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Effects of Higenamine on the Contractility of Aorta and Taenia Coli in Guinea-pigs[+]

Sang Bok Suk, Kwang Wook Ko, Sang Jin Lee,*
Sang-Ik Hwang* and Ki-Whan Kim*

*Department of Pediatrics and Department of Physiology,**
Seoul National University College of Medicine, Seoul 110-460, Korea

=Abstract=**Higenamine isolated from *Aconiti tuber* is known to have positive inotropic action through the adrenergic β-receptors. However, the effects of higenamine on the vascular smooth muscle and taenia coli have not yet been elucidated. The actions of higenamine were investigated on the activated aortic strips of guinea-pigs and rabbits by norepinephrine, high K-Tyrode's solution and electrical field stimulation, and also on the spontaneous contractions of guinea-pig taenia coli. Electrical activities and mechanical contractions were simultaneously recorded using the conventional suction-electrode method and single sucrose gap technique. All experiments were performed in tris-buffered Tyrode's solution which was aerated with 100% O_2 and kept at 35℃.**

Higenamine suppressed dose-dependently the norepinephrine-induced contraction. Propranolol potently antagonized the inhibitory effects of higenamine on rabbit aorta, while less potently on guinea-pig aorta. Higenamine seemed to suppress profoundly the component of intracellular Ca^{2+} mobilization in the contraction curve of norepinephrine in guinea-pig aorta. Higenamine did not seem to suppress the component of Ca^{2+} influx through the potential-sensitive Ca^{2+} channel in K-contracture of guinea-pig aorta. Higenamine suppressed both passive resting tension and active tension (phasic contraction) driven by electrical field stimulation (A.C. 60 Hz, 5-7 V/cm, duration 10 sec, interval 3 min) in guinea-pig aorta. Propranolol also rapidly suppressed both passive resting and active tensions. Higenamine dose-dependently suppressed the frequency and amplitude of spontaneous contraction, resulting in complete abolishment of it above 10^{-6} M concentration in guinea-pig taenia coli. Propranolol almost completely antagonized its effect. Higenamine reduced dose-dependently both burst frequency and spike frequency, and depolarized membrane potential in guinea-pig taenia coli. Propranolol almost completely blocked its effect.

In conclusion the inhibitory action of higenamine to the norepinephrine-induced contraction might be produced by the depression of intracellular Ca^{2+} mobilization and Ca^{2+}-influx through β-adrenoceptors in guinea-pig aorta. The Ca^{2+}-influx through the potential-sensitive Ca^{2+} channel might not be suppressed by higenamine in guinea-pig aorta. The inhibitory effects of higenamine on the spontaneous contractions of guinea-pig taenia coli result from the decrease of burst frequency and spike frequency through the β-adrenoceptors.

Key Words: *Higenamine, Aorta, Taenia coli, Norepinephrine-induced contraction, Electrical field stimulation, K-contracture, Spontaneous contraction*

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INTRODUCTION

Higenamine is a substance which was isolated from *Aconiti tuber* and named by Kosuge *et al.* in 1978. It is now known as $C_{16}H_{17}NO_3 \cdot HCl$ with a structure of dl-1-(4-hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetra-hydroisoquinoline hydrochloride.

The excitatory mechanism of higenamine was reported to be the inhibition of Na-K pump like digitalis (Shin *et al.* 1976). The inotropic action was additively determined by the extracellular calcium concentration (Hong *et al.* 1980). The positive inotropic action of higenamine on the rabbit left atrium was reported to be due to the potentiation of calcium influx through the cell membrane (Chang *et al.* 1981). In the electrophysiological study of rabbit papillary muscle it was reported that higenamine increased the slow inward calcium currents (Kwon *et al.* 1981). The action of higenamine studied on calcium transport and ATPase activity in the sarcoplasmic reticulum (SR) was explained as the inhibition of Mg-dependent Ca-ATPase that is essential for calcium transport into the SR as well as the potentiation of calcium release from the SR, resulting in the increase of the intracellular calcium concentration (Kim *et al.* 1982).

In the study of the effect of higenamine on the adrenaline receptors of the cardiovascular system in rabbits, the inotropic action of higenamine was explained as the stimulation of β-adrenoceptors (Park *et al.* 1984; Kim *et al.* 1986). Higenamine increased the heart rate by increasing sodium current in the SA node of rabbits (Cho *et al.* 1986). Higenamine was also reported to act on the calcium current rather than the potassium current (Rhee *et al.* 1987). In in-vivo study of rabbits (Kim *et al.* 1986) higenamine decreased blood pressure and total peripheral resistance, and increased cardiac output and heart rate. These effects were all blocked by pretreatment with propranolol. Atenolol also lowered cardiac output and heart rate, and hexamethonium lowered the heart rate and increased blood pressure. Most investigations concerning higenamine were focused on its effects on the heart. However, the effects of higenamine on smooth muscle contractility must be elucidated, in order to understand the higenamine-induced responses to the cardiovascular system.

This study was undertaken to clarify the effects of higenamine on the Ca^{2+} transport through the smooth muscle membrane using aorta and taenia coli.

MATERIALS AND METHODS

Albino guinea-pigs of either sex, weighing 300 g, were stunned and bled. Firstly, the taenia coli were excised from the large intestine and then the thoracic aorta from the thoracic cavity. The excised tissues were managed to be used as experimental preparation in phosphate-buffered Tyrode's solution at room temperature. The experiments were done in tris-buffered Tyrode's solution at 35℃.

The ionic compositions of phosphate-buffered and tris-buffered Tyrode's solutions were as follows respectively (mM): phosphate-buffered Tyrode's, NaCl 147, KCl 4, $CaCl_2 \cdot 2H_2O$ 2, $MgCl_2 \cdot 6H_2O$ 1.05, $NaH_2PO_4 \cdot 2H_2O$ 0.42, $Na_2HPO_4 \cdot 12H_2O$ 1.81, glucose 5.5; tris-buffered Tyrode's, NaCl 147, KCl 4, $CaCl_2 \cdot 2H_2O$ 2, $MgCl_2 \cdot 6H_2O$ 1.05, tris · HCl 5, glucose 5.5. The solutions were aerated with 100% O_2 and pH of the solution was maintained at 7.35.

1) The strips of thoracic aorta

Pure thoracic aorta was isolated from peripheral connective tissue in the preparation chamber and was made into a long strip by cutting helically (helical strip). The strip, 10—12 mm long and 1—2 mm wide was recovered at room temperature for 1 hour. The mechanical contractions were recorded in a vertical chamber (capacity, 100 ml) by Grass FT-03 force transducer connected to a Device physiograph.

Before the main experiment the optimal length of the strip was determined from the length-tension curve of the contractions evoked by electrical field stimulation (A.C. 60 Hz, 5-7 V/cm, duration 10 sec, interval 3 min). The basal tension was about 500 mg. High K-Tyrode's solution used in K-contracture experiment was made by replacing sodium ion with potassium ion isotonically.

2) The taenia coli

The taenia coli (longitudinal muscle) was isolated from the remaining circular muscle completely in

the preparation chamber and was trimmed to the experimental strip which was 10 mm long, 2 mm wide and 5 mg in wet weight.

The mechanical contractions were recorded by the same method as the aortic strip except the electrical field stimulation because taenia coli has spontaneous contractions.

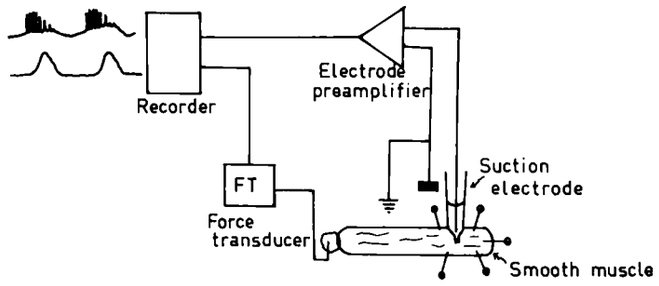

Fig. 1. A schematic representation of the isometric contraction and electrical activity recording system. The isometric contractions were recorded through a tension transducer from the smooth muscle preparation. The electrical activities were measured extracellularly by use of suction electrode.

