Effects of Mazindol on the Contractions in the Rat Vas Deferens

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Effects of mazindol on the contraction induced by nerve stimulation and agonist drugs were investigated to examine the inhibition of catecholamine uptake by mazindol in the vas deferens. Mazindol at 10^{-9} — 10^{-7} M potentiated a single electrical pulse-elicited neurogenic contraction which consisted of a twitch and phasic contraction. This potentiation was almost completely antagonized by prazosin. Mazindol leftwardly shifted the dose-response curve to norepinephrine in a dose-dependent manner; the dose-ratio was 6.2-fold and 83.2-fold at 10^{-9} M and 10^{-7} M mazindol, respectively. The dose-response curves to dopamine and methacholine were also slightly shifted by mazindol. In the dibenamine-treated vas deferens, mazindol did not affect the dose-response curve to methacholine. These results suggest that mazindol potently inhibits the norepinephrine uptake without acting on the smooth muscle cells in the rat vas deferens.

Keywords — mazindol; uptake inhibition; norepinephrine; dopamine; vas deferens

Introduction

Mazindol is an anorectic and has a weak stimulating action on the central nervous system.^{1,2)} It has been reported that the anorexia by mazindol is due to the inhibition of uptake of dopamine, leading to the enhancement of dopaminergic transmission in the neostriatum and other regions in the brain.^{3,4)} In addition, mazindol has been indicated to block the uptake of norepinephrine and serotonin in the synaptosomes prepared from the brain.^{5,6)} A number of *in vivo* and *in vitro* studies have suggested that mazindol binds to the uptake sites of dopamine and norepinephrine in the neurons of the brain.^{3,6,7)} Such binding sites are considered to be distinct from the dopaminergic or noradrenergic receptors, since the affinity of receptor ligands with the binding sites of mazindol was much lower than that for the receptors.^{3,7,8)}

In contrast to the central nervous system, effects of mazindol on the peripheral neurons have scarcely been investigated. To study the actions of mazindol on the peripheral adrenergic neurons, we have investigated the effects of mazindol on the contractions induced by nerve stimulation and catecholamines in the rat vas deferens. The vas deferens is densely innervated by the postganglionic sympathetic nerve and is frequently used to assess the inhibitory action of drugs on norepinephrine uptake.

Materials and Methods

Vas deferens was removed from male Wistar strain rats (250—350 g) and the epididymal portion 10 mm in length was mounted with a load of 0.5 g in an organ bath of 10 ml capacity. The preparation was perfused at a rate of 3 ml/min with the physiological salt solution (PSS) of the following composition: 120 mM NaCl, 6.0 mM KCl, 2.0 mM CaCl_2 , 1.0 mM MgCl_2 , 1.0 mM NaH_2PO_4 , 20.0 mM NaHCO_3 , 5.0 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES) and 14 mM glucose. The bathing solution was maintained at 37 °C and was continuously gassed with 95% O_2 and 5% CO_2 throughout the experiment. The vas deferens was equilibrated for at least 60 min before the experiment. When necessary, 10^{-6} M dibenamine was applied for 15 min during the equilibration period.

The contractile responses were isometrically measured with a strain gauge (Minebia Co., UL-10 GR). For the electrical stimulation of the intramural nerves, two platinum ring electrodes connected to an electric stimulator (Nihon Kohden, MSE-3R) were placed around the top and bottom of the preparation. A single pulse of 1 ms and supramaximal voltage was applied to the vas deferens at an interval of at least 20 min.

The dose-response curves to the agonist drugs were determined by the single dose method. Af-

ter the control responses were obtained, the perfusion solution was switched to the PSS which contained mazindol, and the responses in the presence of mazindol were determined. The peak tensions developed during the 1 min exposure to increasing concentrations of the agonists were used to construct the dose-response curves, in which these values were expressed as percentage of the maximal developed tension under the control condition. The maximal developed tension was defined as the largest tension developed in a series of concentrations of an agonist. When determining pD_2 , the responses in the presence of mazindol were normalized to the maximal response attained at each concentration of mazindol, and pD_2 was calculated according to the linear regression analysis using the responses ranging between 10% and 90%.

