PHARMACOLOGICAL STUDIES OF IMIDAZOLINE DERIVATIVES. III^{1,2)} ACTIONS ON CARDIOVASCULAR SYSTEM AND ACUTE TOXICITY

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(Received September 11, 1985)

The effects of 8 imidazoline derivatives on the cardiovascular system and their acute toxicity were investigated. In anesthetized rats, K-6341 and K-6343 produced relatively long-lasting elevation of blood pressure. This hypertensive response was prevented by prazosin. In contrast, K-4011, K-3827 and K-4300 exerted long-lasting hypotensive actions. Norepinephrine-induced increase in blood pressure was competitively antagonized by these three derivatives. Therefore, the hypertensive and hypotensive actions of imidazoline derivatives are conceivably exerted by activating and blocking α_1 -adrenoceptors. In the isolated guinea-pig atrial preparations, K-4011, K-3827, K-6341 and K-6343 produced positive inotropic responses which were independent of β -adrenoceptor activation. The positive inotropic effect of K-6341, which was the most potent, was specifically attenuated or abolished by diltiazem, suggesting that a change in Ca^{2+} movement across the cell membrane is contributing to K-6341-induced increase in atrial contractions. The main symptoms of mice manifested when injected with imidazoline derivatives were a decrease in spontaneous movement, exophthalmos, an increase in palpebral opening, paralysis of four limbs and respiratory depression. Acute LD_{50} values of imidazoline derivatives in mice (*i.v.*, *i.p.*) were between those of tolazoline and naphazoline.

Keywords — structure-activity relationship; imidazoline derivative; blood pressure; atria; α -adrenoceptor; acute toxicity; rat; guinea-pig; mouse

INTRODUCTION

Since the development of clonidine as an antihypertensive drug, much attention has been given to the effects of imidazoline derivatives on blood pressure.³⁾ Clonidine usually exerts biphasic action on the blood pressure. That is, it first produces a temporal increase in blood pressure and, subsequently, long-lasting hypotension accompanied by a bradycardia. It has been suggested that the long-lasting hypotension is due to a decrease in the tonic activity of peripheral sympathetic nerves which in turn is presumably due to the stimulation of α -adrenoceptor in the vasomotor center of the medulla oblongata by clonidine.^{4,5)}

On the other hand, tolazoline produced complicated responses in blood pressure. Tolazoline has been known as an α -antagonist^{6,7)} and, in contrast, it was shown to potentiate sympathetic nerve stimulation-induced responses. Tolazoline-induced hypotension has been reported in dogs,⁸⁾ cats⁹⁾ and rats.¹⁰⁾ Benfey *et al.*¹¹⁾ observed, however, that tolazoline showed hypertensive action in approximately 50% of

the examined dogs and in all reserpine-pretreated dogs. A hypertensive action of tolazoline was also detected in reserpine-pretreated cats.¹²⁾ Naphazoline, an α -agonist, has been shown to exert hypertensive action in dogs,¹³⁾ cats¹⁴⁾ and rats.¹⁰⁾ Complicated responses to imidazoline derivatives appear to have resulted from their actions on α_1 - and α_2 -adrenoceptors.

In the present study, the effects of 8 newly synthesized imidazoline derivatives on the cardiovascular system were investigated to elucidate the mechanisms of actions relating to their effect on blood pressure in rats. An acute toxicity of imidazoline derivatives were also examined in mice.

MATERIALS AND METHODS

1. Measurement of Blood Pressure — Male Wistar-strain rats (300–500 g in body weight) were placed on a fixing board on their backs under anaesthesia with urethane (1.2 g/kg, *i.p.*). The systemic blood pressure was measured by a pressure transducer (Nihon Koden MPU-0.5) through a polyethylene catheter inserted into

the right carotid artery. The mean blood pressure was also measured using another amplifier through the same pressure transducer. The heart rate was monitored with a pulse rate tachometer (Nihon Kodon, RT-5). The measurement of respiration was performed with a thermistor pick up (Nihon Kodon, TR-612T) placed in a polyethylene tube inserted into the trachea. These were all recorded continuously on an ink-writing oscillograph (Nihon Kodon, RM-85).

