PHARMACOLOGICAL STUDIES OF IMIDAZOLINE DERIVATIVES. II.¹⁾ AGONISTIC AND ANTAGONISTIC ACTIONS ON α -ADRENOCEPTORS IN VARIOUS SMOOTH MUSCLE PREPARATIONS

KOUCIHI TAKEUCHI, KENICHI AKATSUKA* AND YUTAKA KASUYA**

*Department of Pharmacology, Meiji College of Pharmacy, * 1-35-23, Nozawa, Setagaya-ku, Tokyo, 154, Japan and Department of Chemical Pharmacology, Faculty of Pharmaceutical Science, University of Tokyo, ** Bunkyo-ku, Tokyo, 113, Japan*

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The effects of 8 imidazoline derivatives on α -adrenoceptor-mediated contraction or relaxation of various kinds of smooth muscle preparations were investigated. In rabbit aortic strips, K-6341, K-6343 and K-4011 produced α -agonistic action which was antagonized by prazosin. In the study of α -antagonistic action of imidazoline derivatives, *i.e.* antagonism against norepinephrine- or phenylephrine-induced contraction of the rabbit aortic strip, the order of potency series, prazosin > phentolamine > yohimbine > K-4011, K-3827, K-4300 > tolazoline was found. Reserpine pretreatment did not affect the results. In the study of α -antagonistic effect on the relaxing response to norepinephrine of methacholine-contracted rat ileum, the potency series was revealed as follows: prazosin > phentolamine > yohimbine > K-4011, K-3827, K-4300, tolazoline. A similar experiment was performed on histamine-contracted guinea-pig taenia coli, and the results were nearly identical to the case of the rat ileum, except that K-6341 and K-6343 also exhibited α -antagonistic action which was not detected in the rat ileum. This suggests that α_2 -receptor may exist in the postsynaptic α -adrenoceptor in the guinea-pig taenia coli. The results of the present study indicate that the potency of imidazoline derivatives to inhibit norepinephrine-induced contraction of some smooth muscle is almost identical to that which inhibited norepinephrine-induced relaxation of other smooth muscles. Furthermore, K-4300 was found to possess anti-acetylcholine and anti-histamine actions in the guinea-pig taenia coli and anti-serotonin action in the rat fundus strips.

Keywords — imidazoline derivative; α -adrenoceptor; aortic strip; ileum; taenia coli; fundus strip; rabbit; rat; guinea-pig

INTRODUCTION

It has been shown that the introduction of a methylenedioxy radical to the phenyl ring of imidazoline derivatives increased their affinity to α -adrenoceptors in the rat vas deferens.¹⁾ The stimulation of α -adrenoceptor produces contractions in some smooth muscle preparations (vas deferens, arterial strips, *etc.*) on one hand, and relaxation in other smooth muscle tissues (gastrointestinal smooth muscle, *etc.*) on the other.

Recently, α -adrenoceptor was classified into two subtypes, *i.e.* α_1 - and α_2 -adrenoceptors.^{2,3)} However, such subclassification of α -adrenoceptors has been performed in only restricted tissues, *e.g.* vas deferens, arteries, *etc.* The precise distribution of subtypes of α -adrenoceptors and their functional significance are therefore still to be clarified in various tissues adrenergically innervated. Imidazoline derivatives are conceivably useful tools for investigation to elucidate the characteristics and distribution of α_1 - and α_2 -adrenoceptors.

In this study, in an attempt to clarify α -agonistic and α -antagonistic properties of imidazoline derivatives in various tissues other than the vas deferens, pharmacological analysis was performed on the rabbit aortic strips which contract in response to α -adrenoceptor activation and on the rat intestine and guinea-pig taenia coli which relax in response to α -adrenoceptor activation. In addition, antagonistic effects of imidazoline derivatives against acetylcholine and histamine were examined using the guinea-pig ileum and those against serotonin were investigated using the rat fundus strip.

