP (Fig. 10A). However, Meb-induced $[^3]$H release from the $[^3]$HNA-preloaded strip was not affected by 8-bromo cyclic GMP (Fig. 10B).

Discussion

The present study demonstrates that Meb induces a marked contraction of isolated rabbit aorta via the release of endogenous NA from intramural adrenergic nerve terminals. Meb-induced contraction did not involve the
action of serotonin, histamine, acetylcholine and the metabolites of arachidonic acid such as prostaglandins, thromboxane and leukotrienes, since the contractions were not affected by methysergide, diphenhydramine, atropine, indomethacin and nordihydroguaiaretic acid. In contrast, Meb-induced contraction was completely abolished by prazosin, indicating that an $\alpha_1$-adrenergic action is involved in the response to Meb. However, the contraction induced by Meb developed after a longer latency and at a considerably slower rate than that induced by NA. Furthermore, it was remarkable that once the maximal contraction had been induced by Meb, the aortic strips no longer responded to the following applications of Meb, whereas a normal NA-induced contraction could be observed in such Meb-insensitive aortic strips. These results indicate that Meb does not have a direct action on the $\alpha_1$-adrenoceptors in the aortic smooth muscle. In aortic strips from rabbits pretreated with reserpine (3.0 mg/kg, a day), the supersensi-
Activity to exogenously applied NA could be observed, but Meb failed to induce any response, suggesting that the endogenous catecholamine would be involved in Meb-induced contraction. In fact, Meb evoked the release of NA from NA-preloaded aortic strips. There was a good time course relationship between [3H]release and contraction induced by Meb. In addition, HPLC analysis showed that a considerable amount of NA was released during Meb-induced contraction.
of NA was accumulated in the bathing fluid of aortic strips in the presence of Meb. These results suggest that Meb initially interacts with the storage site of endogenous NA and then evokes the release of sufficient amounts of NA to cause contraction of aortic smooth muscle. Over 50 years ago, the effects of Meb on the circulation had been shown by observations that intravenous injection of Meb produced a marked rise of blood pressure, followed by a fall, and that Meb-perfused blood vessels were first contracted and then dilated (14). The present results can well account for the finding of these earlier investigations.

Meb is well-known to inhibit the activation of guanylate cyclase in vascular smooth muscle (15). As Meb has been reported to inhibit both cyclic GMP accumulation and relaxation induced by nitric oxide-yielding vasodilators such as nitroglycerin and nitroprusside, in several vascular smooth muscles (1-5), this action of Meb has contributed remarkably to support the theory that cyclic GMP is involved in regulation of smooth muscle relaxation (16). Results of the present study showed that Meb itself slightly, but significantly, decreased the basal levels of cyclic GMP in aortic strips and that Meb-induced contraction was attenuated by 8-bromo cyclic GMP. However, the release of NA from the aortic preparation by Meb appears to be independent of the decrease in tissue cyclic GMP content, since 8-bromo cyclic GMP could not inhibit the Meb-induced [3H]release from [3H]NA-preloaded aortic strips. Therefore, the decrease in cyclic GMP may not be the trigger in Meb-induced contraction, even though that may augment the muscular contractility (2, 9, 15).

Recent studies have shown that some vasodilators such as acetylcholine and the calcium ionophore A-23187 produce an endothelium-dependent vascular relaxation (17) via the formation of an endothelium-derived relaxing factor (EDRF) which can stimulate guanylate cyclase (18) and increase tissue cyclic GMP levels (5-8, 10, 17). Meb has been shown to be a potent inhibitor of endothelium-dependent vascular relaxation (6, 10). Furthermore, it has been recently reported that Meb antagonizes the action of spontaneously released EDRF and subsequently augments the tone in rabbit aortic rings (10). However, the contractile response to Meb in the present study was not explained by the inhibitory effect of Meb on EDRF, since removal of endothelial cells from the intimal surface of aortic strips did not alter the response to Meb. These results indicate that the release of endogenous NA may play an essential role in Meb-induced contraction.

