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Inhibition of calcium channels by harmaline and other harmala alkaloids in vascular and intestinal smooth muscles

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1 Effects of harmaline and other harmala alkaloids on the contractions induced in the vascular smooth muscle of rabbit aorta and intestinal smooth muscle of taenia isolated from guinea-pig caecum were examined.

2 In rabbit isolated aorta, harmaline inhibited the sustained contraction induced by 65.4 mM K^+ with an IC_{50} (concentration needed for 50% inhibition) of 4.6×10^{-5} M. This inhibitory effect on high K^+-induced contraction was antagonized by raising the concentration of external Ca^{2+} but not by Bay K 8644, a Ca^{2+} channel facilitator. Harmaline also inhibited the sustained contraction induced by noradrenaline (10^{-6} M) with an IC_{50} of 7.6×10^{-5} M. The inhibitory effects on noradrenaline-induced contractions were not antagonized by raising the external Ca^{2+} concentrations or by Bay K 8644.

3 In guinea-pig taenia, harmaline inhibited the 45.4 mM K^+-induced contraction with an IC_{50} of 6.8×10^{-5} M and the carbachol (10^{-6} M)-induced contraction with an IC_{50} of 7.0×10^{-5} M. The inhibitory effects on both high K^+- and carbachol-induced contractions were antagonized by raising the external Ca^{2+} concentrations but not by Bay K 8644.

4 Harmaline, at the concentrations needed to inhibit the muscle contraction, inhibited the increase in $^{45}Ca^{2+}$ uptake induced by high K^+, noradrenaline and carbachol in aorta and taenia.

5 Harmaline did not change the cellular Na^+ and ATP contents in resting and high K^+ stimulated taenia.

6 Other harmala alkaloids also inhibited the contractions in these smooth muscles. The order of the inhibitory potency was 6-methoxyharman = harmine > harmaline = 2-methylharmine = harmane > 6-methoxyharmalan > harmalol = harmol for the contractions induced by high K^+ in aorta and taenia and by carbachol in taenia, and 2-methylharmine > 6-methoxyharman > 6-methoxyharmalan = harmol = harmalol = harmane > harmine > harmaline for the contraction induced by noradrenaline in aorta.

7 These results suggest that harmaline inhibits the contractile response of rabbit aorta and guinea-pig taenia by inhibiting different types of Ca^{2+} channel. The structure-activity relationship indicates that the potency and selectivity of the inhibitory effects on these channels are varied by modification of the structure of this alkaloid.

Introduction

Harmaline, an alkaloid extracted from the plant *Peganum harmala*, is a hallucinogen (Schultes, 1969). It also has an inhibitory effect on the Na^+,K^+-ATPase (Canessa *et al.*, 1973). Further, harmaline and other related harmala alkaloids are known to inhibit contractile responses induced by ouabain and acetylcholine in guinea-pig ileal smooth muscle (Hider *et al.*, 1981a,b). However, the mechanism of the relaxant action in smooth muscle has not been clarified.

In the present study, we have examined the mechanism of the inhibitory action of harmaline on vascular and intestinal smooth muscles. It was found that harmaline has unique inhibitory effects on the dif-

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ferent types of Ca^{2+} channel in these smooth muscles. The potency and selectivity of the inhibitory effects are varied by modifications of the structure of the alkaloid. Some of these experiments have been breifly reported at a Japanese Pharmacological Society meeting (Umeno *et al.*, 1985).

Methods

Tissue preparations

The thoracic aorta was isolated from male rabbits (2–3 kg) and cut into spiral strips of 3–4 mm width and about 15 mm length. The adventitial layer was removed from the strip as described by Karaki & Urakawa (1977) in order to avoid the possible release of endogenous catecholamines (Karaki *et al.*, 1984a). Segments of taenia, approximately 10 mm in length were removed from the caecum of male white guinea-pigs weighing 250–300 g.