MATERIALS AND METHODS

The animals used in this study were male Albino rabbits (Japanese White, 2.5–3.5 kg in body weight), male Wistar-strain rats (150–200 g in body weight) and male Hartley-strain guinea-pigs (350–450 g in body weight). Dissected smooth muscle preparations were prepared in an organ bath of 20 ml capacity contain-

ing a modified Krebs-Ringer's solution of following composition (mM): NaCl, 120; KCl, 4.7; MgCl_2 , 1.2; CaCl_2 , 2.2; NaHCO_3 , 25; KH_2PO_4 , 1.2 and glucose, 14. The bathing solution was continuously aerated with 95% O_2 and 5% CO_2 . The contraction and relaxation of the smooth muscle were isotonicity recorded on an electric kymograph.

1. The Rabbit Aortic Strip — The rabbits were sacrificed by a blow on the head and the thoracic aorta was quickly excised. The aortic strip, 3 mm in width and 25–30 mm in length, was prepared by helically cutting the tissue according to the method of Furchgott *et al.*⁴⁾ The strip was prepared in an organ bath containing modified Krebs-Ringer's solution maintained at $37 \pm 1^\circ\text{C}$. A resting tension of 4.0 g was applied to the tissue, and 4 h were allowed for equilibration.

In the first series of experiments, the direct stimulating effects of imidazoline derivatives on the aortic strip were examined. Dose-response curves were determined for those derivatives which exhibited direct stimulating action. In the second series of experiments, antagonistic effects of imidazoline derivatives against norepinephrine and phenylephrine were examined, and pA_2 values were determined. For some experiments, the rabbits were pretreated with reserpine 48 h before sacrifice.

2. The Rat Ileum and Guinea-Pig Taenia Coli — After sacrificing the rat by a blow on the head, the ileum was excised and the intraluminal materials were removed. An ileal strip of about 4 cm length was suspended in the organ bath containing modified Krebs-Ringer's solution. The bathing medium was maintained at $29 \pm 1^\circ\text{C}$. A resting tension of 0.5 g was applied on the tissue and 30 min was allowed for equilibration. In the presence of 10^{-6} M propranolol, a contraction of the ileum was induced by 10^{-6} M methacholine. When the contraction reached a stable state, the tissue was relaxed by cumulatively applying norepinephrine. After washing the tissue with fresh solution several times, the tissue was again contracted by methacholine, one of imidazoline derivatives was applied for 10 min and its effect was observed. Subsequently, in the presence of imidazoline derivatives, norepinephrine was again applied cumulatively to induce relaxation. From the first and second dose-response curves to norepinephrine, pA_2 values of imidazoline derivatives were calculated.

A preparation of the guinea-pig taenia coli, approximately 2 cm in length, was prepared similarly to the rat ileum. A resting tension of 0.5 g was applied to the tissue, and 30 min was allowed for equilibration. After the tissue contraction in response to 10^{-6} M histamine attained a steady state, norepinephrine was cumulatively applied. The subsequent experiments were performed similarly to those for the rat ileal preparation as described above.

3. The Guinea-Pig Ileum and Rat Fundus Strip — The guinea-pig was sacrificed, and the ileum was quickly removed by abdominal incision. A strip of about 3 cm length was suspended in the organ bath containing the modified Krebs-Ringer's solution maintained at $29 \pm 1^\circ\text{C}$. A resting tension of 0.5 g was applied and the tissue was incubated for 30 min for equilibration. The dose-response curves to histamine and acetylcholine were obtained by cumulative application and the effects of imidazoline derivatives on the dose-response curves were investigated.

The rat fundus preparation was made by cutting an oblong strip according to the method of Vane,⁵⁾ and prepared as described above. The bathing medium was maintained at $36 \pm 1^\circ\text{C}$. A resting tension of 1.0 g was applied to the tissue, and 60 min was allowed for equilibration. The effects of imidazoline derivatives on the contractile responses to serotonin, applied cumulatively, were examined.

Materials — The structural formulas of newly synthesized imidazoline derivatives were illustrated in the preceding paper.¹⁾ The following drugs were used in the present study: tolazoline hydrochloride (Fluka); yohimbine hydrochloride (Wako); phentolamine mesylate (CIBA-Geigy); prazosin hydrochloride (Pfizer); propranolol hydrochloride (Inderal® Sumitomo); L-phenylephrine (Tokyo kasei); L-norepinephrine bitartrate (Wako); acetylcholine chloride (Tokyo kasei); methacholine chloride (Tokyo kasei); histamine hydrochloride (Nakarai); serotonin (Wako).