When the experiment was performed in vagotomized rats, it was started at least 1 h after bilaterally cutting the vagus nerves at the cervical level.

Imidazoline derivatives at doses of 0.03, 0.1, 0.3 and 1.0 mg/kg were administered, and their effects on the blood pressure were observed. Those derivatives showing hypertensive actions were examined as to whether an antagonism by prazosin (0.1 mg/kg) was exhibited. The antagonistic effects of imidazoline derivatives against blood pressure-increasing action of norepinephrine were studied as follows: norepinephrine at each dose (1, 3, 10 and 30 μ g/kg) was first administered at random at an interval of 10 min. About 30 min later, the administration of norepinephrine was repeated in the presence of one dose of imidazoline derivatives (0.3 or 1.0 mg/kg).

The concentrations of administered solution of drugs including imidazoline derivatives were adjusted to a final volume of 0.5 ml/kg with saline. The drug solution was intravenously administered through a polyethylene catheter inserted into the femoral vein.

2. Studies on the Guinea-Pig Atrial Preparation

— Male Hartley-strain guinea-pigs (350–450 g in body weight) were killed by a blow on the head, and the heart was immediately dissected out. In an oxygenated solution, the atrial preparation consisting of both right and left atria was made by carefully detaching from the ventricle. The spontaneously beating atrial preparation was prepared in an organ bath of 20 ml capacity containing a modified Krebs-Ringer's solution of the 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 medium was maintained at $37 \pm 1^\circ C$ and aerated with 95% O_2 and 5% CO_2 . A resting tension of 1.0 g was applied to the tissue, and 1 h was allowed for equilibration. Isometric contraction of the

atria was recorded on an ink-writing oscillograph (Nihon Kodon, RM-85) through a force-displacement transducer (Nihon Kodon, TB-611T), and the heart rate was monitored by means of a pulse rate tachometer (Nihon Kodon, RT-5).

The chronotropic and inotropic effects of imidazoline derivatives (10^{-5} M) were first examined and, next, the effects on the action of isoproterenol (10^{-9} – 10^{-7} M). The influence of diltiazem was examined on the effect of imidazoline derivatives which showed relatively potent positive inotropic action.

3. *Acute Toxicity* — For the study of acute toxicity, male ddY-strain mice (20 g in body weight) were used. Imidazoline derivatives were administered intravenously in one series and intraperitoneally in another series of experiments. After drug administration, the behavior of animals was carefully observed for up to 3 d. LD_{50} values were determined according to the up and down method.¹⁵⁾

Materials — The structural formula of newly synthesized imidazoline derivatives were illustrated in the preceding paper.¹⁾ The following drugs were used in the present study: tolazoline hydrochloride (Fluka); prazosin hydrochloride (Pfizer); yohimbine hydrochloride (Wako); L-norepinephrine bitartrate (Wako); isoproterenol hydrochloride (Tokyo kasei); nephazoline hydrochloride (Ciba-Geigy); histamine hydrochloride (Nakarai), and diltiazem hydrochloride (Herbesser® Tanabe).

Statistical Methods — Student's *t*-test was used for statistical evaluation of the data.

RESULTS

1. Effects on Rat Blood Pressure

K-6341 and K-6343 increased and K-4011, K-3827 and K-4300 decreased the rat blood pressure. These responses were rapid and persisted for 20–30 min after *i.v.* injection. As typical examples, hypertensive response to K-6341 and hypotensive response to K-4011 are presented in Fig. 1. The heart rate was slightly decreased by K-6343 and increased by K-4011. No influence on the respiration was detected.

The hypertensive effects of K-6341 and K-6343 were dose-dependent, and were almost completely prevented by prazosin (Fig. 2).