Solutions

Normal physiological salt solution contained (mM): NaCl 136.9, KCl 5.4, $CaCl_2$ 1.5, $MgCl_2$ 1.0, $NaHCO_3$ 23.8 and glucose 5.5. High K^+ solution was made by replacing 60 mM NaCl with equimolar KCl for aorta or by adding 40 mM KCl to normal solution for taenia. The concentration of $CaCl_2$ was changed to 0.3 mM or 7.5 mM in some experiments. The solution was continuously bubbled with a 95% O_2 and 5% CO_2 mixture at 37°C. The pH of the solution was 7.3 ± 0.1.

Muscle tension

Muscle strips were suspended in an organ bath (20 ml) under resting tension of 1 g for aorta and 0.5 g for taenia and the contractions were recorded isometrically with a strain gauge transducer (Nihon-Koden). The muscles were equilibrated in normal solution for at least 2 h until the contractile response to the high K^+ solution became stable. After equilibration, the concentration of external Ca^{2+} was changed in some experiments and 30 min later, muscle strips were stimulated by 65.4 mM K^+ or 10^{-6} M noradrenaline in aorta or by 45.4 mM K^+ or 10^{-6} M carbachol in taenia. Muscle contraction reached a steady level within 30–40 min and then harmaline or related alkaloids were cumulatively added at 15–20 min intervals, as shown in Figure 1. In some experiments, a single concentration (2×10^{-4} M) of harmaline was added during the sustained contraction to induce almost complete relaxation followed by an addition of 7.5 mM Ca^{2+} or 10^{-7} M Bay K 8644, and recovery of muscle contraction was observed, as described by Spedding & Berg (1984).

Na^+ content

For the measurement of tissue Na^+ content, muscle strips were weighed, tied to a glass rod with silk string and equilibrated in normal solution for a minimum of 2 h before the start of experimental procedures. After incubation with a test solution, the muscles were blotted on filter paper and transferred to a quartz test-tube containing 0.5 ml of a mixture containing equal volumes of HNO_3 (61%) and $HClO_4$ (60%) and heated overnight at 180–240°C. Dried samples were dissolved in 0.01 N HCl shortly before the determination of Na^+. CsCl (1 g l^{-1}) was added to the standard and diluted solutions. The Na^+ concentration of the diluted samples was measured with a flame photometer (Hitachi, Type 208).

$^{45}Ca^{2+}$ uptake

Cellular $^{45}Ca^{2+}$ content was measured by a cold lanthanum wash method described by Karaki & Weiss (1979). After a 5 min incubation with a solution labelled with $^{45}Ca^{2+}$ (0.5 μCi ml^{-1}), the muscles were washed with a solution containing La^{3+} ($LaCl_3$ 73.8 mM, glucose 5.5 mM and Tris 23.8 mM adjusted to pH 6.9 with maleic acid at 0°C) for 30 min in order to remove extracellular bound $^{45}Ca^{2+}$. The muscles were then placed in scintillation vials containing 0.5 ml of tissue solubilizer (Lumasolve, Lumac) and were digested overnight at 50–60°C. The solubilized samples were then mixed with 5 ml scintillator (Lumagel, Lumac) and radioactivity was determined in a liquid scintillation spectrometer (Tri-Carb 3380, Packard).

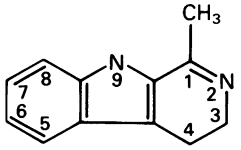
ATP content

The ATP content of the muscle was measured by the method originally described by Strehler & McElroy (1957) and modified by Karaki *et al.* (1982). After incubation in the chosen medium, muscle strips were boiled for 5 min in 2 ml hot water and ATP was extracted for 5 min. The extract was cooled to 0°C and the amount of ATP in the extract was determined with a photon counter (Lumac Biometer) with luciferine-luciferase reagent.