Statistical Methods — The pA_2 and pD_2 values were determined according to the method of van Rossum⁶⁾ Student's *t*-test was used for statistical evaluation of the data.

RESULTS

1. Effects on the Rabbit Aortic Strip

Imidazoline derivative-induced contractile

responses of the aortic strips obtained from normal and reserpine-pretreated rabbits are summarized in Fig. 1. K-6341, K-6343, K-4011, K-3827 and tolazoline exerted contractile effects on the rabbit aortic strip. The effect of K-6341 was the most potent, and its intrinsic activity was 0.84 when the intrinsic activity of norepinephrine was taken as 1. The intrinsic activities of K-6343 and K-4011 were 0.52 and 0.32, respectively. The effects of K-3827 and tolazoline

were only slight. The results obtained on reserpine-treated tissue were qualitatively identical to those with the normal tissues, except for the several-fold leftward shift of the dose-response curves by reserpine treatment.

Prazosin, an α_1 -antagonist, produced a parallel rightward shift of the dose-response curves for K-6341 and K-6343. The effect of prazosin was dose-dependent (Fig. 2).

K-4011, K-3827 and K-4300 showed com-

FIG. 1. Comparison of Imidazoline Derivatives-Induced Contractions in the Isolated Rabbit Aortic Strips. Ordinate: percent of the maximum contraction induced by norepinephrine. Abscissa: molar concentration of agonists. O, norepinephrine; X, phenylephrine; □, K-6341; △, K-6343; ■, K-4011; ▲, K-3827; ●, tolazoline. The results are the mean of 8 experiments; vertical bars show S.E. of the mean.

FIG. 2. Effects of Prazosin on K-6341- and K-6343-Induced Contractions in the Isolated Rabbit Aortic Strips.

Ordinate: percent of the maximum contraction induced by K-6341 or K-6343. Abscissa: molar concentration. O, control; □, prazosin 10^{-9} M; ●, 10^{-8} M; ■, 10^{-7} M. The results are the mean of 7 experiments; vertical bars show S.E. of the mean.

petitive antagonistic action against norepinephrine. The results obtained on the aortic strips from normal and reserpine-pretreated rabbits are presented in Fig. 3 and 4, respectively.

The antagonistic effects of imidazoline derivatives against phenylephrine were almost identical to those against norepinephrine. The potency series as α -antagonists was as follows: prazosin > phentolamine > yohimbine > K-4011, K-3827, K-4300 > tolazoline (Table I). K-6341, K-6343, K-4010, K-6342 and K-4299 showed no appreciable antagonistic effects.

2. Effects on the Rat Ileum and Guinea-Pig *Taenia Coli*

Imidazoline derivatives except for K-6342

produced a weak relaxation in the rat ileal preparation. The effects of tolazoline and K-4300 were approximately 20% and 10% of the maximum relaxing effect of norepinephrine, respectively. The other derivatives showed lesser effects. In the guinea-pig taenia-coli, imidazoline derivatives produced complicated responses, which are roughly classified into 4 patterns as follows: 1) relaxation (K-6341 > K-4011 > K-6343), 2) relaxation followed by contraction (tolazoline, K-3827), 3) contraction (K-4300), and 4) no response (K-4010, K-6342, K-4299).

In the rat ileum, K-4011, K-3827 and K-4300 exerted antagonistic action against norepinephrine: the effects on the dose-response curves are illustrated in Fig. 5. A potency series

FIG. 3. Effects of Imidazoline Derivatives on the Dose-Response Curves to Norepinephrine in the Isolated Rabbit Aortic Strips

Ordinate: percent of the maximum contraction induced by norepinephrine. Abscissa: molar concentration of norepinephrine; norepinephrine alone (○) and in the presence of 10^{-6} M (●), 3×10^{-6} M (□), 10^{-5} M (■) and 3×10^{-5} M (△) imidazoline derivatives. Each point is the mean of 6 experiments.

as α -antagonist prazosin > phentolamine > yohimbine > K-4011, K-3827, K-4300, tolazoline was detected. The potencies of imidazoline derivatives as α -antagonists were almost identical and only slight in extent (Table II).

