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Vasorelaxant effect of harman

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Abstract

The in vivo cardiovascular effect and in vitro vasorelaxant effect of harman, one of harmala alkaloids isolated from *Peganum harmala*, were examined in this study. Harman (1–10 mg/kg, i.v.) dose-dependently produced transient hypotension and long-lasting bradycardia in pentobarbital-anesthetized rats, which were attenuated by N^G-nitro-L-arginine pretreatment. In isolated rat endothelium-intact thoracic aortic rings, harman concentration dependently relaxed phenylepherine-induced contractions with an IC_{50} value around 9 μM. This vasorelaxant effect was attenuated by endothelium removal or N^ω-nitro-L-arginine methyl ester pretreatment, but not by tetraethylammonium or indomethacin pretreatment. In cultured rat aortic endothelial cells, harman (1–100 μM) concentration dependently increased nitric oxide (NO) release, which was dependent on the presence of external Ca^{2+}. Harman pretreatment (3–30 μM) also concentration dependently inhibited the contractions induced by phenylephrine, 5-hydroxytryptamine (5-HT), and KCl in endothelium-denuded aortic rings in a non-competitive manner. In addition, harman pretreatment reduced both phasic and tonic phases of phenylephrine-induced contractions. Receptor binding assays further indicated that harman (K_i values around 5–141 μM) interacted with the cardiac α_1-adrenoceptors, brain 5-HT_2 receptors, and cardiac 1,4-dihydropyridine binding site of L-type Ca^{2+} channels. Therefore, the present results suggested that the vasorelaxant effect of harman was attributed to its actions on the endothelial cells to release NO and on the vascular smooth muscles to inhibit the contractions induced by the activation of receptor-linked and voltage-dependent Ca^{2+} channels. The vasorelaxant effect may be involved in the hypotensive effect of harman.

Keywords: Harman; Vasorelaxant; Endothelium; Nitric oxide (NO)

1. Introduction

Harman (1-methyl-β-carboline), first isolated from *Peganum harmala* L., and related alkaloids are distributed widely in medicinal plants and found endogenously in mammalian tissues (Airaksinen and Kari, 1981; Beck and Faull, 1986). These harmala alkaloids have a wide spectrum of pharmacological actions, including monoamine oxidase inhibition (Fuller et al., 1986; Rommelspacher et al., 1994; Adell et al., 1996), binding to benzodiazepine receptors (Muller et al., 1981; Rommelspacher et al., 1981, 1985; Baum et al., 1996), convulsive or anticonvulsive actions (Loew et al., 1985), tremorogenesis (Lutes et al., 1988), anxiolytic and behavioral effects (Barbaccia et al., 1986; Adell et al., 1996), antioxidative action (Tse et al., 1991), and immunomodulatory effects (Li, 1996; Wang et al., 1996b). There were also some reports concerning the cardiovascular actions of harmala alkaloids. For example, Aarons et al. (1977) have reported that harmine reduces systemic arterial blood pressure and total peripheral vascular resistance; harmaline-evoked decreases are frequently followed by a secondary increase; and the effects of harmalol on these two parameters are inconsistent. However, there has been no report concerning the cardiovascular effect of harman. Therefore, the present study was carried out to examine the cardiovascular effects of harman, especially the vasorelaxant effects and its underlying mechanisms of action in isolated rat thoracic aortic preparations. The effect of harman on nitric oxide (NO) production by cultured rat aortic endothelial cells was also examined. A receptor-binding assay was also performed to further identify its action at receptor level.

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2. Materials and methods

2.1. Blood pressure and heart rate measurement

Males Sprague–Dawley rats (180–280 g) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The right femoral artery was cannulated for the recording of arterial blood pressure and heart rate with a Gould model 3400S polygraph (Valley View, OH) via a P23XL pressure transducer (Viggo-Spectramed, Oxnard, CA), and the femoral vein was cannulated for drug administration (i.v.). To study the role of NO in the harman's effect, a nitric oxide synthase (NOS) inhibitor N^G-nitro-L-arginine was pretreated 30 min prior to harman administration.