Saponin-skinned muscle

Skinned smooth muscle was prepared by the methods described by Saida & Nonomura (1978). A thin muscle strip, approximately 0.1 mm wide and 2 mm long, was treated with saponin (100 μg ml^{-1}) for 60 min in a relaxing solution of the following composition: KCl 136.9 mM, $MgCl_2$ 5 mM, Tris-maleate 20 mM, ATP-Na_2 5 mM, EGTA 2 mM, pH 6.8 at 22–24°C. Ca^{2+} concentration was calculated using binding constant for EGTA with Ca^{2+} of 10^6 M^{-1} (Harafuji & Ogawa, 1980).

Table 1 Structure of harmala alkaloids



	2	3–4	6	7	*IC_{50} for K^+-induced contraction*[1]	*Selectivity*[2]
6-Methoxyharman		=	$-OCH_3$	$-H$	0.28	0.46
Harmine		=	$-H$	$-OCH_3$	0.48	0.37
Harmaline		–	$-H$	$-OCH_3$	1.00	0.61
2-Methylharmine	$-CH_3$	=	$-H$	$-OCH_3$	1.17	3.60
Harmane		=	$-H$	$-H$	1.39	1.42
6-Methoxyharmalan		–	$-OCH_3$	$-H$	1.80	2.24
Harmalol		–	$-H$	$-OH$	3.48	4.21
Harmol		=	$-H$	$-OH$	4.78	5.79

[1]Relative concentration needed for 50% inhibition (IC_{50}) of the high K^+-induced contraction in rabbit aorta taking the IC_{50} of harmaline as 1.00. [2]Ratio of IC_{50} value for high K^+-induced contraction to that for noradrenaline-induced contraction in rabbit aorta. Data are taken from Table 3.

Drugs and chemicals

Harmaline hydrochloride (Nakarai Chemicals), harmine hydrochloride (Aldrich Chemical), harmol hydrochloride (Sigma Chemical), harmalol hydrochloride (Sigma), harmane hydrochloride (Sigma), 2-methylharmine (Sigma), 6-methoxyharman (Sigma), 6-methoxyharmalan (Sigma), saponin (ICN), (−)-noradrenaline bitartrate (Wako Pure Chemicals), histamine hydrochloride (Wako), Bay K 8644 (Schramm *et al.*, 1983; donated by Bayer AG), ATP-Na_2 (Sigma) and $^{45}CaCl_2$ (New England Nuclear) were used.

The chemical structures of harmala alkaloids used in the present experiments are shown in Table 1.

Statistics

Results of the experiments are expressed as mean ± s.e.mean. Student's t test was used for statistical analysis and $P < 0.01$ was taken as significant.

Results

High K^+-induced contractions

Cumulative application of harmaline inhibited the sustained contractions induced by high-K^+ in rabbit aorta and guinea-pig taenia (Figure 1). Figure 2 shows the concentration-response relationships for the inhibitory effect of harmaline at three different concentrations of external Ca^{2+}. The concentration-response curves were shifted to the right by raising the concentration of external Ca^{2+} from 0.3 to 1.5 or 7.5 mM in both aorta and taenia.

Interaction between harmaline and Bay K 8644, a Ca^{2+} channel facilitator (Schramm *et al.*, 1983), was examined as described by Spedding & Berg (1984). In both aorta and taenia, addition of 2×10^{-4} M harmaline almost completely inhibited the high K^+-induced contractions and addition of 7.5 mM Ca^{2+} recovered the muscle tension. However, addition of 10^{-7} M Bay K 8644 did not change the muscle tension inhibited by harmaline (data not shown).