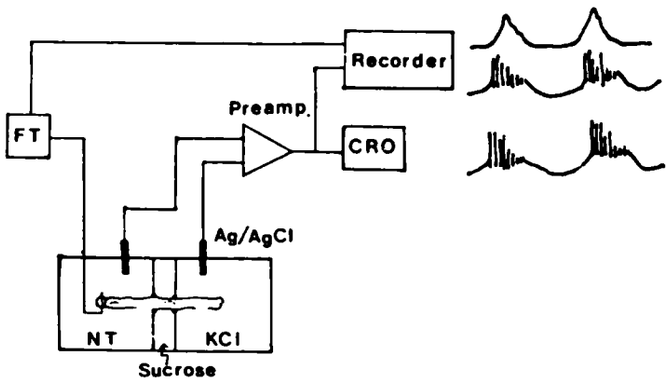

Fig. 2. Diagram of the single sucrose-gap apparatus. The muscle chamber consisted of three compartments; NT for perfusion of normal Tyrode's or test solution, sucrose compartment for perfusion of isoosmotic sucrose solution, and KCl compartment for perfusion of isotonic KCl Tyrode's solution. A muscle strip is mounted as shown, one end (KCl compartment) fixed and the other (NT compartment) connected to a force transducer. The width of sucrose-gap is 1 mm. Electrical activities are recorded through the two Ag/AgCl recording electrodes connected to a preamplifier, CRO and recorder. Mechanical contractions are measured through the force transducer (FT) connected to a recorder.

In the recording of electrical activities two methods were used; extracellular (Fig. 1) and intracellular recording using the single sucrose gap technique (Fig. 2). The extracellular recording was done in the horizontal chamber (capacity, 3 ml), where the strip was pinned out at one end for the recording of action potentials and was connected to the force transducer via a thread ring at the other end for the simultaneous recording of contraction. The suction electrode was used for the extracellular recording of action potentials, attached to the serosal side of the strip. The perfusion rate of tris-buffered Tyrode's solution was 6 ml/min and the optimal length for the strip was determined by the length-tension diagram. The experimental chamber for the single sucrose-gap method consisted of three compartments: the normal Tyrode's compartment where the isotonic contractions were recorded by force transducer, the sucrose compartment of 1 mm in width and the isotonic KCl compartment where the electrical activity was recorded through Ag/AgCl electrode.

Drugs used were as follows:
atropine sulfate (Sigma)
norepinephrine (Arterenol, Hochst)
phentolamine (Regitine, CIBA)
dl-propranolol (Sigma)
tetrodotoxin (TTX, Sankyo)

In this experiment no statistics were used because the result analysis was a qualitative comparison between data groups, and the figures cited in this article were representative ones selected after confirmation of the same results.

RESULTS

1. The effect of higenamine on the contractility of the aortic smooth muscle

Having no spontaneous contractions the strip of aorta was induced to contraction by norepinephrine, high K-Tyrode's solution or electrical field stimulation.

1) The effect of higenamine on the norepinephrine-induced contraction

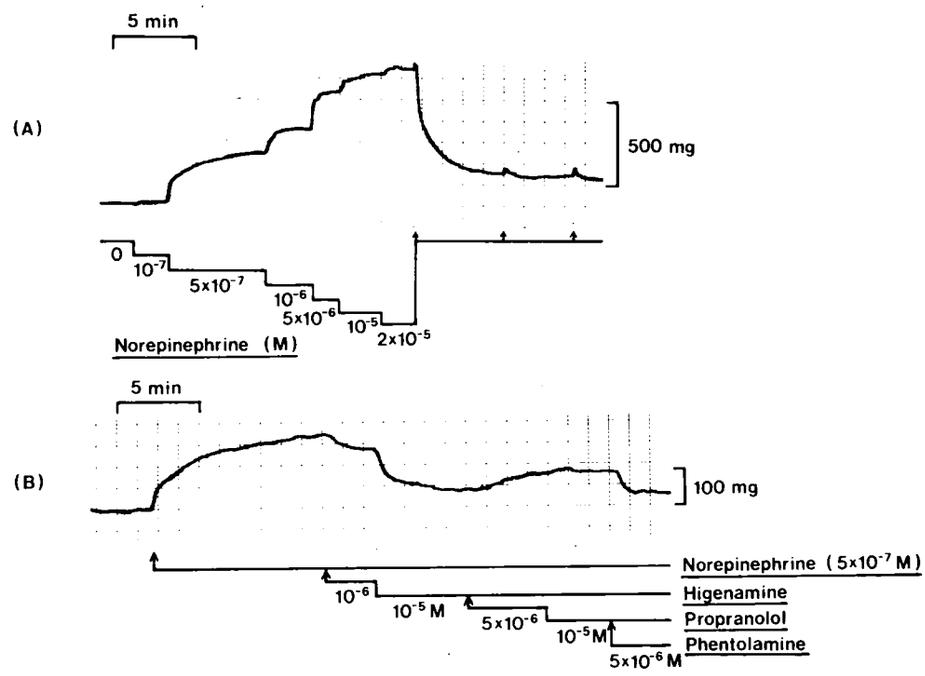

Fig. 3. Dose-dependent contractile responses to norepinephrine (A) and the relaxing effects of higenamine on norepinephrine-induced contraction in guinea-pig aorta (B).
(A) Norepinephrine was administered cumulatively to bathing Tyrode's solution. Sustained contraction was developed initially at the concentration of 10^{-7} M and maximum tension was induced at 10^{-5} M. (B) Higenamine suppressed the norepinephrine-induced contraction in a dose-dependent manner and propranolol blocked the relaxing effects of higenamine.

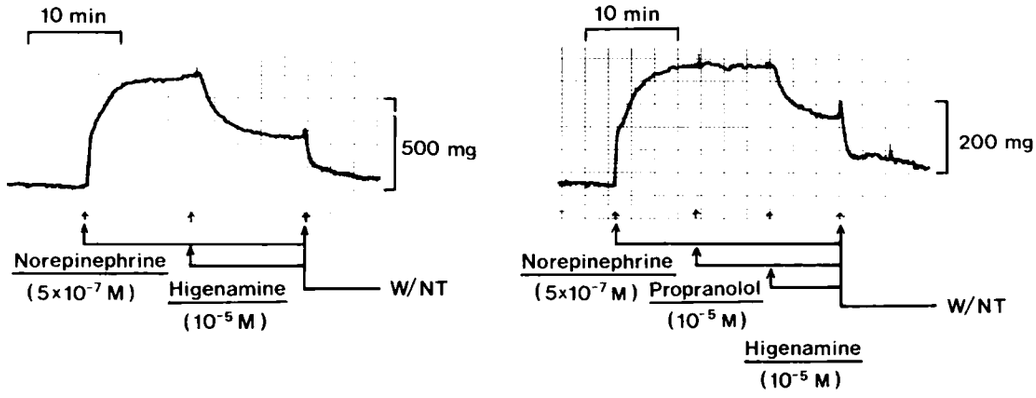

Fig. 4. Effect of pretreated propranolol on the relaxation response of higenamine to norepinephrine-induced contraction in guinea-pig aorta. The sustained contraction induced by norepinephrine (5×10^{-7} M) was relaxed by 56% after higenamine (10^{-5} M) was added to the bathing Tyrode's solution (left), but in the same propranolol-pretreated preparation higenamine relaxed the contraction by 41% (right).

When norepinephrine was administered cumulatively to the bathing Tyrode's solution sustained contraction developed initially at the concentration of 10^{-7} M with maximum tension at 10^{-5} M. The value of ED_{50} was 2×10^{-6} M (Fig. 3A). Higenamine suppressed the contraction induced by norepinephrine (5×10^{-7} M) in a dose-dependent manner and propranolol blocked the relaxing effect of higenamine (Fig. 3B).