In addition to the derivatives which were shown to have α -antagonistic action in the rat ileum (K-4011, K-3827, K-4300), K-6341 and K-6343 were detected to have α -antagonistic action in the guinea-pig taenia coli (Fig. 6). The order of potency series was as follows: prazosin > phentolamine > yohimbine, K-6341, K-4011, K-4300, K-3827, K-6343, tolazoline. In the guinea-pig taenia coli, as in the case of the rat ileum, the α -antagonistic effects of imidazoline derivatives were not potent, and among the

derivatives, the extent of potency was similar (Table II).

3. Antagonism against Acetylcholine, Histamine and Serotonin

Figure 7 shows the antagonistic action of K-4300 against acetylcholine and histamine in the guinea-pig ileum and against serotonin in the rat fundus strip. Against acetylcholine, K-4300 showed no effect at a concentration of 10^{-5} M, but exerted noncompetitive type antagonistic action at 10^{-4} M. Against histamine, 10^{-5} and 10^{-4} M K-4300 showed competitive antagonistic action. In the rat fundus strip, 10^{-4} M K-4300 exerted competitive antagonistic action against serotonin. The other imidazoline derivatives showed no antagonistic actions against the

FIG. 4. Effects of Imidazoline derivatives on the Dose-Response Curves to Norepinephrine in the Isolated Aortic Strips from Reserpinized Rabbit

Ordinate: percent of the maximum contraction induced by norepinephrine. Abscissa: molar concentration of norepinephrine; norepinephrine alone ($\circ$) and in the presence of 10^{-6} M ($\bullet$), 3×10^{-6} M, ($\square$), 10^{-5} M ($\blacksquare$) and 3×10^{-5} M, ($\triangle$) imidazoline derivatives. Each point is the mean of 6 experiments.

agonists.

DISCUSSION

In the present study, it was found that some imidazoline derivatives (K-6341, K-6343, K-4011 and K-3827) induced contraction of the rabbit aortic strips. Their effect was not attenuated but potentiated, *i.e.* the dose-response curves were shifted to the left, by pretreatment with reserpine which caused a depletion of endogenous norepinephrine.⁷⁾ Therefore, it is conceivable that the contractile responses of the rabbit aortic strips were elicited by the direct muscletropic action of imidazoline derivatives, and not through the release of endogenous norepinephrine. The leftward shift of the dose-response curve is likely due to supersensitivity of the smooth muscle of the rabbit aorta induced by reserpine pretreatment.⁸⁾ Because their muscletropic action was competitively antagonized by prazosin, the action of imidazoline derivatives is thought to be exerted through activation of postsynaptic α_1 -adrenoceptor.

K-4011, K-3827 and K-4300 showed competitive antagonistic action against norepinephrine and phenylephrine, relatively specific α_1 -agonists.⁹⁾ Their antagonistic action was not affected by reserpine pretreatment, indicating that presynaptic α -adrenoceptor or neurotransmitter do not participate in their antagonistic action.

The results suggest that α -antagonistic action of imidazoline derivatives is also manifested specifically at the level of postsynaptic α_1 -adrenoceptor.

It has been reported that the depolarization, membrane excitation and contraction of the arterial smooth muscle in response to exogenously applied norepinephrine was abolished by α_1 - and α_2 -adrenoceptor antagonists, whereas the electrical events on the cell membrane induced by intramural nerve stimulation were not affected by these antagonists.¹⁰⁾ Thus, they¹⁰⁾ proposed classification of α -adrenoceptors in the arterial smooth muscle into two groups as follows: extrajunctional receptors (α_1 - and α_2 -receptors) and an intrajunctional receptor (tentatively γ -receptor). However, further studies are needed to substantiate this classification.