Noradrenaline- and carbachol-induced contractions

As shown in Figure 1, harmaline inhibited the 10^{-6} M noradrenaline-induced contraction of rabbit aorta and the 10^{-6} M carbachol-induced contraction of taenia. A similar effect was obtained on the contraction of the aorta induced by histamine (10^{-5} M) (data not shown). Figure 3 shows the concentration-response relationship for the inhibitory effect of harmaline at three different concentrations of external Ca^{2+}. The inhibitory effect of harmaline on the noradrenaline-induced contraction in the aorta was not modified by raising the external Ca^{2+} concentration from 1.5 to 7.5 mM whereas the inhibitory effect was slightly augmented by decreasing the Ca^{2+} concentration to 0.3 mM. In contrast, the inhibitory effect on the carbachol-induced contraction in taenia was

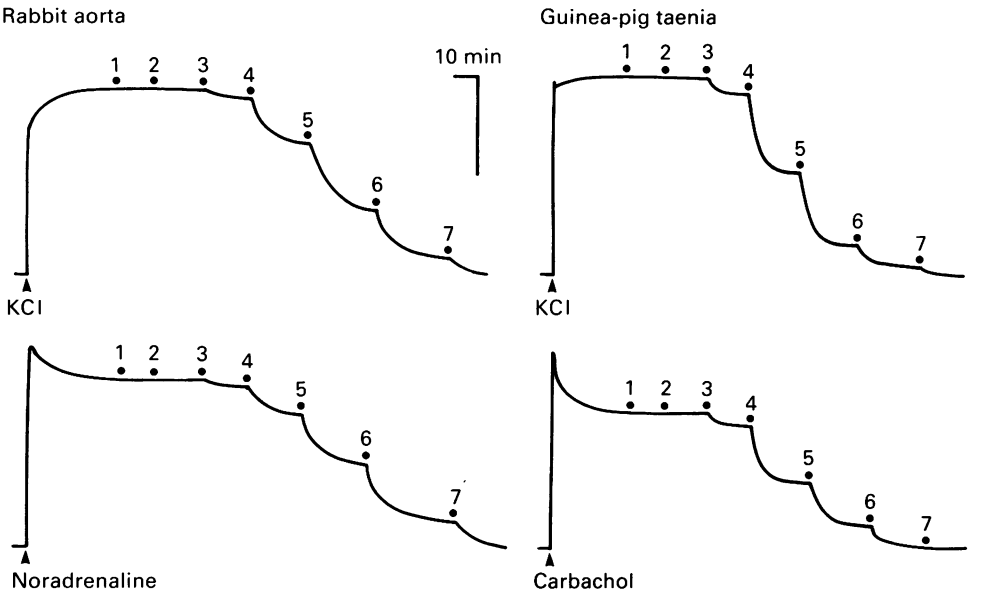


Figure 1 Inhibitory effect of harmaline on the contractions induced by 65.4 mM K^+ and 10^{-6} M noradrenaline in rabbit aorta (left) and 45.4 mM K^+ and 10^{-6} M carbachol in guinea-pig taenia (right). Harmaline was added cumulatively during the sustained contractions in these muscles (shown by dots). Concentration of harmaline was as follows. (1) 5×10^{-6} M, (2) 10^{-5} M, (3) 2×10^{-5} M, (4) 5×10^{-5} M, (5) 10^{-4} M, (6) 2×10^{-4} M and (7) 5×10^{-4} M. Horizontal bar indicates 10 min and vertical bar indicates 1 g tension for aorta and 5 g tension for taenia.