The sustained contraction induced by norepinephrine (5×10^{-7} M) was relaxed by 56% after higenamine (10^{-5} M) was added to the bathing Tyrode's solution (Fig. 4, left). But in the same preparation pretreated with propranolol (10^{-5} M) higenamine

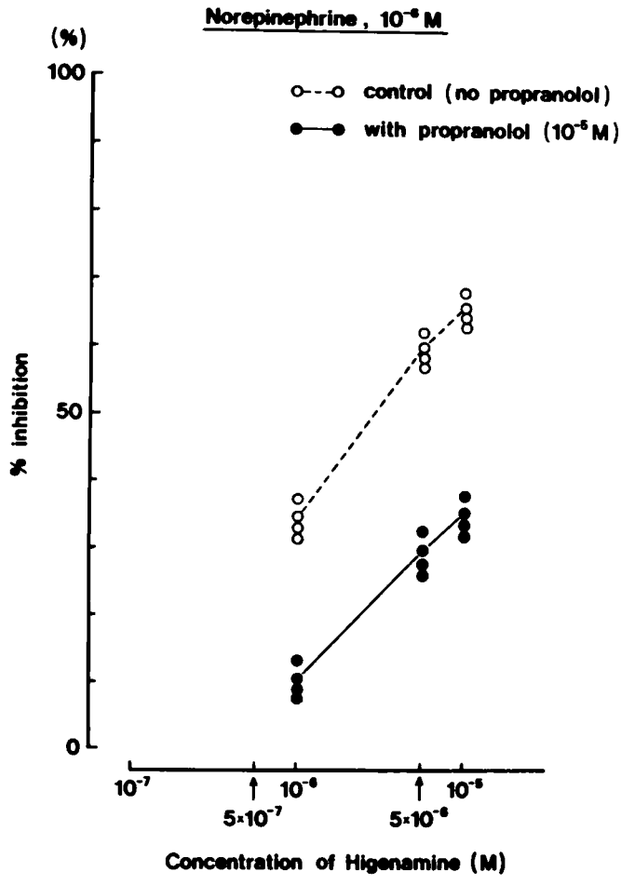

Fig. 5. Dose-dependency of the relaxant effects of higenamine on norepinephrine-induced contraction, and the blocking effects of pretreated propranolol on the relaxing response of higenamine.

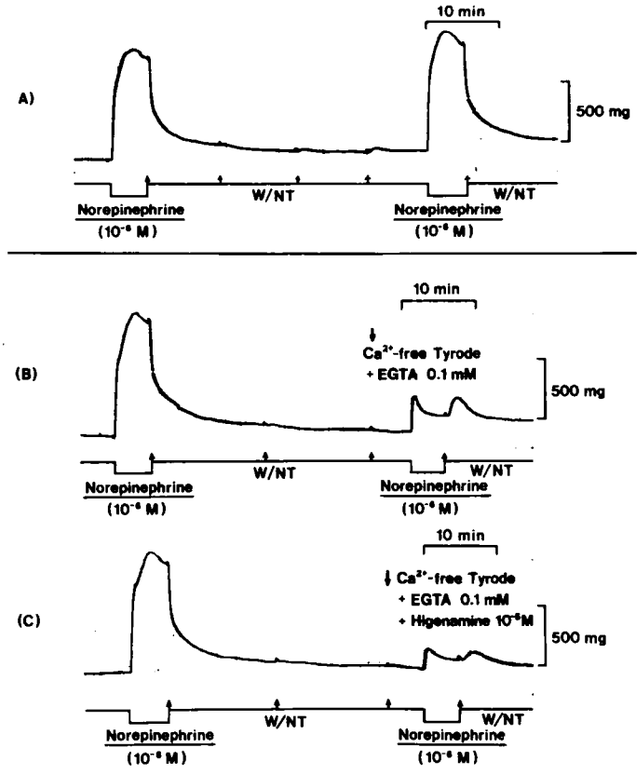

Fig. 6. Suppression of norepinephrine-induced contractions of guinea-pig aortic strip by higenamine in a Ca^{2+}-free Tyrode's solution. Repeated exposures of aortic strip to norepinephrine developed contractions with same amplitude; No tachyphylaxis was observable on the response of norepinephrine (A). In the presence of higenamine within the media, the component of contraction curve produced by norepinephrine-induced intracellular Ca^{2+} mobilization (C) was much suppressed, compared with that in the absence of higenamine (B).

relaxed norepinephrine-induced contraction by 41 % (Fig. 4, right).

The dose-dependency of the relaxing effect of higenamine on norepinephrine-induced contraction was compared to that in cases pretreated with propranolol (Fig. 5). Higenamine administered in the concentrations of 10^{-6} M, 5×10^{-6} M and 10^{-5} M produced blocking effects of 35%, 60% and 65% respectively, while in the cases pretreated with propranolol those effects were 10%, 30% and 35% respectively.

2) The effect of higenamine on the intracellular calcium mobilization

Repeated exposures of the aortic strip to norepinephrine did not diminish the amplitude of contractions (no tachyphylaxis phenomenon) (Fig. 6A). Norepinephrine induced more small contraction in the Ca-free Tyrode's solution containing higenamine than in pure Ca-free Tyrode's solution (Fig. 6B, C). Therefore, it was suggested that the relaxing effect of higenamine through β-receptors was not only related to the inhibition of Ca influx through receptor-operated Ca channel, but also related to the intracellular calcium mobilization.

3) Comparison between the effects of higenamine on guinea-pig aorta and those on rabbit aorta

The response of the aortic smooth muscle to norepinephrine was compared between guinea-pigs and rabbits (Fig. 7). The value of ED_{50} was 2×10^{-6} M in the guinea-pig aorta, whereas it was 10^{-7} M in the rabbit aorta, showing different sen-

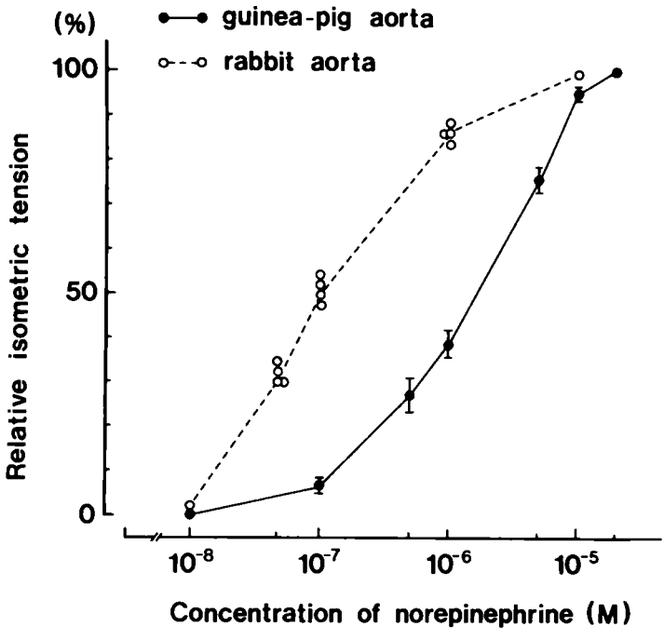

Fig. 7. Dose-dependency of norepinephrine-induced contractions in guinea-pig aorta and rabbit aorta. Developed tension was expressed as a percentage of maximum response induced at the concentration of 2×10^{-5} M norepinephrine in guinea-pig aorta. Each point shows the mean±S.E.M. for pooled data from 9 preparations. ED_{50} was at a concentration of 2×10^{-6} M norepinephrine in guinea-pig aorta, whereas that of rabbit aorta was at a concentration of 10^{-7} M norepinephrine.

sitivity between animal species. Sustained contractions developed dose-dependently as the concentration of norepinephrine increased. These contractions were suppressed more severely in rabbits than in guinea-pigs and propranolol blocked completely the relaxing effect of higenamine (Fig. 8). The effects of pretreated and post-treated propranolol were shown in Fig. 9. Higenamine (10^{-6} M) relaxed the contraction induced by norepinephrine (10^{-7} M) by 78%. Complete recovery was observable after the administration of propranolol (10^{-6} M). Pretreated propranolol (10^{-6} M) blocked completely the relaxing effect of higenamine (10^{-6} M).