The existence of α_2 -adrenoceptor, which is now well known to exist on sympathetic nerve terminals (presynaptic α_2 -adrenoceptor), has also been demonstrated in peripheral effector organs, *e.g.* smooth muscles, blood platelet, *etc.* It has been suggested that postsynaptic α_1 - and α_2 -adrenoceptors exist in the rat aorta.^{11,12)} On the other hand, the presence of a postsynaptic α_2 -adrenoceptor has been negated in the rabbit aorta, pulmonary artery and portal vein.^{13,14)} Thus, interpretation concerning the distribution of postsynaptic α_2 -adrenoceptor in the arterial

TABLE I. Comparison of α -Adrenergic Action of Imidazoline Derivatives in Isolated Rabbit Aortic Strips

Compounds	Normal				Reserpinized ^{a)}			
	N ^{b)}	i.a. ^{c)}	pD ₂	pA ₂	N	i.a.	pD ₂	pA ₂
Norepinephrine	1.00				1.00			
Tolazoline	14	+	—	5.70±0.057	19	+	—	5.86±0.076
K-4010	10	—	—	—	8	—	—	—
K-6342	19	—	—	—	20	—	—	—
K-4011	15	0.32±0.046	—	6.22±0.053 ^{d)}	15	0.36±0.025	—	6.24±0.084 ^{d)}
K-6341	13	0.84±0.061	5.68±0.129	—	24	0.86±0.032	6.15±0.068	—
K-3827	11	+	—	6.06±0.072 ^{d)}	15	+	—	6.05±0.068
K-6343	16	0.52±0.041	4.40±0.094	—	24	0.67±0.021	5.07±0.143	—
K-4299	10	—	—	—	13	—	—	—
K-4300	15	—	—	6.01±0.075 ^{d)}	16	—	—	6.09±0.097
Yohimbine	11	—	—	6.65±0.087 ^{d)}	15	—	—	6.71±0.070 ^{d)}
Phentolamine	12	—	—	7.72±0.158 ^{d)}	15	—	—	7.63±0.050 ^{d)}
Prazosin	12	—	—	8.76±0.141 ^{d)}	13	—	—	9.10±0.041 ^{d)}

a) Animal were injected intraperitoneally with reserpine (1.5 mg/kg) 48 h and (1.0 mg/kg) 24 h before the experiments. b) Number of experiments. c) i.a.: intrinsic activity. d) $p < 0.01$ when compared with tolazoline. +: small contraction.

smooth muscle is still controversial and requires classification.¹⁵⁾

The agonistic and antagonistic effects of imidazoline derivatives on α -adrenoceptors in the rabbit aortic strips in the present study were almost identical to those only on postsynaptic α_1 -adrenoceptors in the rat vas deferens.¹⁾ It can be assumed therefore that the rabbit aorta is a preparation exhibiting only or preferentially α_1 -adrenoceptor. Thus, the results of our study are in agreement with those reported by Docherty *et al.*^{13,14)}

From the action of imidazoline derivatives on α_1 -adrenoceptor in the rabbit aortic strips, the following structure-activity relationship was depicted: 1) the derivatives in which the methyl-

enedioxy radical is introduced to the 2' and 3' positions of their phenyl ring possess more potent α_1 -antagonistic effect than those having the methylenedioxy radical at the 3' and 4' positions, and 2) the derivatives become more potent α_1 -antagonists when the imidazoline ring is connected with a phenyl ring *via* a methylene residue, compared with an amino residue. This structure-activity relationship was virtually similar to that obtained in the rat vas deferens.¹⁾

K-4011, K-4300 and K-3827 exerted competitive antagonizing action against norepinephrine in the rat ileum and guinea-pig taenia coli presumable on the postsynaptic α_1 -adrenoceptor, because these derivatives were shown to have α_1 -antagonistic action in the rabbit aorta

FIG. 5. Effects of Imidazoline derivatives on the Dose-Response Curves to Norepinephrine in the Isolated Rat Ileum

Ordinate: percent of the maximum relaxation by norepinephrine of methacholine (10^{-6} M)-contracted rat ileum. Propranolol (10^{-6} M) presented in all experiments. Abscissa: molar concentration of norepinephrine; norepinephrine alone ($\circ$) and in the presence of 3×10^{-5} M ($\blacksquare$) and 10^{-4} M ($\square$) imidazoline derivatives. Each point is the mean of 6–8 experiments.