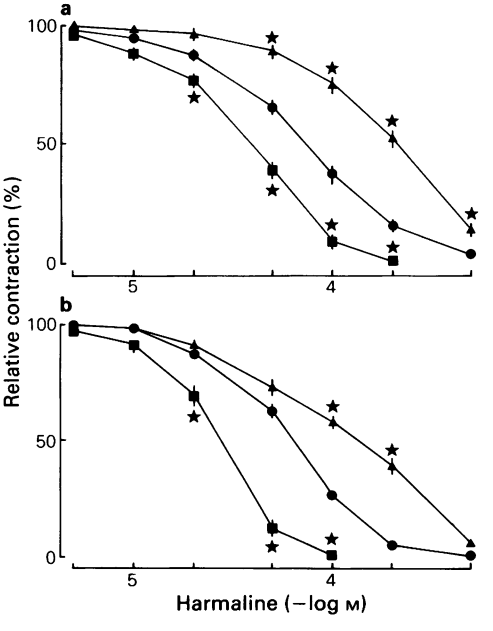


Figure 2 Inhibitory effects of harmaline on the contractions induced by 65.4 mM K^+ in rabbit aorta (a) and 45.4 mM K^+ in guinea-pig taenia (b) in the presence of 0.3 mM Ca^{2+} (■), 1.5 mM Ca^{2+} (●) and 7.5 mM Ca^{2+} (▲). Muscle strips were incubated with different concentrations of Ca^{2+} for 30 min before the addition of stimulant. Harmaline was cumulatively applied during sustained contraction induced by stimulant, as shown in Figure 1. 100% represents the muscle tension before the addition of harmaline. Each point represents mean of 4 to 6 experiments and s.e.mean is shown by vertical bar when it is greater than the symbol. *Significantly different ($P < 0.01$) from the value in 1.5 mM Ca^{2+} solution.

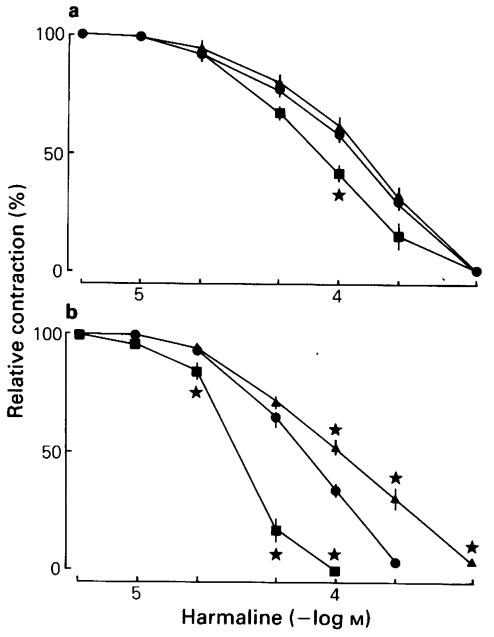


Figure 3 Inhibitory effects of harmaline on the contractions induced by 10^{-6} M noradrenaline in rabbit aorta (a) and 10^{-6} M carbachol in guinea-pig taenia (b) in the presence of 0.3 mM Ca^{2+} (■), 1.5 mM Ca^{2+} (●) and 7.5 mM Ca^{2+} (▲). For further details, see Figure 2.

augmented by the decrease and reduced by the increase in external Ca^{2+} concentrations.

Both the noradrenaline-induced contraction in aorta and the carbachol-induced contraction in taenia were almost completely inhibited by 2×10^{-4} M harmaline. Addition of 7.5 mM Ca^{2+} recovered muscle tension in taenia but not in aorta. In contrast, addition of 10^{-7} M Bay K 8644 did not reverse the inhibitory effect of harmaline in aorta or taenia (data not shown).

Na^+ and ATP contents

As shown in Table 2, 10^{-4} M harmaline did not change the tissue Na^+ content, although 10^{-5} M ouabain, a specific inhibitor of Na^+,K^+-ATPase, increased the Na^+ content in taenia.

Table 2 also shows that 10^{-4} M harmaline had no effect on the ATP content of taenia in normal and high K^+ solutions.

Table 2 Changes in Na^+ and ATP content of guinea-pig taenia

	Na^+	*ATP*
	(mmol kg^{-1} wet weight)	
Control	7.8 ± 0.3 (6)	1.35 ± 0.09 (6)
Ouabain 10^{-5} M	48.1 ± 2.5 (6)	–
Harmaline 10^{-4} M	7.5 ± 1.2 (6)	1.14 ± 0.08 (6)
45.4 mM K^+	–	1.05 ± 0.07 (6)
45.4 mM K^+ + harmaline 10^{-4} M	–	1.14 ± 0.10 (6)

Muscle strips were incubated with the high K^+ and/or inhibitors for 60 min. –: not determined.