2.2. Isolated thoracic aorta preparations

The isolated aortic rings were prepared from the thoracic aortas of male Sprague–Dawley rats (180–280 g) according to Chiou et al. (1996). In brief, the aorta was cut into approximately 3–4-mm-long ring segments and endothelium-denuded aortic rings were obtained by gentle rubbing with the fingertip. The aortic ring was suspended under basal tension of 1 g in a 10-ml organ bath containing Krebs' solution continuously aerated with a gas mixture of 95% O_2 and 5% CO_2 and maintained at 37°C. The composition of Krebs' solution was as follows (mM): NaCl (118), KCl (4.7), $NaHCO_3$ (25), KH_2PO_4 (1.2), $MgSO_4 \cdot 7H_2O$ (1.2), $CaCl_2$ (2.5), and glucose (11.1). Isometric tension change was measured with a Grass FT03 force transducer and recorded on a four-channel polygraph (Gould RS3400 polygraph). Before the start of experiment, all preparations were allowed to equilibrate for 60 min, during which time Krebs' solution were replaced at least twice.

2.3. Vasorelaxant effect of harman

The vasorelaxant effect of harman was examined in phenylephrine (0.3 μM)-induced sustained contractions of endothelium-intact and endothelium-denuded aortic preparations. This concentration of phenylephrine induced approximately 85 ± 3% of the maximal contraction. Lack of functional vascular endothelium was confirmed by the loss of relaxant response to 3 μM acetylcholine before the experiment began. After the contraction induced by phenylephrine had reached a stable plateau, cumulative concentrations of harman were added. The harman-evoked vasorelaxant effect was expressed as a percentage of relaxation and the IC_{50} (the concentration to produce a 50% maximal relaxation) value was determined from the concentration–response curve by data fitting with computer software GraFit (Erithacus Software, Staines, Middlessex, UK)

The involvement of the mediator for endothelium-related vasorelaxation induced by harman was examined with the pretreatment of N^{ω}-nitro-L-arginine methyl ester (a NOS inhibitor), tetraethylammonium (a K^+ channel blocker), or indomethacin (a cyclooxygenase inhibitor).

2.4. NO measurement

The culture of rat aortic endothelial cells and the measurement of NO in the medium were according to the methods of Wang et al. (1996a, 1999). In brief, endothelial cells were grown in 35-mm^2 dishes in 1 ml of Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and antibiotics. Upon reaching confluence in about 4 days, the medium was changed to 1 ml of Hanks' balanced salt solution (HBSS) with L-arginine (100 μM) and added $CaCl_2$ (to 2.5 mM). The changing over to HBSS was necessary because it provided the least interference in the assay. However, additional Ca^{2+} was required to make the final concentration comparable to that in normal Krebs' solution. The cells were then equilibrated for 60 min at 37°C. Aliquots (250 μl) of the supernatant were collected for analysis of nitrite by chemiluminescence, and the total content of NO in the medium before drug treatment was calculated and taken as basal 100%. Vehicle (HBSS without Ca^{2+}) or harman (1, 10, 100 μM) was then added for 30 min and the cell supernatants (100 μl) were collected for analysis of nitrite to examine the change of NO content. Similar experiments also were carried out in Ca^{2+}-free HBSS to examine the role of external Ca^{2+}. Samples (100 μl) containing nitrite were measured by adding a reducing agent (0.8% VCl_3 in HCl) to the purge vessel to convert nitrite to NO, which was then carried by a flow of helium to the NO analyzer (Model 280, Sievers Research, Boulder, CO). Nitrite concentrations were calculated by comparison with standard solution of sodium nitrite.

2.5. Inhibition of phenylephrine-, 5-hydroxytryptamine (5-HT)-, or KCl-induced contractions

A series of experiments was designed to assess the involvement of α_1-adrenoceptors, 5-HT receptors, or 1,4-dihydropyridine binding site of L-type Ca^{2+} channels in the vasorelaxant effect of harman in endothelium-denuded aortic preparations. Various concentrations of harman (3, 10, 30 μM) were added 10 min before the construction of cumulative concentration–response curves with phenylephrine, 5-HT, or KCl. The results are expressed as the percentage of the maximum contractile tension to phenylephrine, 5-HT, or KCl before and after harman treatment.