4) The effect of higenamine on K-contracture

Higenamine suppressed the K-contracture produced by 25 mM K-Tyrode's solution dose-dependently (Fig. 10A) and pretreatment with propranolol (5×10^{-6} M) and phentolamine (5×10^{-6} M) completely blocked the effect of higenamine on K-contracture (Fig. 10B). Therefore, it was suggested that the relaxing effect of higenamine was developed not through the potential-sensitive Ca-channel, but through the receptor-operated Ca-channel.

5) The effect of higenamine on the contraction induced by electrical field stimulation

The strip was stimulated regularly with A.C. 60 Hz, 7 V/cm for 10 sec every 2.5—3 min. Both passive resting and active tensions were rapidly decreased by the addition of higenamine (10^{-6} M and 10^{-5} M) to bathing Tyrode's solution, and gradually recovered after washout with normal Tyrode's solution (Fig. 11A). But the phasic contractions suppressed by higenamine were not restored to normal level by the addition of propranolol to bathing Tyrode's solution (Fig. 11B).

The phasic contractions disappeared rapidly after the administration of 3 blockers (TTX 3×10^{-7} M, phentolamine 10^{-6} M, and atropine 10^{-6} M), and reappeared gradually after washout with normal Tyrode's solution (Fig. 12A). The phasic contraction driven by electrical field stimulation was suppressed by higenamine and completely disappeared after the administration of phentolamine (Fig. 12B).

The phasic contractions evoked by electrical field stimulation were thought to be due to the release of neurotransmitters, especially norepinephrine from the tissue nerve endings. But the absence of the effect of propranolol and the decrease of passive resting tension could not be explained.

2. The effect of higenamine on the spontaneous contractions of the taenia coli

1) The inhibitory effect of higenamine on the spontaneous contractions

Higenamine suppressed the amplitude of spontaneous contractions and this effect was reversed by washout with normal Tyrode's solution (Fig. 13). Higenamine exerted an inhibitory action on the amplitude and frequency of spontaneous contraction from the concentration of 10^{-8} M dose-dependently and completely abolished it at the concentration of 10^{-6} M (Fig. 14). The depressed contractility induced by higenamine (10^{-7} M) was antagonized by propranolol (10^{-6} M). However, phentolamine had no effect at all (Fig. 15).

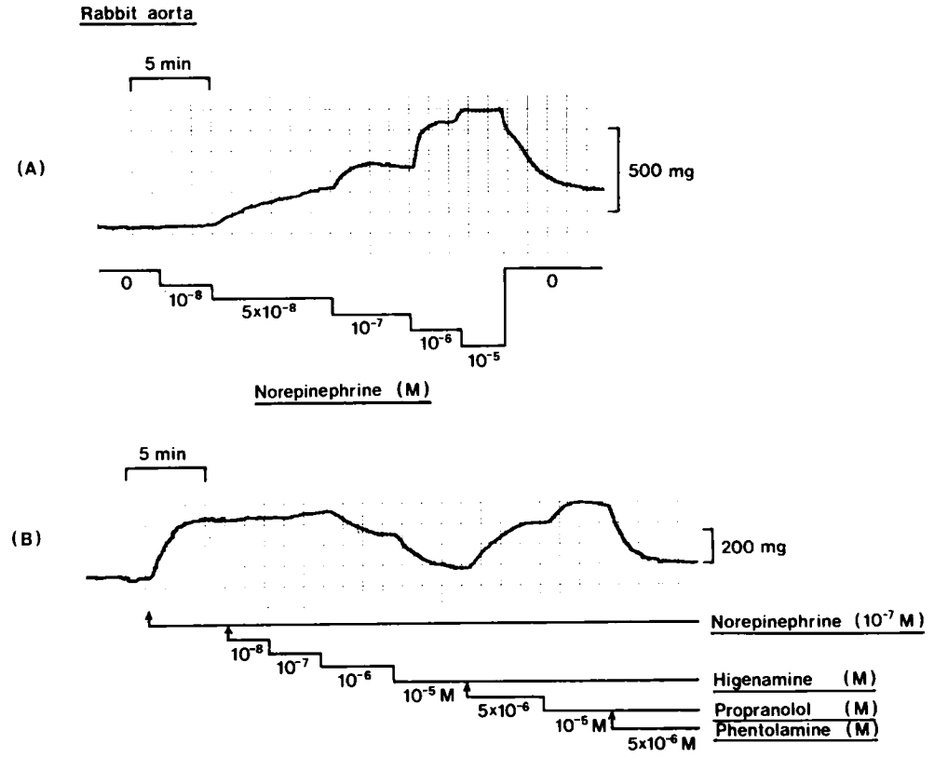

Fig. 8. Dose-dependent contractile responses to norepinephrine (A) and the relaxing effects of higenamine on norepinephrine-induced contraction in rabbit aorta (B).
(A) The sustained contractions were potentiated dose-dependently in parallel with the increase in norepinephrine concentration administered cumulatively to bathing Tyrode's solution. ED_{50} was observed at the concentration of 10^{-7} M and maximum tension developed at 10^{-5} M norepinephrine.
(B) Higenamine suppressed the norepinephrine-induced contraction in a dose-dependent manner, and post-treated propranolol blocked completely the relaxing effects of higenamine.

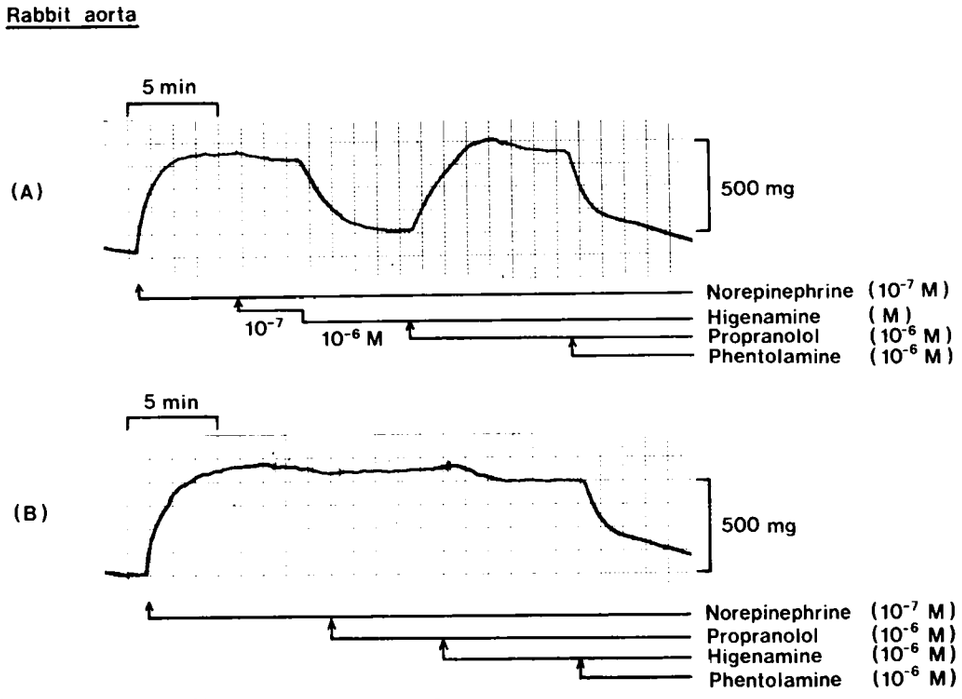

Fig. 9. Effects of post-treated (A) and pretreated (B) propranolol on the relaxation response of higenamine to norepinephrine-induced contraction in rabbit aorta. Higenamine (10^{-6} M) relaxed the contraction induced by norepinephrine (10^{-7} M) by 78%, and complete recovery was observable after the administration of propranolol (10^{-6} M) additionally (A). Pretreated propranolol blocked completely the relaxation response of higenamine to norepinephrine-induced contraction (B).