Mazindol (Sandoz) was dissolved in ethanol and diluted in a perfusing solution to obtain an appropriate concentration. Final concentration of ethanol in the perfusing solution was 0.01%. Other drugs used were: *l*-norepinephrine (Nakarai), methacholine chloride (Nakarai),

prazosin hydrochloride (Pfizer), dibenamine hydrochloride (Nakarai), and tetrodotoxin (Sigma).

Results

Effects of Mazindol on Neurogenic Contraction

Electrical stimulation with a single pulse (supramaximal voltage, 1 ms duration) evoked a twitch contraction in the epididymal portion of the rat vas deferens (Fig. 1 control). Following the twitch, a few phasic contractions with amplitudes just above the detectable level were developed in some preparations (3 out of 7). Both the twitch and phasic contractions were abolished by 10^{-6} M tetrodotoxin. Mazindol at 10^{-9} – 10^{-7} M dose-dependently increased the amplitudes of and elongated the duration of the twitches (Figs. 1, 2). Mazindol also enhanced the phasic contractions occurring after the twitch. Treatment with 10^{-8} M mazindol caused development of the phasic contractions in all the preparations. As shown in Fig. 1, their amplitudes and persistence increased as the concentra-

Fig. 1. Effects of Mazindol on the Neurogenic Contraction

Intramural nerves of the vas deferens were stimulated by a single electrical pulse (supramaximal voltage, 1 ms duration) at the dots. The vas deferens was exposed to increasing concentrations of mazindol (10^{-9} – 10^{-7} M) in the perfusion solution, after which 10^{-6} M prazosin was applied in the presence of 10^{-7} M mazindol. Upper panel illustrates a twitch and following phasic contractions at a slow recording speed. Lower panel illustrates the twitch contraction at a fast recording speed. Note the different calibration scale in the two panels.

Fig. 2. Effects of Mazindol and Prazosin on the Amplitude of the Twitch Contraction

The mean of percent increase in the amplitude of twitch contraction obtained in 6 preparations is shown in the ordinate. Vertical bars represent standard errors of the mean.

tion of mazindol was raised. The potentiating effects of mazindol on the twitches and the phasic contractions were almost completely antagonized by 10^{-6} M prazosin (Figs. 1, 2).

Effects of Mazindol on the Dose-Response

Curve to Norepinephrine and Dopamine

Mazindol at 10^{-9} – 10^{-7} M shifted the dose-response curve to norepinephrine to the left (Fig. 3 (A)). The dose-response curves in the presence of mazindol were almost parallel to the control curve, and the magnitude of the shift was increased in a dose-dependent manner. During the perfusion with the PSS containing more than 10^{-8} M mazindol, small phasic contractions were repetitively induced in some preparations. The maximal response to norepinephrine was not affected by mazindol up to 10^{-8} M, but was slightly enhanced by 10^{-7} M mazindol. Table I summarizes the effects of mazindol at various concentrations on pD_2 , dose-ratio and the maximal developed tension to norepinephrine.

The dose-response curve to dopamine was also shifted by mazindol to the left, but the shift was much smaller than that of norepinephrine (Fig. 3 (B), Table I). There was no clear dose-dependence observed in the extent of the shift. Unlike the case of norepinephrine, mazindol rarely produced repetitive phasic contractions in a series of applications of dopamine. The maximal response to dopamine was slightly enhanced

Fig. 3. Effects of Mazindol on the Dose-Response Curves to Norepinephrine and Dopamine

The responses are expressed as the mean of percent of the maximal contraction in the absence of mazindol (control). Vertical bars represent standard errors of the mean. The number of preparations tested for each concentration of mazindol is 6 or 7; control curves are the mean of 18 and 13 preparations for norepinephrine and dopamine, respectively.