2.6. Effect on phenylephrine-induced biphasic contraction

The contractile response of endothelium-denuded aortic ring to phenylephrine can be separated into phasic and tonic components according to the method described by Chiou et al. (1992) with slight modifications. The phasic

contraction was first initiated with phenylephrine (30 μM) in Ca^{2+}-free Krebs' solution (containing 2.5 mM EGTA) and the tonic contraction was then induced by further addition of 2.5 mM $CaCl_2$. Harman was pretreated 10 min prior to phenylephrine addition.

2.7. Receptor binding assay

According to previous reports (Liao et al., 1995; Yu et al., 1995; Chiou et al., 1996), the interaction of harman with 5-HT_2 receptors was assessed in mouse cerebral cortex membrane preparations and that with α_1-adrenoceptors or 1,4-dihydropyridine binding site of L-type Ca^{2+} channels in rat heart membrane preparations. In brief, binding assays were initiated by the addition of a receptor membrane preparation in an appropriate buffer containing the specific radioligand for the tested receptors or binding site. After incubation, bound ligands were separated from free ligands by vacuum filtration through a 24-mm glass fiber filter (Whatman GF/C). The radioactivity of bound radioligand was then counted. Harman was tested on the listed receptors in various concentrations from 0.1 to 1000 μM. The competition binding curve was analyzed to determine the IC_{50} (concentration of the tested drug to compete 50% of specifically bound radioligand) using the computer software GraFit (Erithacus Software) The K_i value was calculated from the IC_{50} value using the Cheng–Prusoff equation (Cheng and Prusoff, 1973).

2.8. Drugs and reagents

Harman, sodium pentobarbital, N^G-nitro-L-arginine, phenylephrine hydrochloride, acetylcholine chloride, N^ω-nitro-L-arginine methyl ester, tetraethylammonium chloride, indomethacin, calcium chloride dihydrate, L-arginine, 5-HT hydrochloride, potassium chloride, EGTA, sodium chloride, magnesium sulfate heptahydrate, monopotassium phosphate, sodium bicarbonate, and D-glucose were purchased from Sigma (St. Louis, USA). Dulbecco's modified Eagle's medium, fetal calf serum, and HBSS were pur-