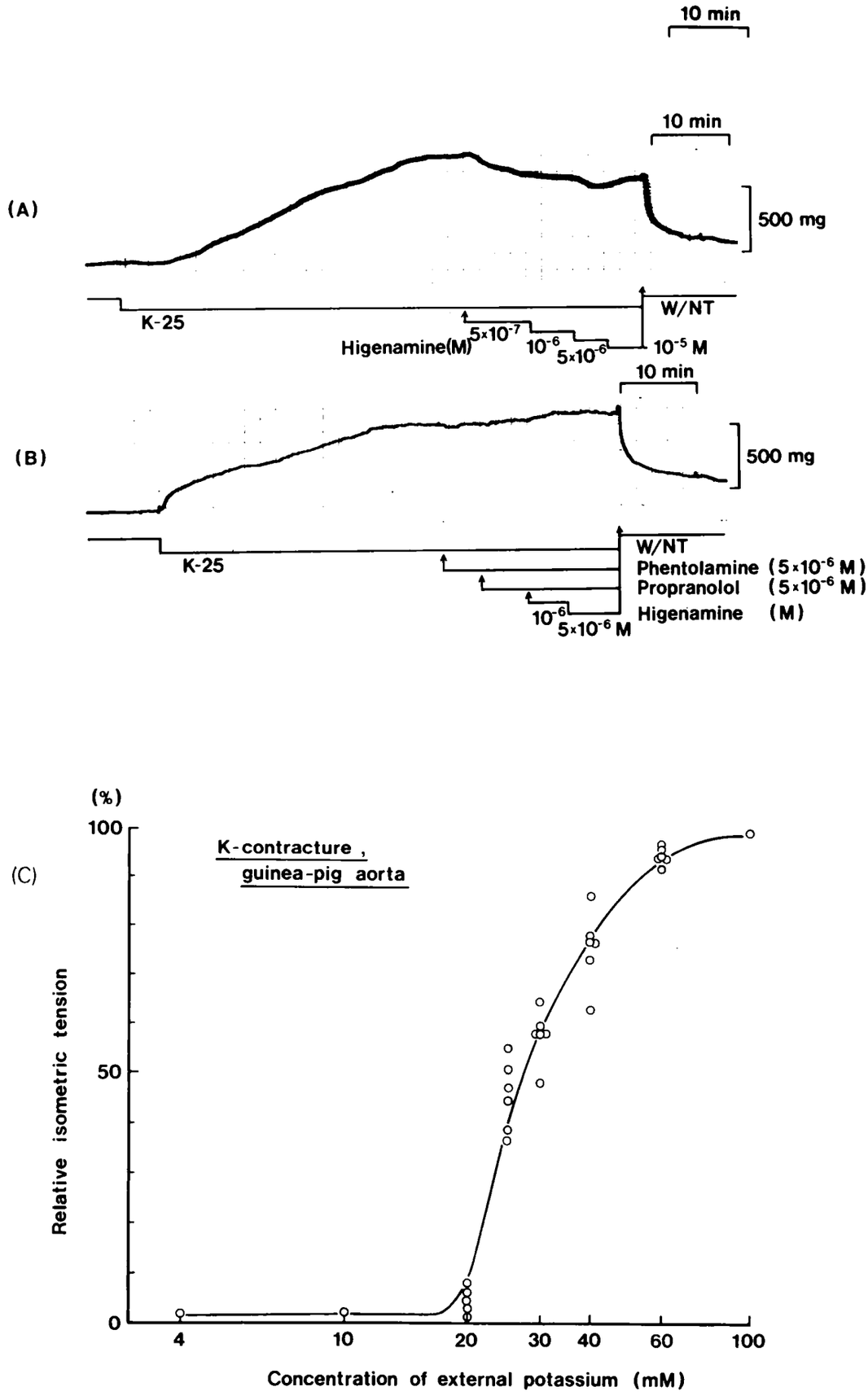

Fig. 10. Effect of higenamine on K-contracture developed at 25 mM K-Tyrode's solution in guinea-pig aorta. Higenamine suppressed the K-contracture dose-dependently (A), whereas pretreated propranolol blocked completely the higenamine effect on K-contracture (B). Dose-dependency of K-contracture in guinea-pig aorta (C).

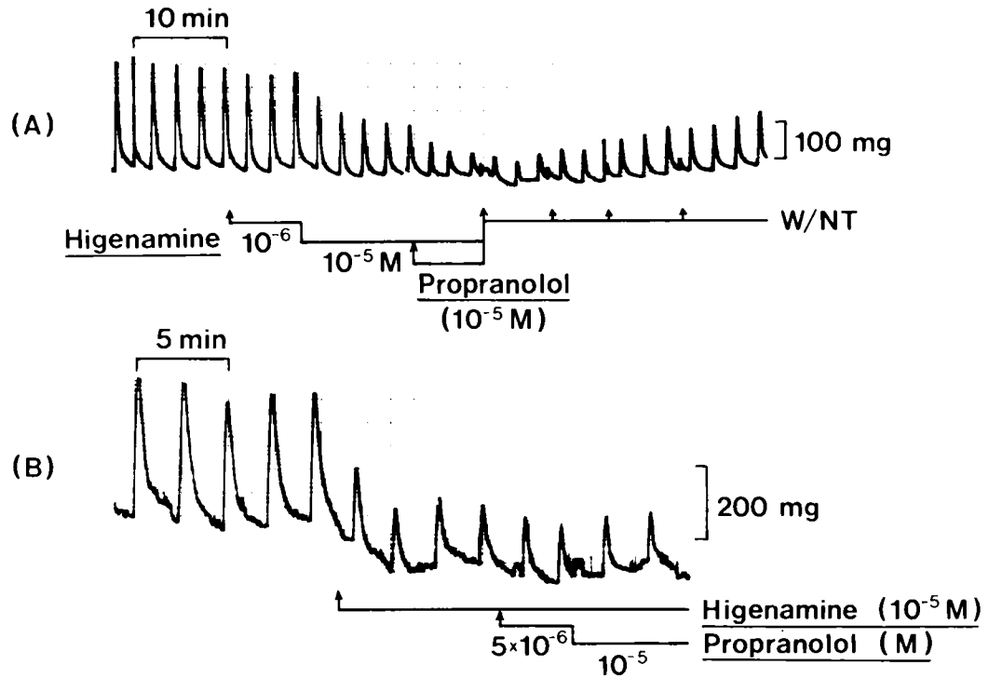

Fig. 11. Effect of higenamine on the phasic contractions induced by electrical field stimulation in guinea-pig aortic strip. The strip was stimulated regularly with A.C., 60 Hz, 7 V/cm for 10 sec every 2.5 minutes. Both passive resting tension and active tension were rapidly decreased by the addition of higenamine to bathing Tyrode's solution, and gradually recovered after washout with normal Tyrode's solution (A). The phasic contractions suppressed by higenamine were not restored to normal level by the addition of propranolol to bathing Tyrode's solution (A, B).