FIG. 6. Effects of Imidazoline Derivatives on the Dose-Response Curves to Norepinephrine in the Isolated Guinea Pig *Taenia Coli*

Ordinate: percent of the maximum relaxation by norepinephrine of histamine (10^{-6} M)-contracted tissue. Abscissa: molar concentration of norepinephrine; norepinephrine alone (○) and in the presence of 10^{-5} M (●) and 10^{-4} M (□) imidazoline derivatives. Each point is the mean of 6–8 experiments.

TABLE II. Summary of pA_2 Values of Imidazoline Derivatives

Compounds	<i>N</i> ^{a)}	Mean $\pm$ S.E. (α -Blocking activity)		
		Ileum ^{b)}	<i>N</i>	<i>Taenia coli</i> ^{c)}
Tolazoline	7	4.67 ± 0.162	15	5.07 ± 0.116
K-4010	6	—	18	—
K-6342	8	—	16	—
K-4011	6	5.04 ± 0.066	14	5.34 ± 0.102 ^{d)}
K-6341	6	—	16	5.44 ± 0.077 ^{d)}
K-3827	6	5.03 ± 0.085	16	5.24 ± 0.076
K-6343	6	—	17	5.07 ± 0.053
K-4299	7	—	18	—
K-4300	8	4.73 ± 0.062	14	5.25 ± 0.101
Yohimbine	8	5.35 ± 0.064 ^{d)}	10	5.53 ± 0.094 ^{d)}
Phentolamine	8	6.49 ± 0.057 ^{d)}	15	6.95 ± 0.076 ^{d)}
Phazosin	8	7.30 ± 0.062 ^{d)}	15	7.89 ± 0.082 ^{d)}

a) Number of experiments. b) Propranolol (10^{-6} M) present in all experiments. pA_2 values calculated from relaxation of norepinephrine on methacholine (10^{-6} M) induced contraction in isolated rat ileum. c) pA_2 values calculated from relaxation of norepinephrine on histamine (10^{-6} M) induced contraction in isolated guinea pig *taenia coli*. d) $p < 0.01$ when compared with tolazoline.

FIG. 7. Antagonism of K-4300 against Acetylcholine (a), Histamine (b) and Serotonin (c) in the Guinea Pig Ileum (a, b) and the Rat Fundus Strip (c)

Ordinate: percent of the maximum contraction induced by each agonist. Abscissa: molar concentration of agonist; agonist alone ($\circ$) and in the presence of 10^{-5} M ($\bullet$) and 10^{-4} M ($\square$) K-4300. The results are the mean of 6–9 experiments; vertical bars show S.E. of the mean.

(above-described) and the rat vas deferens.¹¹ Taking antimuscarinic and anti-histamine action of K-4300 (*vide infra*) into consideration, α_1 -antagonistic effect of K-4300 might be underestimated to some extent. It is interesting to note that K-6341 and K-6343 also exerted competitive antagonizing action against norepinephrine in the guinea-pig taenia coli. These two derivatives were found to exert α_2 -antagonistic, but not α_1 -antagonistic, action in the rat vas deferens.⁹ Some part of the action of K-3827 might also be exerted on α_2 -adrenoceptor, because it was shown in the rat vas deferens that K-3827 was a considerably selective α_2 -antagonist. Therefore, it can be suggested that, in the guinea-pig taenia coli, postsynaptic α_2 -adrenoceptor may coexist with α_1 -adrenoceptor. This is supported by our preliminary experiment, where it was detected that clonidine, a selective α_2 -agonist, produced relaxation of histamine-contracted taenia-coli dose-dependently and was competitively antagonized by yohimbine, a selective α_2 -antagonist (unpublished observation).

In addition to the effects on presynaptic and postsynaptic α -adrenoceptors, K-4300 showed antagonistic action against acetylcholine and histamine in the guinea-pig taenia coli and against serotonin in the rat fundus strip. Thus, K-4300 was shown to exhibit a broad spectrum of actions in various smooth muscle preparations. The investigation concerning structure-activity rela-

tionship for the action of imidazoline derivative on a variety of drug receptors is an intriguing subject to be elucidated in the future.

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