$^{45}Ca^{2+}$ uptake

As shown in Figure 4, high K^+ and noradrenaline increased the $^{45}Ca^{2+}$ uptake during a 5 min $^{45}Ca^{2+}$ incubation period in rabbit aorta. In guinea-pig taenia coli, high K^+ and carbachol also increased the $^{45}Ca^{2+}$ uptake. Although the resting $^{45}Ca^{2+}$ uptake was not changed by 5×10^{-4} M harmaline in aorta and by 2×10^{-4} M harmaline in taenia. The increments induced by high K^+, noradrenaline and carbachol were completely inhibited by these concentrations of harmaline.

Saponin-treated muscle

In the saponin-treated taenia, 10^{-6} M Ca^{2+} induced a sustained contraction. Addition of 2×10^{-4} M harmaline during this sustained contraction decreased muscle tension by 3.6% and 2×10^{-3} M harmaline by 14.3% ($n = 2$).

Effects of other harmala alkaloids

Cumulative application of harmala alkaloids, i.e., 6-methoxyharman, harmine, 2-methylharmine, harmane, 6-methoxyharmalan, harmalol and harmol, inhibited the sustained contractions in aorta and taenia. Concentrations of these alkaloids needed to induce 50% inhibition of the contractions (IC_{50}) in aorta and taenia are listed in Table 3. The order of the inhibitory potency for the contraction induced by high K^+ in aorta was 6-methoxyharman = harmine > harmaline = 2-methylharmine = harmane > 6-methoxyharmalan > harmalol = harmol. The order of the inhibitory potency for the contraction of taenia induced by either high K^+ or carbachol was almost the same. On the noradrenaline-induced contraction in aorta, in contrast, these alkaloids showed a different order of inhibitory potency; 2-methylharmine > 6-methoxyharman > 6-methoxyharmalan = harmol = harmalol = harmane > harmine > harmaline.

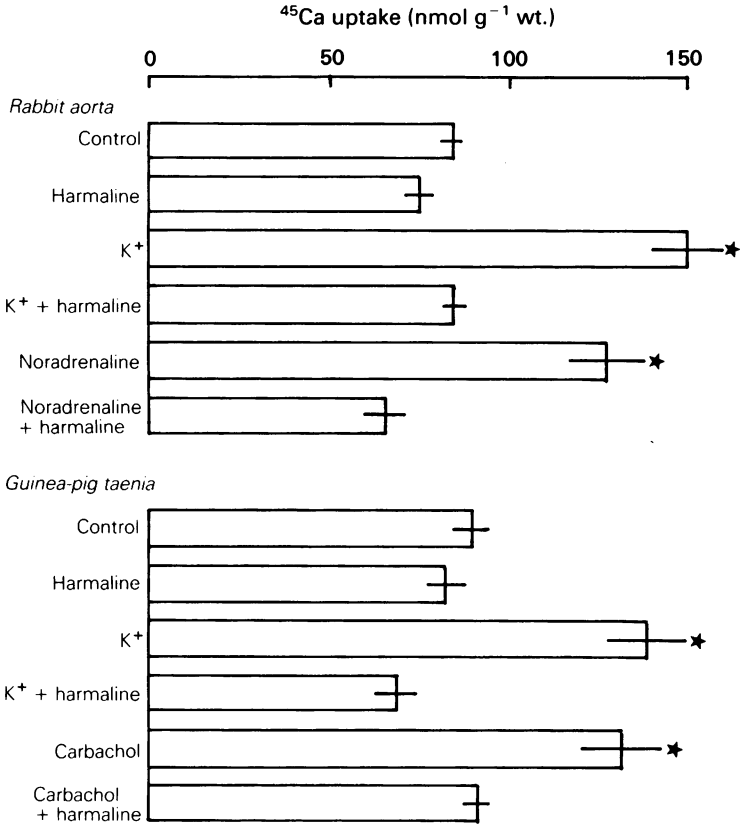


Figure 4 Effects of harmaline on $^{45}Ca^{2+}$ uptake in rabbit aorta and guinea-pig taenia. Harmaline at concentrations of 5×10^{-4} M and 2×10^{-4} M was used for aorta and taenia, respectively. Harmaline was added 30 min before the addition of $^{45}Ca^{2+}$ with or without stimulant. Each bar represents mean of 6 experiments and s.e.mean is shown by horizontal error bar. *Significantly greater ($P < 0.01$) than the respective control.