○, control; ■, 10^{-9} M mazindol; □, 10^{-8} M mazindol; ●, 10^{-7} M mazindol.

TABLE I. Effects of Mazindol on pD_2 and Maximal Tension in Response to Norepinephrine and Dopamine

		Control	Mazindol		
			10^{-9} M	10^{-8} M	10^{-7} M
Norepinephrine	$pD_2^{a)}$	6.00 (5.86—6.15)	6.79 (6.57—7.02)	7.38 (7.02—7.74)	7.92 (7.41—8.43)
	Max ^{b)}	2.09 ± 0.06	2.13 ± 0.12	2.19 ± 0.14	2.34 ± 0.16
	D/R ^{c)}		6.2-fold	24.0-fold	83.2-fold
	$n^{d)}$	(18)	(6)	(6)	(6)
Dopamine	pD_2	4.64 (4.58—4.71)	ND ^{e)}	4.90 (4.78—5.00)	4.84 (4.76—4.92)
	Max	2.43 ± 0.07	ND	2.59 ± 0.17	2.70 ± 0.09
	D/R		ND	1.8-fold	1.6-fold
	n	(13)		(6)	(7)

a) 95% confidence limits in parentheses, b) maximal developed tension (g), mean $\pm$ standard error, c) dose-ratio, d) the number of preparations, e) not determined.

by 10^{-7} M mazindol (Table I).

Effects of Mazindol on the Dose-Response Curve to Methacholine

Effects of mazindol on the methacholine-induced contractions were investigated to study the postjunctional action of mazindol on the smooth muscle cells of the rat vas deferens. In the intact preparation, mazindol at 10^{-8} — 10^{-7} M shifted the dose-response curve to methacholine to the left and slightly enhanced the maximal response to methacholine (Fig. 4 (A));

however, there was no dose-dependence in these changes. When the vas deferens pretreated with dibenamine was used, mazindol at 10^{-7} M did not significantly affect the dose-response curve to methacholine as shown in Fig. 4 (B). Presented in Table II are the effects of mazindol on pD_2 , dose-ratio and the maximal tension to

Fig. 4. Effects of Mazindol on the Dose-Response Curve to Methacholine

Dose-response curves were determined in the intact (A) and the dibenamine-treated vas deferens (B). The number of preparations used for each concentration of mazindol is 6. Control curves are the mean of 12 and 6 for the intact and dibenamine-treatment, respectively.

○, control; □, 10^{-8} M mazindol; ●, 10^{-7} M mazindol.

TABLE II. Effects of Mazindol on pD_2 and Maximal Tension to Methacholine in Intact and Dibenamine-Treated Vas Deferens

		Control	Mazindol	
			10 ⁻⁸ M	10 ⁻⁷ M
Methacholine (Intact)	pD ₂ ^{a)}	4.94 (4.84—5.04)	5.58 (5.42—5.75)	5.22 (5.09—5.35)
	Max ^{b)}	2.59 ± 0.09	2.89 ± 0.14	2.89 ± 0.11
	D/R ^{c)}		4.4-fold	1.9-fold
	n ^{d)}	(12)	(6)	(6)
Methacholine (Dibenamine)	pD ₂	4.78 (4.70—4.86)	ND ^{e)}	4.95 (4.86—5.05)
	Max	2.30 ± 0.09	ND	2.38 ± 0.06
	D/R			1.5-fold
	n	(6)		(6)

a) 95% confidence limits in parentheses, b) maximal developed tension (g), mean $\pm$ standard error, c) dose-ratio, d) the number of preparations, e) not determined.

methacholine in the intact and dibenamine-treated rat vas deferens.