K-4011, K-3827 and tolazoline produced a rapid fall in the blood pressure, which continued

for a considerably long time. Compared with the hypotensive effects of these 3 compounds, the effect of K-4300 was a slightly weaker. These effects were dose-dependent and were not affected by vagotomy (Fig. 3). The hypotensive effect of yohimbine is also shown in Fig. 3.

The antagonistic action of imidazoline derivatives against hypertensive action of norepinephrine was examined, and the effects on the dose-response curves to norepinephrine are presented

in Fig. 4. As can be seen in this figure, the dose-response curve was shifted in parallel to the right. The effect of K-4011 appeared to be most potent.

The other imidazoline derivatives, K-4010, K-6342 and K-4299 showed neither effects on the blood pressure nor on hypertensive response to norepinephrine.

2. Effects on the Guinea-Pig Atria

When imidazoline derivatives, K-4011,

FIG. 1. Typical Records Showing the Influence of K-4011 (1 mg/kg i.v.) and K-6341 (0.3 mg/kg) on Blood Pressure (B.P.), Heart Rate (H.R.) and Respiration (Resp.) of the Anesthetized Rat
M.B.P.: mean systemic blood pressure.

FIG. 2. Effects of Prazosin (0.1 mg/kg i.v.) on Hypertensive Action of K-6341 and K-6343 in Vagotomized Rats

Hypertensive actions of norepinephrine (Nor-E), K-6341 and K-6343 before (○) and after (●) treatment with prazosin. Ordinate: mean blood pressure (mmHg). Abscissa: dose of Nor-E and K-6341 or K-6343. Each point represents the mean blood pressure obtained from more than 6 experiments and the vertical bars represent standard error.

K-3827, K-6341, K-6343 and tolazoline (10^{-5} M) were applied to the isolated guinea-pig atria, the contractile force of the atrial muscle was increased by about 0.5 g tension without change in heart rate. These effects were not affected by propranolol (10^{-6} M), indicating that the positive inotropic actions of these imidazoline derivatives were not exerted through β -adrenoceptor activation. In the presence of diltiazem (10^{-7} M), a potent calcium antagonist, the positive inotropic effects on imidazoline derivatives were abolished almost completely, whereas positive inotropic and chronotropic effects of isoproterenol were scarcely affected. Typical examples of the traces with K-6341 are shown in Fig. 5. Diltiazem at higher concentrations (10^{-6} M) suppressed the cardiac function as well as the effects of isoproterenol (Fig. 5). In the presence of 10^{-5} M diltiazem, the contraction and heart rate were further suppressed and the atria were finally arrested (data not shown).

None of imidazoline derivatives (10^{-5} M) showed antagonistic action against both positive chronotropic and inotropic effects of isoproterenol.

3. Acute Toxicity

LD₅₀ values of imidazoline derivatives in mice following intravenous and intraperitoneal administration are collectively shown in Table I.

In every case, the death of animals occurred within 30 min and 60 min after intravenous and intraperitoneal administration, respectively. The

death was most probably due to severe respiratory depression. The other main toxic symptoms detected in mice were a decrease in spontaneous movement, exophthalmos, increase in palpebral opening, and paralysis of four limbs. Other than these symptoms, a tremor was observed after K-6342 administration and, further, abnormal gait and piloerection were seen after naphazoline administration.

The surviving animals were in a sedated state at around 60 min, but exhibited almost normal behavior 6 h after the administration of imidazoline derivatives. During the observation period for up to 3 d, there detected no death or abnormal behavior occurred.