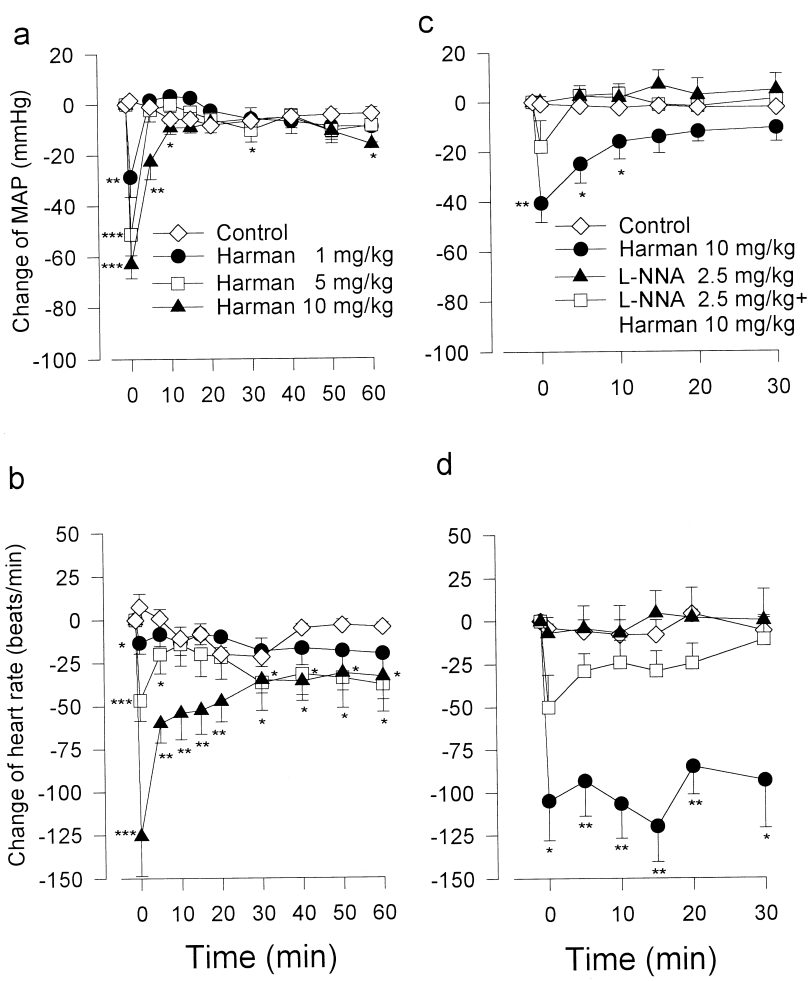

Fig. 1. The cardiovascular effects of harman in the absence and presence of N^G-nitro-L-arginine pretreatment in pentobarbital-anesthetized rats. After harman (1, 5, or 10 mg/kg, i.v.) was administered (a and b) or harman (10 mg/kg, i.v.) was administered after pretreatment with N^G-nitro-L-arginine (L-NAA, 2.5 mg/kg, i.v.) for 30 min (c and d), the change of mean arterial blood pressure (MAP) (a, c) and heart rate (b, d) were recorded. Data are means ± S.E.M. ($n = 5–7$). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with the corresponding control.

chased from Gibco Life Technologies (Grand Island, NY). Vanadium chloride was purchased from Aldrich (Milwaukee, USA).

2.9. Data analysis

Data are expressed as means ± S.E.M. and were analyzed by Student's *t*-test or one-way analysis of variance followed by Newman–Keuls test with a significance level of $P < 0.05$.

3. Results

3.1. Effect on blood pressure and heart rate in vivo

As shown in Fig. 1a and b, harman (1, 5, and 10 mg/kg, i.v.) dose dependently produced transient hypotension (< 15 min) and long-lasting bradycardia (at least 60 min at 10 mg/kg) in pentobarbital-anesthetized rats. As shown in Fig. 1c and d, the hypotensive and bradycardic effects of harman (10 mg/kg) were attenuated by a 30-min pretreatment of N^G-nitro-L-arginine (2.5 mg/kg, i.v.). N^G-nitro-L-arginine itself produced slight hypertension (7 ± 6 mmHg increase) and no significant effect on the heart rate.