Table 3 Concentrations of harmala alkaloids needed to induce 50% inhibition of the contraction induced in rabbit aorta and guinea-pig taenia

	Contractions induced by			
	high K^+ in aorta	*noradrenaline in aorta*	*high K^+ in taenia*	*carbachol in taenia*
	($\times 10^{-5}$ M)			
6-Methoxyharman	1.3	2.8	0.6	1.3
Harmine	2.2	6.0	2.3	2.3
Harmaline	4.6	7.6	6.8	7.0
2-Methylharmine	5.4	1.5	6.4	9.0
Harmane	6.4	4.5	5.0	4.9
6-Methoxyharmalan	8.3	3.7	5.5	6.0
Harmol	16.0	3.8	31.0	41.0
Harmalol	22.0	3.8	22.0	43.0

Harmala alkaloids were cumulatively added during the sustained contraction induced by 65.4 mM K^+ or 10^{-6} M noradrenaline in aorta or 45.4 mM K^+ or 10^{-6} M carbachol in taenia. Values are the mean of 6 to 11 experiments. S.e.means, which were less than 10% of the respective means, are not shown.

Discussion

Harmaline inhibited all the contractions examined in rabbit aorta and guinea-pig taenia. Such nonspecific effects suggest that harmaline does not inhibit a specific receptor for an agonist. Further, harmaline inhibited only slightly the Ca^{2+}-induced contraction of the saponin-treated taenia, suggesting that the inhibitory effect of harmaline is not attributable to inhibition of the contractile proteins in smooth muscle. Harmaline inhibits glucose uptake in intestinal epithelial cells by interfering with the Na^+-dependent sugar transport (Sepulveda *et al.*, 1977). Inhibition of glucose transport results in an ATP-deficiency and inhibition of contraction in smooth muscle (Suzuki *et al.*, 1980; Karaki *et al.*, 1982). However, this possibility is not likely because the ATP content of taenia coli was not affected by harmaline. Harmaline also inhibits Na^+, K^+-ATPase in various tissues (Canessa *et al.*, 1973; Becker & Willis, 1983). In smooth muscle, inhibition of Na^+, K^+-ATPase leads to accumulation of cellular Na^+ and inhibition of contraction (Bose, 1975; Kishimoto *et al.*, 1980). However, harmaline did not change the Na^+ content.

Further examination showed that the inhibitory effects of harmaline on high K^+-induced contractions in aorta and taenia and carbachol-induced contraction in taenia were antagonized by the external Ca^{2+} concentrations whereas the inhibition of noradrenaline-induced contraction in aorta was not. During these contractions, $^{45}Ca^{2+}$ uptake of the muscle increased and harmaline inhibited the increase in $^{45}Ca^{2+}$ uptake in these smooth muscles. It has been suggested that sustained contractions in smooth muscle is largely dependent on the influx of external Ca^{2+} (Cauvin *et al.*, 1983; Karaki & Weiss, 1984), and only initial transient contractions induced by noradrenaline in rabbit aorta (Deth & van Breemen, 1977; Karaki *et al.*, 1979) and by carbachol in guinea-pig taenia (Ohashi *et al.*, 1974; Brading & Sneddon, 1980) are attributable to release of cellular Ca^{2+}. Inhibition by harmaline of sustained contraction and associated $^{45}Ca^{2+}$ influx suggest that harmaline inhibits the pathways of Ca^{2+} influx which are activated by high K^+ and receptor agonists.