DISCUSSION

In the present study, K-6341 and K-6343 showed a blood pressure-elevating action which was long-lasting. In the rabbit aortic strips, a preferential action of K-6341 and K-6343 on postsynaptic α_1 -adrenoceptor *in vitro* has been shown.²⁾ Furthermore, because K-6341 was shown to have a fairly potent antagonistic action on presynaptic α_2 -adrenoceptor in the rat vas deferens,¹⁾ an acceleration of norepinephrine release from vascular adrenergic nerves is conceivable and in part contributing to its hypertensive action. In either case, K-6341- and K-6343-induced elevation in blood pressure was abolished by prazosin, a selective α_1 -adrenoceptor antagonist. This indicates that the hypertensive

FIG. 3. Comparative Illustration of Hypotensive Actions of K-4011, K-3827, K-4300, Tolazoline and Yohimbine in Anesthetized Normal and Vagotomized Rats

Each column represents the decrease in the mean blood pressure obtained from more than 7 experiments. □, tolazoline; ▤, K-4011; ▨, K-3827; ▩, K-4300; ▨, yohimbine. Ordinate: mean blood pressure in mmHg. Abscissa: dose of K-4011, K-3827, K-4300, tolazoline and yohimbine (mg/kg i.v.). Standard errors of the mean are shown as vertical bars.

action of these imidazoline derivatives was exerted through direct and indirect activation of postsynaptic α_1 -adrenoceptor.

In the isolated guinea-pig atrial preparation, K-6341 and K-6343 exerted a positive inotropic action which was independent of β -adrenoceptor stimulation. Although we did not examine the isolated rat heart, positive inotropic effect was not detected in the blood pressure measurement in the rat, *i.e.* only a decrease in pulse pressure was seen following administration of K-6341 (Fig. 1). This was presumably due to the counteraction by the negative inotropic effect of the parasympathetic nerve which was by reflex activated by the increase in blood pressure. This argument is in agreement with the

fact that K-6341-attenuated pulse pressure was increased by vagotomy (data not shown). A decrease in heart rate following K-6341 administration also supports this view.

In the isolated guinea-pig atrial preparations, K-4011, K-3827, K-6341 and K-6343 produced positive inotropic responses which were independent of β -adrenoceptor activation. The positive inotropic effect of K-6341, which was the most potent, was specifically attenuated or abolished by diltiazem, suggesting that a change in Ca^{2+} movement across the cell membrane contributes to K-6341-induced increase in atrial contractions.

K-4011 and K-3827 exerted a potent and long-lasting hypotensive action in rats. Their action was scarcely affected by vagotomy, indicating that parasympathetic nerve excitation was not involved with the hypotensive effect of these imidazoline derivatives. These results are rather surprising, because K-4011 and K-3827 were demonstrated in the rat vas deferens to exert a more selective antagonistic action on presynaptic α_2 -adrenoceptor compared with postsynaptic α_1 -adrenoceptor. The ratio in K_B values were 1 or 2 orders in magnitude.¹⁾ From this relative selectivity, it can be assumed that K-4011 and K-3827 may increase norepinephrine release from sympathetic nerve terminals by their prominent blocking action on α_2 -adrenoceptor which is an important site for negative feedback regulation of norepinephrine release. Nevertheless, the situation *in vivo* seems quite different, *i.e.* the hypotensive effect appeared predominantly, or even be exclusively, due to postsynaptic α_1 -antagonistic and/or α_2 -antagonistic action of the imidazoline derivatives. This is also true for the case of yohimbine, a well known selective α_2 -antagonists. It can be speculated, therefore, that the blocking effect of postsynaptic α_1 -adrenoceptor and/or α_2 -adrenoceptor, especially on the blood pressure, ultimately overcomes the other various effects. Similar arguments can be applied to the case of K-4300 and tolazoline.