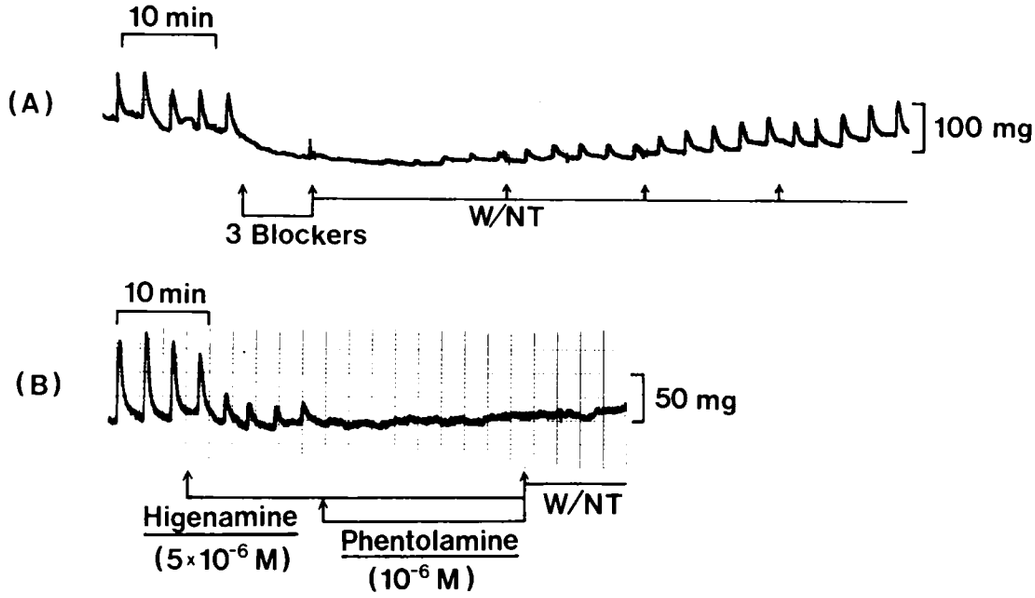

Fig. 12. Characteristics of the phasic contractions induced by electrical field stimulation in guinea-pig aorta. Phasic contractions were induced regularly with A.C., 60 Hz, 7 V/cm for 10 sec every 3 minutes. The phasic contractions disappeared rapidly after the administration of 3 blockers (TTX 3×10^{-7} M, phentolamine 10^{-6} M, and atropine 10^{-6} M), and reappeared gradually after washout with normal Tyrode's solution (A). The phasic contractions driven by electrical field stimulation, which were suppressed by higenamine, disappeared rapidly after the administration of phentolamine (B).

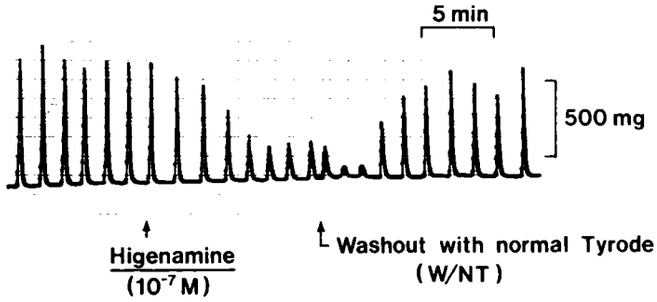

Fig. 13. Effects of higenamine on the spontaneous contractions of guinea-pig taenia coli. Higenamine suppressed the amplitude of spontaneous contractions, and this action of higenamine was reversible; The higenamine-induced depression of contractility disappeared after washout with normal Tyrode's solution.

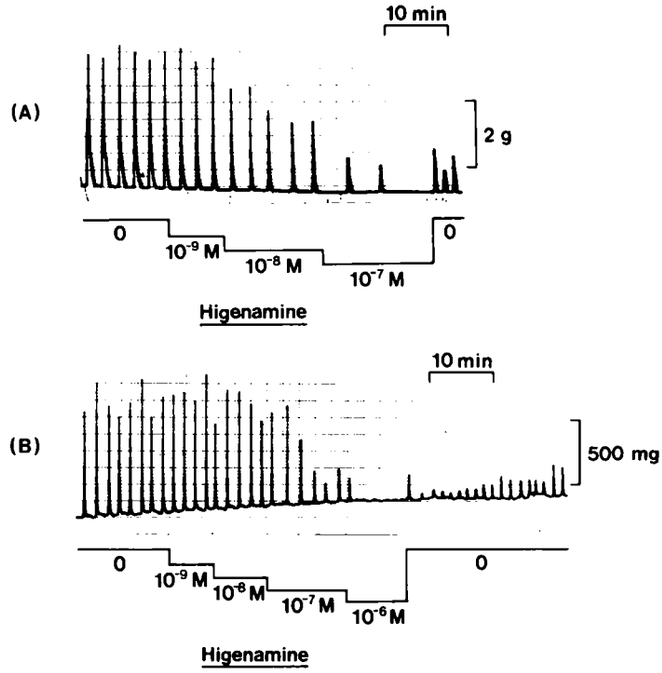

Fig. 14. Dose-dependency of higenamine effects on the spontaneous contractions of guinea-pig taenia coli. Higenamine suppressed the frequency and the amplitude of spontaneous contractions dose-dependently, and blocked completely at the concentration of 10^{-6} M.

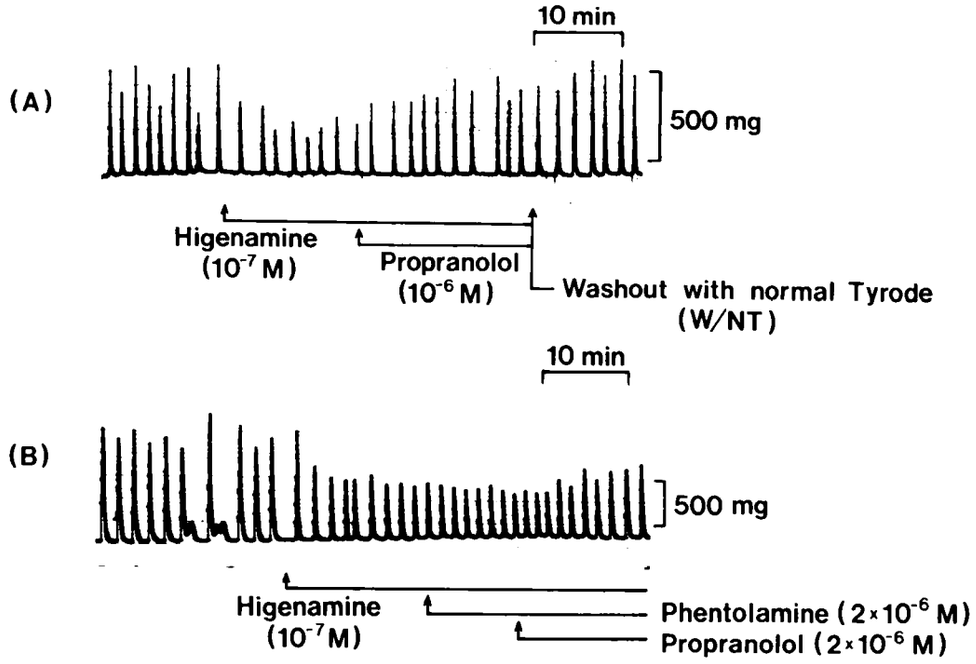

Fig. 15. Effects of propranolol on the inhibitory action of higenamine to spontaneity in guinea-pig taenia coli. The depressed contractility induced by higenamine (10^{-7} M) was antagonized by the addition of propranolol (10^{-6} M) to the bathing Tyrode's solution (A). However, phentolamine had no effects on the higenamine-induced depressed contractility of the taenia coli (B).