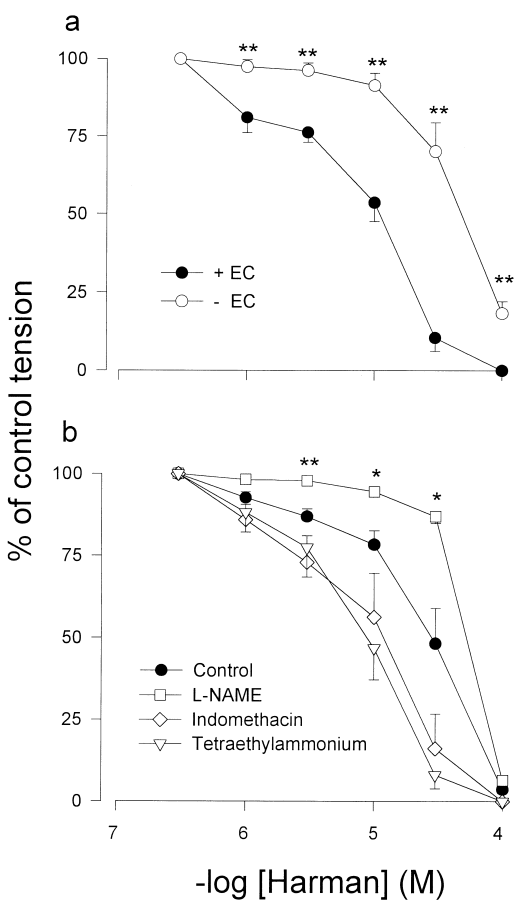

Fig. 2. Effects of endothelium removal, N^{ω}-nitro-L-arginine methyl ester, tetraethylammonium, or indomethacin pretreatment on the vasorelaxant action of harman. (a) Vasorelaxant effects of harman on phenylephrine-induced contractions in isolated endothelium-intact (+EC) and -denuded (−EC) rat thoracic aortic preparations. (b) Effects of N^{ω}-nitro-L-arginine methyl ester (L-NAME, 500 μM for 10 min), tetraethylammonium (10 mM for 60 min), or indomethacin (30 μM for 45 min) pretreatment in endothelium-intact aortic preparations. When the phenylephrine (0.3 μM)-induced contraction reached a plateau, harman was cumulatively added. Data are means ± S.E.M. ($n = 5$–12). $^{*}P < 0.05$, $^{**}P < 0.01$, as compared with the +EC (a) or the control (b).

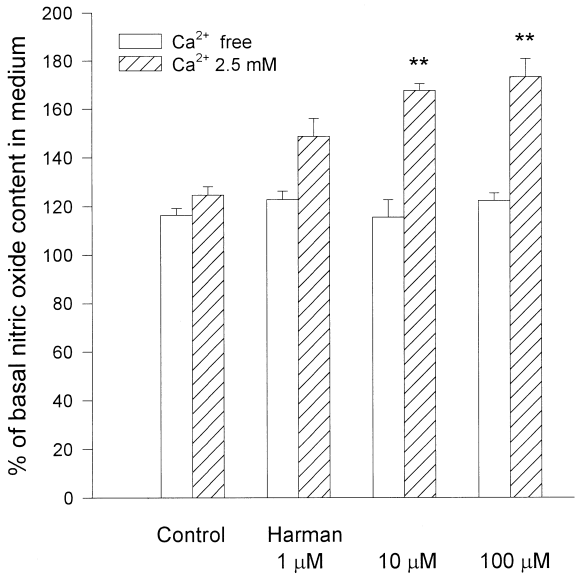

Fig. 3. Effect of harman on NO production in cultured rat aortic endothelial cells. NO content in the medium was quantified before (as basal) and after cells were treated with vehicle control or harman (1, 10, or 100 μM) for 30 min in 2.5 mM Ca^{2+} medium or Ca^{2+}-free medium. Data are means ± S.E.M ($n = 3$–10). $^{**}P < 0.01$, as compared with the corresponding control.

3.2. Vasorelaxation effect in vitro

As shown in Fig. 2a, harman (1–100 μM) concentration dependently relaxed the endothelium-intact aortic rings precontracted with phenylephrine (0.3 μM), producing a 100% relaxation at the concentration of 100 μM with an IC_{50} value of 9 ± 1 μM ($n = 7$). The effect of harman was reversible and not desensitized (data not shown).

The vasorelaxant response to harman was significantly depressed in endothelium-denuded preparations with an IC_{50} value of 48 ± 8 μM ($n = 8$) (Fig. 2a). At concentrations below 10 μM, the vasorelaxant effect of harman was almost completely blocked with endothelium removal. As shown in Fig. 2b, the vasorelaxant effect of harman was similarly attenuated by pretreatment with N^{ω}-nitro-L-arginine methyl ester (500 μM, 10 min), but not by tetraethylammonium (10 mM, 60 min) or indomethacin (30 μM, 45 min) in endothelium-intact aortic preparations.

3.3. Effect on NO production in cultured endothelial cells

As shown in Fig. 3, the NO content in medium was changed from basal 100% to 116.5 ± 2.8% (in Ca^{2+}-free

HBSS) or 124.7 ± 3.5% (in HBSS containing 2.5 mM Ca^{2+}) in the vehicle control group. Harman (1–100 μM) concentration dependently stimulated NO production from endothelial cells in HBSS containing 2.5 mM Ca^{2+} but not in Ca^{2+}-free HBSS (Fig. 3).