The effects of K-6341 were quite unique in that it acted as an agonist on α_1 -adrenoceptors but as an antagonist on α_2 -adrenoceptor.^{1,2)} It can generally be considered that currently known α -adrenoceptor antagonists exerted antagonistic action on α_1 - and/or α_2 -adrenoceptor with no agonistic action and furthermore, α -

FIG. 4. The Inhibitory Effects of K-4011, K-3827, K-4300, Tolazoline and Yohimbine (1 mg/kg *i.v.*) on the Pressor Response to Norepinephrine in an Anesthetized Rat

The compounds and norepinephrine were given through the femoral vein. $\circ$, control; $\bullet$, tolazoline; $\square$, K-4011; $\times$, K-3827; $\triangle$, K-4300; $\blacksquare$, yohimbine. Ordinate: increase in mean blood pressure (mmHg). Abscissa: dose of norepinephrine ($\mu\text{g/kg}$ *i.v.*). Each point represents the mean value obtained from more than 7 experiments and the vertical bars represent standard error.

adrenoceptor agonists exerted only agonistic rather than antagonistic action on α_1 - and/or α_2 -adrenoceptor. It has been reported recently that cirazoline showed α_1 -agonistic and α_2 -antagonistic action.¹⁶⁾ However, these results were not obtained from the same tissue, and Docherty *et al.*¹⁷⁾ negated this in the experiment on the rat vas deferens. Chapleo *et al.*¹⁸⁾ have

reported that RX 77171 acted as an antagonist on presynaptic (α_2) adrenoceptor and as an agonist on postsynaptic (α_1) adrenoceptor. These authors also reported that RX 801074 acted as α_2 -agonist in the rat vas deferens, whereas it acted as α_2 -antagonist in the guinea-pig ileum, although its action was variable depending on the tissues examined. Furthermore, Goldstein *et*

FIG. 5. The Effects of K-6341 on the Atrial Contractile Force and on the Action of Isoproterenol (Upper) and the Effects of Diltiazem on the Increase in Atrial Contractile Force (A.C.F.) Induced by K-6341 and Isoproterenol (Lower) in Isolated guinea-Pig Atria
H.R.: heart rate. Iso., isoproterenol.

TABLE I. Acute Toxicity of Imidazoline Derivatives in male Mice

Compounds	LD ₅₀ value ^{a)} (mg/kg)	
	<i>i.v.</i>	<i>i.p.</i>
K-4010	74.1	98.2
K-6342	53.1	65.3
K-4011	44.4	119.1
K-6341	41.7	120.3
K-3827	31.2	100.0
K-6343	33.0	124.5
K-4299	59.2	195.0
K-4300	36.7	88.9
Tolazoline	56.7	142.6
Naphazoline	18.4	87.8

a) Up and down method.

*al.*¹⁹⁾ have demonstrated that RX 781094 showed clonidine-like α_2 -agonistic action at relatively low concentrations and α_2 -antagonistic action at high concentrations. The subject of the agonistic and antagonistic effects of imidazoline derivatives on α_1 - and α_2 -adrenoceptors in various tissues would be an interesting to pursue to develop drugs possessing a new spectrum of pharmacological actions.

The acute toxicity of imidazoline derivatives used in the present study was generally low, that is, the LD₅₀ values (*i.v.*) of these derivatives were between those of tolazoline and naphazoline.

Among the imidazoline derivatives examined, K-6341 is particularly noteworthy because it directly stimulates postsynaptic α_1 -adrenoceptor, leading to a vasoconstriction, and blocks presynaptic α_2 -adrenoceptor, resulting in an increase in norepinephrine release. Therefore, further investigation of the mechanisms of action of such drugs as K-6341 will provide important information for therapeutic control of hypotension.