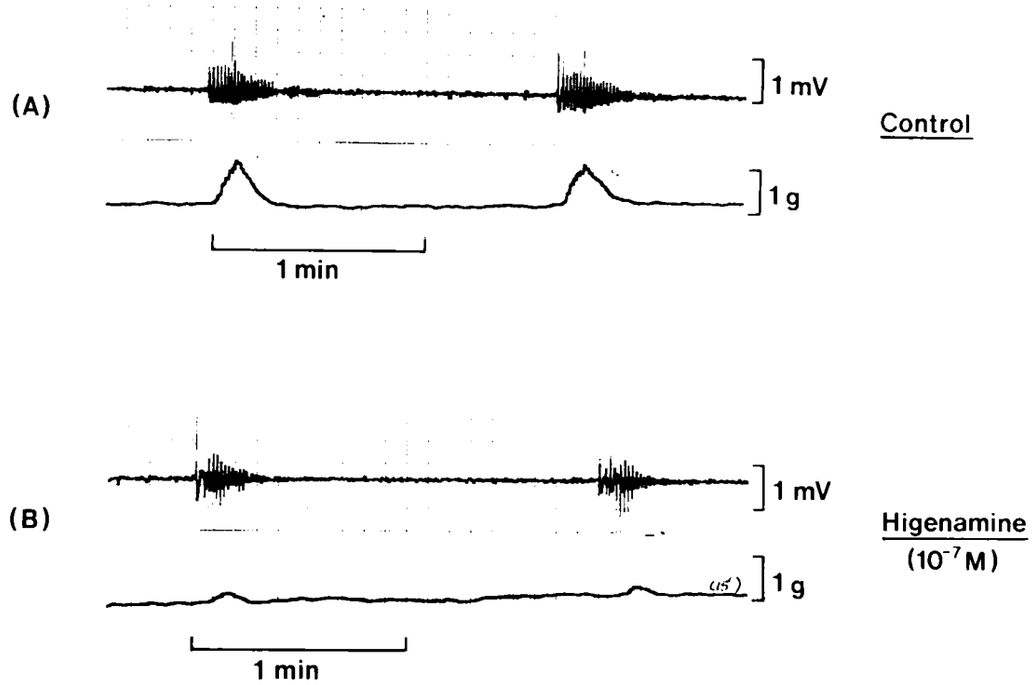

Fig. 16. Effects of higenamine on spontaneous electrical activity and contraction at the concentration of 10^{-7} M in guinea-pig taenia coli. The parameters of electrical activity during the control period with respect to the interval between bursts of spike discharge and the spikes in a burst were compared with those obtained 15 minutes after higenamine administration. The burst interval increased from control 98.4 seconds to 112.8 seconds. The number of spikes in a burst (spike frequency), however, was reduced from control 21 spikes to 11 spikes. The frequency and the amplitude of spontaneous contractions decreased as the burst interval and spike frequency were altered.

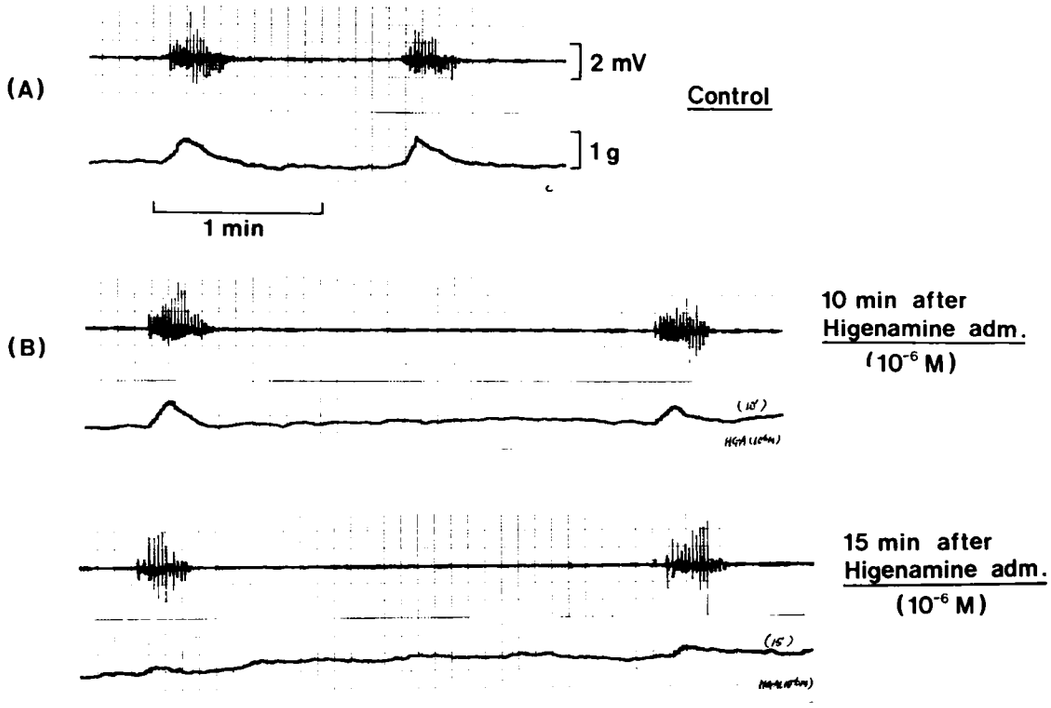

Fig. 17. Effects of higenamine on spontaneous electrical activity and contraction at the concentration of 10^{-6} M in guinea-pig taenia coli. The burst interval was increased from control 85.2 seconds to 182.4 and 189.6 seconds, respectively, 10 and 15 minutes after the administration of higenamine (10^{-6} M). The spike frequency was decreased from control 20 spikes to 18 and 14 spikes respectively. The frequency and the amplitude of spontaneous contractions decreased as the electrical parameters were changed.

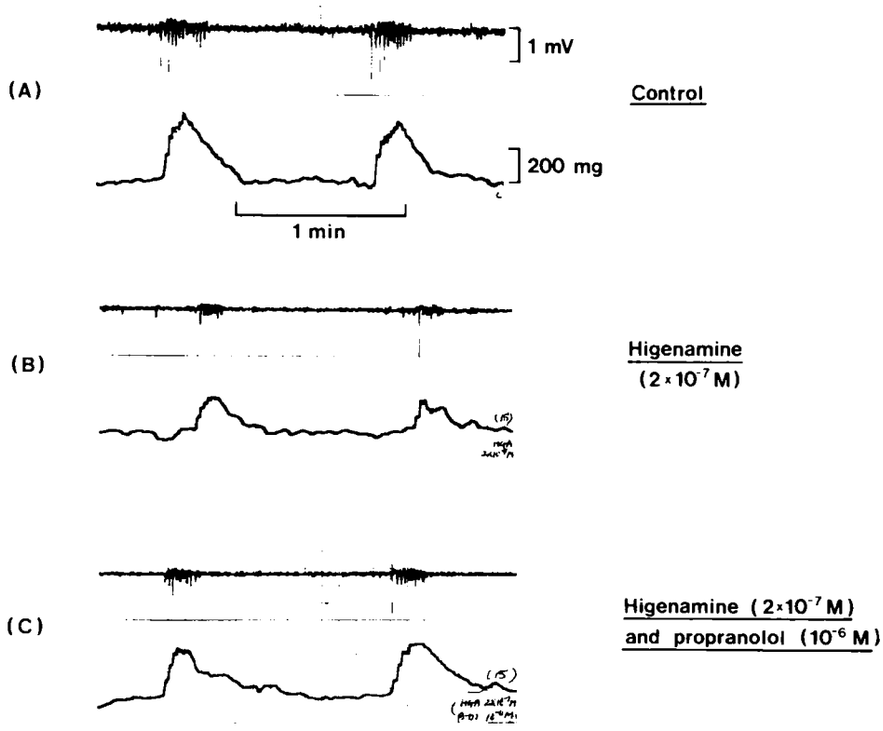

Fig. 18. Effects of propranolol on the higenamine-induced suppression of spontaneity in guinea-pig taenia coli. The spike frequency in a burst was decreased from the control 13 spikes to 8 spikes after higenamine (2×10^{-7} M) administration, and the depressed activity was recovered to 11 spikes by the addition of propranolol (10^{-6} M). However, the slightly increased burst interval was nearly the same. The depressed amplitude of spontaneous contraction was increased after the addition of propranolol to higenamine-containing bathing Tyrode's solution.