In smooth muscle, there are two types of Ca^{2+} channels, voltage-dependent and receptor-linked channels (Bolton, 1979; van Breemen *et al.*, 1979). In rabbit aorta, these Ca^{2+} channels have unique characteristics in that the voltage-dependent channels are selectively inhibited by the organic Ca^{2+} antagonists like verapamil whereas the receptor-linked channels are not sensitive to Ca^{2+} antagonists but are inhibited by sodium nitroprusside. In contrast, both types of Ca^{2+} channel in guinea-pig taenia are inhibited by the organic Ca^{2+} antagonists (although the voltage-dependent channels are more strongly inhibited than the receptor linked channels) but not by sodium nitroprusside. The inhibitory effects of the organic Ca^{2+} antagonists but not sodium nitroprusside may be antagonized by raising the external Ca^{2+} concentrations (Karaki & Weiss, 1984; Karaki *et al.*, 1984b).

Comparing these and the present results, harmaline has inhibitory effects similar to those of the organic Ca^{2+} antagonists; it inhibited high K^+-induced contractions in aorta and taenia and carbachol-induced contraction in taenia and the effects were antagonized by external Ca^{2+}. In addition, harmaline inhibited the noradrenaline-induced contraction which is not affected by external Ca^{2+} concentrations. Thus, harmaline seems to inhibit both the Ca^{2+} antagonist-sensitive and insensitive (but sodium nitroprusside-sensitive) Ca^{2+} channels in smooth muscle.

The inhibitory effect of harmaline was not reversed by Bay K 8644, a dihydropyridine which stimulates Ca^{2+} entry through Ca^{2+} channel (Schramm *et al.*, 1983). Spedding & Berg (1984) found that the inhibitory effect of verapamil, diltiazem and dihydropyridine Ca^{2+} antagonists were antagonized by Bay K 8644 whereas the inhibitory effects of diphenyl alkylamine Ca^{2+} antagonists were not, and proposed the use of Bay K 8644 for classification of Ca^{2+} antagonists. Thus, the site of action of harmaline on Ca^{2+} channels seems to be different from that of verapamil, diltiazem and dihydropyridines.

Although harmaline inhibited these Ca^{2+}-channels nonselectively, some of the harmala alkaloids seem to have more selective effects on one of these two types of Ca^{2+} channels. 6-Methoxyharman and harmine inhibited the Ca^{2+} antagonist-sensitive contraction relatively selectively whereas harmol and harmalol had less effect on this type of contraction (Tables 1 and 3). These results support the idea that the pathways of Ca^{2+} influx for high K^+- and noradrenaline-induced contractions are different.

Comparing the chemical structures and the inhibitory effects on smooth muscle contractions of the harmala alkaloids (Tables 1 and 3), it was concluded that; (1) the presence of 6- or 7-methoxy group increases the inhibitory effect on the Ca^{2+} antagonist-sensitive channels but not on the Ca^{2+} antagonist-insensitive channels; (2) 7-OH group decreases the inhibitory potency on the Ca^{2+} antagonist-sensitive channels; (3) 3–4 double bond selectively increases the inhibitory potency on the Ca^{2+} antagonist-sensitive Ca^{2+} channels although this effect is not seen in the presence of 7-OH group; and (4) 2-methyl group seems to increase the inhibitory potency on the Ca^{2+} antagonist-insensitive channels. The lower inhibitory potency of the compounds with 7-OH group on the Ca^{2+} antagonist-sensitive channels may be due to the decreased lipophilicity. However, since the inhibitory potency on the Ca^{2+} antagonist-insensitive Ca^{2+} channels is not decreased by the 7-OH group, the role

of the 7-OH group may not be explained solely by the change in lipophilicity. Although Ca^{2+} antagonists are lipophilic in nature, their pharmacological potency does not necessarily parallel their lipophilicity (Spedding, 1985).

In conclusion, it is suggested that harmaline inhibits the contractile responses of rabbit aorta and guinea-pig taenia by inhibiting different types of Ca^{2+} channel. The structure-activity relationship indicated that the potency and selectivity of the inhibitory effects on these channels are varied by modifications of the structure of this alkaloid.

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