3.4. Effects on phenylephrine-, 5-HT-, and KCl-induced contractions

As shown in Fig. 4, the cumulative concentration–response curves to phenylephrine, 5-HT, or KCl were shifted to the right and the maximum responses were concentration dependently depressed by harman (3–30 μM). These results indicated a non-competitive antagonism.

3.5. Effects on the phenylephrine-induced biphasic contraction

Both the phasic contraction produced by phenylephrine in calcium-free medium with EGTA and the tonic contraction resulting from the reintroduction of $CaCl_2$ to the medium were all concentration dependently attenuated with harman (3–30 μM) pretreatment (data not shown). Harman was more potent to inhibit phasic contraction (IC_{50} = 9 ± 2 μM) than to inhibit the tonic contraction (IC_{50} = 17 ± 4 μM).

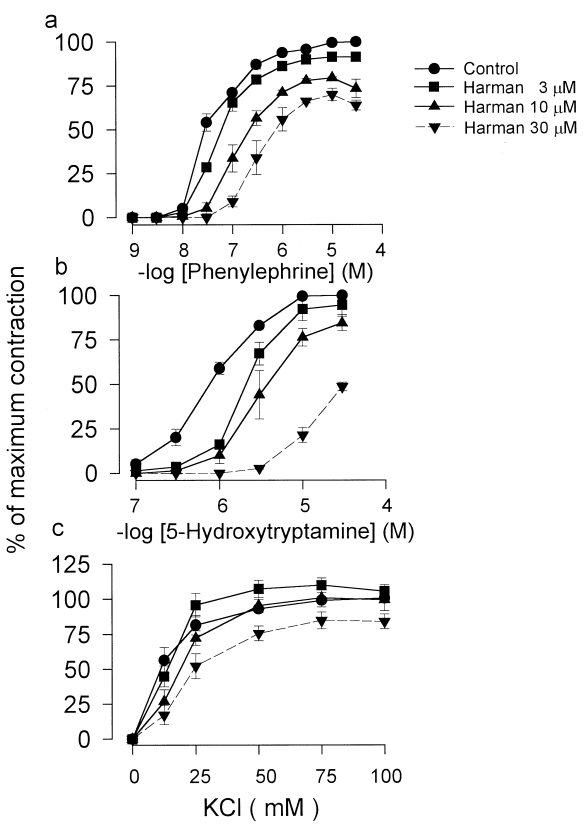

Fig. 4. Effects of harman on the concentration–response curves of phenylephrine, 5-HT, and KCl in endothelium-denuded aortic preparations. Harman (3, 10, or 30 μM) was added 10 min prior to construction of the concentration–response curve of phenylephrine (a), 5-HT (b), or KCl (c). Data are means ± S.E.M. (n = 4–7).

3.6. Receptor binding assay

Receptor binding assays indicated that harman interacted with the cardiac α_1-adrenoceptors, brain 5-HT_2 receptors, and cardiac 1,4-dihydropyridine binding site of L-type Ca^{2+} channels. The affinity of harman for 5-HT_2 receptors (K_i = 5 ± 1 μM) was higher than that for α_1-adrenoceptors (K_i = 39 ± 9 μM) and 1,4-dihydropyridine binding sites (K_i = 141 ± 6 μM).

4. Discussion

In anesthetized rats, harman (1–10 mg/kg, i.v.) dose dependently produced transient hypotension and long-lasting bradycardia. This cardiovascular effect of harman was similar to other harmala alkaloids such as harmine (Aarons et al., 1977). Similar to harmine (Aarons et al., 1977), the hypotensive effect of harman can probably be attributed to its vasorelaxant effect, as harman produced concentration-dependent vasorelaxant effect in the presence of phenylephrine and inhibited the contractions evoked by phenylephrine, 5-HT, and KCl in isolated rat thoracic aortic rings. In this study, we also demonstrated that both hypotensive and bradycardic effects of harman were attenuated with a NOS inhibitor N^G-nitro-L-arginine, indicating the involvement of NO. The underlying mechanism of bradycardic action of harman has not been further studied and is worth studying in the future.