REFERENCES

- 1) K. Takeuchi, K. Akatsuka, K. Goto and Y. Kasuya: Pharmacological studies of imidazoline derivatives. I. A comparison of the effect on pre- and postsynaptic α -adrenoceptors in the rat vas deferens, *J. Pharmacobio-Dyn.*, **9**, 375–384 (1986).
- 2) K. Takeuchi, K. Akatsuka and Y. Kasuya: Pharmacological studies of imidazoline derivatives. II. Agonistic and antagonistic actions on α -adrenoceptors in various smooth muscle preparations, *J. Pharmacobio-Dyn.*, **9**, 385–394 (1986).
- 3) J. A. Wilber: Clonidine, an introduction, *J. Cardiovas. Pharmacol.*, **2**, S1–S3 (1980).
- 4) P. B. M. W. M. Timmermans and P. A. Van Zwieten: Central and peripheral α -adrenergic effects of some imidazolines, *Eur. J. Pharmacol.*, **45**, 229–236 (1977).
- 5) W. Kobinger and L. Pichler: Investigation into some imidazoline compounds, with respect to peripheral α -adrenoceptor stimulation and depression of cardiovascular centers, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **291**, 175–191 (1975).
- 6) R. P. Ahlquist, R. A. Huggins and R. A. Woodbury: The pharmacology of benzyl-imidazoline (Priscol), *J. Pharmacol. Exp. Ther.*, **89**, 271–288 (1947).
- 7) M. Nickerson: The pharmacology of adrenergic blockade, *Pharmacol. Rev.*, **1**, 27–101 (1949).
- 8) M. Hartman and H. Isler: Chemische Konstitution und pharmacologische Wirksamkeit von in 2-Stellung substituierten Imidazoline, *Arch. Exp. Pathol. Pharmacol.*, **192**, 141–154 (1939).
- 9) D. Chess and F. F. Yonkman: Adrenolytic and sympatholytic actions of priscol (benzylimidazoline), *Proc. Soc. Exp. Biol. Med.*, **61**, 127–130 (1946).
- 10) H. H. Maling, A. K. Cho, Z. Horakova and M. A. Williams: The pharmacologic effects of ST-155 (Catapres) and related imidazolines in the rat, *Pharmacology*, **2**, 337–351 (1969).
- 11) B. G. Benfey and D. R. Varman: On the hypertensive action of tolazoline and hydergine, *Can. J. Biochem. Physiol.*, **41**, 941–946 (1963).
- 12) B. G. Benfey and D. R. Varman: Vasoconstrictor action of tolazoline, *Br. J. Pharmacol. Chemother.*, **22**, 66–71 (1964).
- 13) B. N. Craver, H. F. Chase and F. F. Yonkman: Pharmacologic studies of a new vasoconstrictor: 2-Naphthyl-(1')-methyl-imidazoline hydrochloride (Privine or naphazoline). II. Vascular and respiratory reactions in the anesthetized dog, *J. Pharmacol. Exp. Ther.*, **82**, 275–287 (1944).
- 14) F. F. Yonkman, B. Rennick and H. L. Schwerma: Pharmacologic studies of a new vasoconstrictor: 2-Naphthyl-(1')-methyl-imidazoline hydrochloride (Privine or naphazoline). III. An attempt to localize the seat of action in terms of adrenergic and cholinergic foci, *J. Pharmacol. Exp. Ther.*, **84**, 197–202 (1945).
- 15) K. Takahashi and N. Okuda: Acute toxicity test, "Environmental Toxicology: Method and Safety Evaluation," ed. by Y. Shirasu and O. Mathuoka, Softscience, Inc., Tokyo, 1975, pp. 131–161.
- 16) R. R. Ruffolo and J. E. Waddell: Receptor interactions of imidazoline IX. Cirazoline is an α_1 -adrenergic agonist and an α_2 -adrenergic antagonist, *J. Pharmacol. Exp. Ther.*, **222**, 29–36 (1982).
- 17) J. R. Docherty and J. C. McGrath: The actions of cirazoline on the rat vas deferens, *Br. J. Pharmacol.*, **82**, 9–14 (1984).
- 18) C. B. Chapleo, J. C. Doxey, L. W. Frank, P. L. Meyers, A. G. Roach, C. F. C. Smith and N. K. Virdee: Comparison of the α -adrenoceptor profiles of clonidine and two oxygenated arylamino imidazolines, *Eur. J. Pharmacol.*, **91**, 123–128 (1983).
- 19) J. M. Goldstein, L. C. Knobloch and J. B. Malick: Electrophysiological demonstration of both α_2 -agonist and antagonist properties of RX 781094, *Eur. J. Pharmacol.*, **91**, 101–105 (1983).