It is also found in the present study that the pretreatment with Meb blocked the active uptake of [3H]NA by aortic strips, and inhibited the contractile response to electrical nerve stimulation, which were mediated by NA. The contractile response of aortic strip to electrical stimulation was first augmented and then declined in the presence of 10 μM Meb, and it was completely abolished after the treatment with 50 μM Meb. The response to electrical stimulation suppressed by Meb was not recovered even after the treatment with 1 μM NA. These results suggest that Meb initially stimulates the release of endogenous NA and gradually caused a depletion of the stored NA. The ability of intramural adrenergic nerves to store NA seemed to be irreversibly lost by Meb. This notion seems to well explain the reason why after the maximal response to Meb, the aortic strips lost the contractility selectively to the following application of Meb. The mechanism by which Meb produced a depletion of stores of NA is unclear at present. It has been established that the release of neurotransmitter is initiated by the arrival of the action potential at the nerve terminal, which is associated with the selective increase in the permeability of the neuroplasmic membrane to sodium ions (19). Tetrodotoxin is well-known to block the release of neurotransmitter in response to nerve impulse as the result of inhibiting the sodium currents in the neuroplasmic membrane (20). This toxin had no effect on Meb-induced contraction. In addition, calcium is thought to play an essential role in coupling the nerve impulse with the release of NA from granules in adrenergic nerve terminals (21). Meb-induced contraction could be observed even when calcium in the bathing fluid was exchanged with 1 mM EGTA. Therefore, the
release of NA induced by Meb may be independent of the excitation of adrenergic fibers in aortic strips.

It is well recognized that sympathomimetic amines such as tyramine, produce most of their effect indirectly by releasing NA from the nerve-endings (22). The action of Meb seems to be different from that of sympathomimetic amines, since Meb-induced contraction could be observed even when the pool of NA available for displacement with tyramine had been depleted in the presence of guanethidine. Tyramine-induced contractions in the aortic strips were abolished after the action of Meb (I. Matsuoka et al., unpublished data). Guanethidine, an adrenergic neuron blocking agent, is known to initially release NA from adrenergic nerve terminals and then decrease gradually the tissue stores of NA (23, 24). Guanethidine blocked the response to electrical stimulation of adrenergic nerve terminals and sometimes produced a slight contraction probably via the release of NA. In any case, Meb-induced contraction was little affected by the treatment with guanethidine. In contrast, the aortic strips from rabbits pretreated with reserpine for a day, showed no response to Meb. It seems that tyramine (22) and guanethidine (23) under the conditions used in the present study produce only minor changes of total tissue content of NA, whereas the treatment with reserpine reduced tissue NA to negligible levels (24). Meb, therefore, appears to have a potent depleting effect and to cause the release of a greater part of the NA stored in adrenergic nerve terminals.

It is thought that a considerable part of NA released from adrenergic nerve terminals by drugs such as guanethidine and reserpine is deaminated by intraneuronal monoamineoxidase (22, 24, 25). The pharmacological effects of the released NA are minimized through such a metabolism. In HPLC analysis, we found not only the peak of NA but also two unidentified peaks on the chromatograms for the substances released from aortic strips in response to Meb. This result indicated that some part of the released NA had been converted to several metabolites. Meb was, however, reported to inhibit intracellular amineoxidase (26). This inhibitory action of Meb might contribute at least partially to the release of a sufficient amount of unchanged NA to cause marked contraction of aortic smooth muscle.

Reserpine is often used to deplete catecholamines stored in many organs. For this purpose, it is necessary to administer reserpine to animals in vivo because the reserpine-induced depletion of catecholamines is developed very slowly (24, 25), and at least partially dependent on nerve activity, since it is reduced by preganglionic denervation (27, 28) or ganglionic blockade (28). In contrast to reserpine, Meb seems to be able to cause depletion of NA in vitro by an hour without changing the characteristics of postsynaptic adrenoceptors. In addition, Meb could induce contraction in several arterial and venous preparations (I. Matsuoka et al., unpublished data). Although further investigations are need to clarify the mechanism of action of Meb, it is suggested that Meb might be a useful pharmacological tool as an adrenergic neuron blocking agent in vitro.

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