The vasorelaxant effect of harman was associated with the actions on both endothelial cells and vascular smooth muscles. At concentrations below 10 μM, the vasorelaxant effect of harman was mostly dependent on the endothelium because endothelium removal completely blocked the vasorelaxant effect. At concentrations higher than 10 μM, the vasorelaxant effect of harman was partially attenuated by endothelium removal, indicating a direct action on the smooth muscles.

The use of different blockers (N^ω-nitro-L-arginine methyl ester, tetraethylammonium, and indomethacin) to examine the involvement of a mediator in the harman-induced endothelium-related vasorelaxation indicated that NO, but not endothelium-derived hyperpolarizing factor or prostacyclin, was involved. The action of harman on endothelial cells to release NO was confirmed by the experiment directly on the cultured aortic endothelial cells. The involvement of NO in the action of harman was consistent in vitro and in vivo. Although the underlying mechanism of action was unclear, the external Ca^{2+} was necessary for

the action of harman to increase NO release from endothelial cells.

Harman (3–30 μM) pretreatment also inhibited phenylephrine-, 5-HT-, and KCl-induced contractions in endothelium-denuded aortic rings, supporting a direct action on the vascular smooth muscles. The cumulative concentration–response curves to these spasmogens were shifted to the right with the maximum response attenuated by harman, indicating that the antagonistic effects of harman on certain targets (i.e., α_1-adrenoceptors, 5-HT_2 receptors, and L-type Ca^{2+} channels) were not competitive. However, in a similar concentration range, harman was able to interact with cardiac α_1-adrenoceptor, brain 5-HT_2 receptors, and cardiac 1,4-dihydropyridine binding sites of L-type Ca^{2+} channels with K_i values around 5–141 μM as shown by the receptor binding assays. The affinity of harman for brain 5-HT_2 receptors was consistent with that reported by Baum et al. (1996). Due to the differences of receptor subtypes (for example, α_{1B}-adrenoceptor in rat heart but α_{1D}-adrenoceptor in rat aorta; Graham et al., 1996) or tissue specificity, it should be determined whether a similar interaction of harman with these receptors or binding sites occurs in vascular smooth muscles. However, these results suggested that the actions of harman on vascular smooth muscles were multiple.

It has been suggested that there are biphasic responses, including the fast and slow components in the vasoconstriction induced by α_1-adrenoceptor agonist norepinephrine (Heaslip and Rahwan, 1982; Cauvin and Malik, 1984; Wilson et al., 1987). The fast (phasic) phase is due to the release of intracellular Ca^{2+}, and the slow (tonic) phase is largely dependent on the influx of external Ca^{2+}. The results showed that harman inhibited the phasic contraction produced by phenylephrine in calcium-free medium and the tonic contraction resulting from the reintroduction of $CaCl_2$ to the medium. Therefore, harman had effects on both phenylephrine-sensitive intracellular Ca^{2+} release and phenylephrine-induced Ca^{2+} influx and was more potent in the former. Although other mechanisms of action (i.e., post-receptor action) cannot be excluded, the actions of harman probably involve α_1-adrenoceptors and the 1,4-dihydropyridine binding site of L-type Ca^{2+} channels, as revealed by the receptor binding assays and the inhibitory effects of harman on phenylephrine- and KCl-induced contractions. Karaki et al. (1986) has reported that harmaline, a harman-related harmala alkaloid, seems to inhibit both the Ca^{2+} antagonist-sensitive and insensitive (but sodium nitroprusside-sensitive) Ca^{2+} channels in smooth muscles.

In conclusion, the present findings suggest that the vasorelaxant effect of harman can be attributed to its actions on endothelial cells to release NO and on vascular smooth muscles to inhibit the contractions induced by the activation of receptor-linked and voltage-dependent Ca^{2+} channels. This vasorelaxant effect of harman may be involved in its hypotensive effect.

Acknowledgements

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