Discussion

Many reports have demonstrated that in the vas deferens electrical stimulation provoked the release of multiple transmitters simultaneously from the adrenergic nerve terminals (see 9 for references).⁹⁾ Although there has been much debate regarding the co-transmitters, norepinephrine has been widely accepted as the transmitter in the vas deferens. Under the present experimental condition, a single pulse stimulation elicited a twitch contraction and phasic contractions, although the phasic contractions were not observed in all preparations. Mazindol enhanced both types of contractions and the potentiating effects of mazindol were eliminated by prazosin. These results suggest that norepinephrine is involved in the generation of the twitch and phasic contractions and that mazindol enhances these contractions by inhibiting the uptake of norepinephrine in the peripheral adrenergic nerve terminals.

Mazindol shifted the dose-response curve to norepinephrine to the left. Except for the responses to low concentrations of norepinephrine, the shift in the dose-response curves was parallel to the control curve and the magnitude of the shift was increased with the increase of mazindol concentration. The small phasic con-

tractions induced by the presence of mazindol could be involved in the contractile responses to norepinephrine and may more influentially affect the contractions in response to low concentrations of norepinephrine. This may account for the deviant responses to low concentrations of norepinephrine in the presence of mazindol. At a low concentration of 10^{-9} M, mazindol increased pD_2 by 0.8 (Table I), which corresponds to a 6.2-fold dose-ratio. At 10^{-8} M mazindol, the dose-ratio reached 24.0-fold. Compared with other agents which are used to inhibit the uptake of norepinephrine, the potency of mazindol was high. Park *et al.*¹⁰⁾ have reported that the dose-ratio in response to norepinephrine was 9.0-fold and 12.0-fold for 10^{-6} M cocaine and 10^{-7} M desipramine, respectively, in the rat vas deferens. Nomifensin at 10^{-7} M, a compound structurally related to mazindol, was shown to induce 10-fold increase in the rat vas deferens (Y. Ishida, personal communication). In view of its high potency and the fact that mazindol did not have significant direct effects on the smooth muscle cells (see below), mazindol can be viewed as a useful drug to inhibit the uptake of norepinephrine in the adrenergically innervated organs.

Mazindol potentiated the methacholine-induced contractions in the intact vas deferens, but not in the dibenamine-treated vas deferens, indicating that mazindol did not have postjunctional action on the smooth muscle cells and that endogenous norepinephrine was involved in the

potentiation. Since dibenamine treatment did not substantially affect the methacholine-induced contractions (see pD₂ and the maximal response in Table II), an indirect action of methacholine can be ruled out. It seems possible that the potentiation by mazindol at high concentration is partially due to the norepinephrine released, since mazindol above 10⁻⁶ M was reported to have the releasing action of catecholamines in the vas deferens¹¹⁾ and the synaptosomes from the brain.³⁾ Alternatively, even though mazindol did not release norepinephrine, mazindol can sensitize the smooth muscle cells of vas deferens by facilitating the action of norepinephrine which is leaked spontaneously from the adrenergic nerve terminals.

An interesting result in the present study was that the leftward shift in the dose-response curves to dopamine was very small. Similar minimal effect by cocaine on the dopamine response was reported in the rat vas deferens.¹⁰⁾ The small shift in the dose-response curve to dopamine may not be due to the fact that dopamine was not taken up into the adrenergic nerve terminals. In fact, Eisenhofer *et al.*¹²⁾ have recently demonstrated that in the rat vas deferens, most of the exogenously administered [³H]dopamine was converted to [³H]norepinephrine, indicating that [³H]dopamine was taken up by noradrenergic nerves and then into synaptic vesicles for metabolism by dopamine-β-hydroxylase. Hence, it is supposed either that dopamine is taken up by a different transport system from that for norepinephrine, or that the rate of dopamine transport is much slower than that of norepinephrine.

In summary, the present study has shown that mazindol has a high potency to inhibit the uptake of norepinephrine in the rat vas deferens. Since mazindol did not have significant action on the smooth muscle cells, mazindol can be used as an agent to selectively inhibit the uptake of norepinephrine in peripheral organs.

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