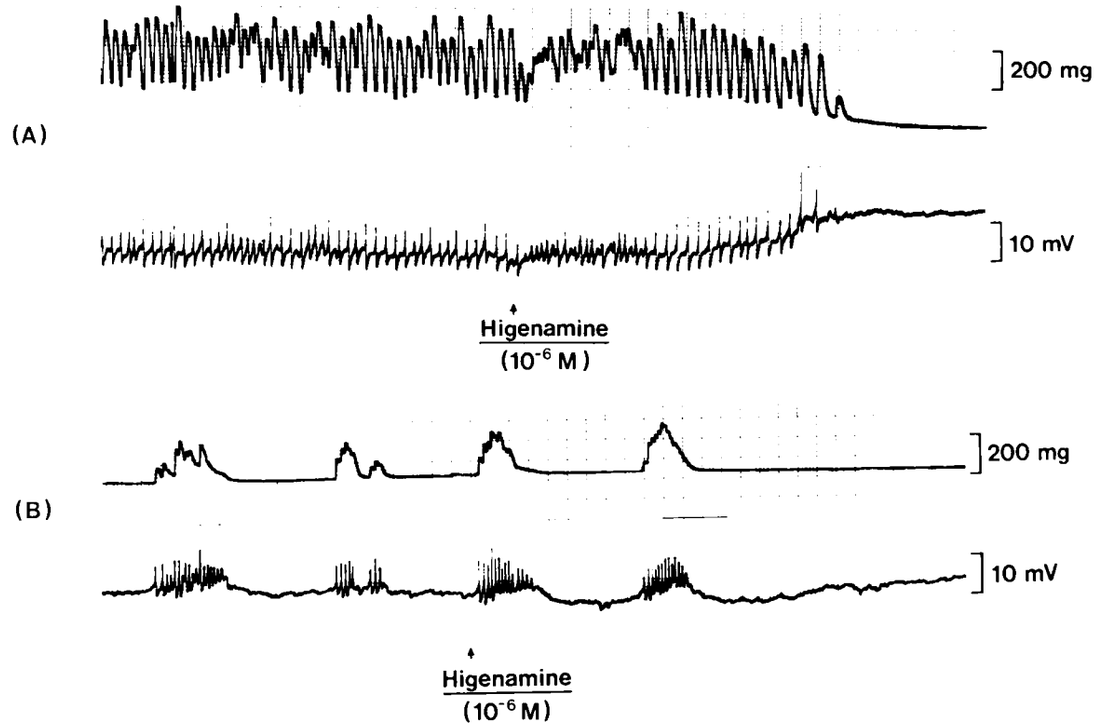

Fig. 19. Effects of higenamine on spontaneous electrical activity and contraction in guinea-pig taenia coli. Spontaneous electrical activity and contraction were simultaneously measured by use of the single sucrose-gap technique. Intentional overstretch induced continuous Ca^{2+}-spike discharge (A). Higenamine (10^{-6} M) blocked completely the continuous spike discharge (A), and regular spontaneous activities were also completely blocked by the addition of higenamine and membrane potential was gradually depolarized.

2) The effect of higenamine on the spontaneous electrical activity and contraction

Two parameters of the electrical activity were measured during the control period and 15 min after the administration of higenamine (Fig. 16). The burst interval increased from control 98.4 sec to 112.8 sec and the number of spikes in a burst (spike frequency) was reduced from control 21 spikes to 11 spikes. The frequency and the amplitude of spontaneous contractions were decreased as the burst interval and the spike frequency of the spontaneous electrical activity decreased respectively. In the same way the burst interval was increased from control 85.2 sec to 182.4 sec and 189.6 sec, 10 and 15 min after the administration of higenamine (10^{-6} M) respectively, and the spike frequency was decreased from control 20 spikes to 18 and 14 spikes respectively (Fig. 17). The effect of higenamine was partially antagonized by propranolol (Fig. 18). The spike frequency which was decreased from control 13 spikes to 8 spikes by higenamine was recovered to 11 spikes by the addition of propranolol (10^{-6} M). The depressed amplitude of spontaneous contraction was increased in parallel with the increased spike frequency. However, the burst interval which was slightly increased was nearly the same as before.

3) The effect of higenamine on the membrane potential

The spontaneous electrical activity was recorded simultaneously with the contraction using the single sucrose-gap technique (Fig. 19). Intentional overstretch of the strip induced continuous spike discharges which were completely blocked by the addition of higenamine (10^{-6} M) with development of the depolarization of the membrane potential. In normal regular spontaneous activities higenamine ceased contractions and action potentials with gradual membrane depolarization.

DISCUSSION

After the discovery of higenamine as a cardiac stimulant, lots of investigations have been made of this agent. Under treatment with lanthanum and verapamil in rabbit atrium higenamine recovered depressed cardiac contractility dose-dependently, suggesting that a part of the cardiotonic action of higenamine was potentiation of calcium influx through the cell membrane (Chang *et al.* 1981).

Higenamine increased slow inward current, just like catecholamine, in the papillary muscle of rabbits (Kwon *et al.* 1981) and in the heart of pigs (Wang *et al.* 1982). In the sarcoplasmic reticulum higenamine suppressed the reuptake of calcium by inhibiting the activity of Ca-ATPase necessary for Ca^{2+} transport and potentiated its release (Kim *et al.* 1982). Higenamine was supposed to act on the adrenoceptors, considering the fact that the value of pA_2 of higenamine to propranolol was similar to that of epinephrine to propranolol as being 8.58 and 7.50 respectively in the left atrium of rabbits (Park *et al.* 1984).

In in-vivo experiments with rabbits higenamine decreased blood pressure and total peripheral resistance, and increased cardiac output and heart rate. These effects were all blocked by propranolol (Kim *et al.* 1986). From the above results higenamine was supposed to act on β-adrenoceptors.

The contraction of the smooth muscle is known to be generated in three mechanisms; excitation-contraction coupling (electrical activation), depolarization-induced contraction and pharmaco-mechanical coupling (non-electrical activation) by contracting agents such as norepinephrine, angiotensin II, histamine and vasopressin and so on (Haeusler 1972; Mekata and Nin 1972; Bohr 1973; Fleckenstein 1977; Kim *et al.* 1978; Bolton 1979).

In this experiment, the contractions of the aortic smooth muscle were generated by norepinephrine, depolarization driven by high K-Tyrode's solution and electrical field stimulation.

Norepinephrine was reported to generate contractions without membrane depolarization in the pulmonary artery (Su *et al.* 1964) and in the carotid artery and the aorta of rabbits (Mekata *et al.* 1972; Mekata 1974). In accordance with the above results norepinephrine generated contraction without depolarization below the concentrations of 10^{-8} M in the pulmonary artery of rabbits. However, it generated large contraction with depolarization and decrease of membrane resistance above the concentrations of 2×10^{-8} M.

Norepinephrine was supposed to generate

contractions by calcium influx into the cell at low concentration and by calcium release from intracellular calcium stores as well as calcium influx from outside the cell at high concentration (Casteels *et al.* 1977). It was also reported that norepinephrine induced contraction by releasing calcium from the cell membrane or intracellular calcium stores in the beginning of contraction and required calcium supply through calcium influx for the maintenance of it. Norepinephrine (10^{-7} M~10^{-5} M) produced steady tonic contractions (Fig. 3 and Fig. 4) and it (10^{-6} M) generated small contraction even in Ca-free Tyrode's solution, supposedly through calcium release from intracellular stores (Fig. 6). Higenamine was proved to act on two components of the contraction, that is, the one maintained by Ca influx and the one induced initially by intracellular Ca release.

The relation between higenamine and the potential-sensitive Ca channel was investigated (Fig. 10). The contraction induced by 25 mM K-Tyrode's solution was relaxed dose-dependently by higenamine. But the pretreatment with phentolamine and propranolol completely blocked this effect. Therefore, it was supposed that higenamine did not affect directly membrane potential depolarized by K-Tyrode's solution, but affected a process of releasing neurotransmitters from nerve endings embedded in tissue. Higenamine was, thus, estimated not to act on the potential-sensitive Ca channel.

Electrical field stimulation was supposed to induce the release of neurotransmitters from nerve endings because the phasic contraction induced by it was abolished by phentolamine (Fig. 12). Higenamine decreased both passive resting and active tensions and this effect was not recovered by propranolol.

There are slow rhythmic fluctuations of membrane potential called slow wave at intervals of 1~3 min in the taenia coli of guinea-pigs. At the top of the fluctuation spike potentials are generated at the frequency of 1 spike/sec. The more the strip was stretched the narrower became the interval between the bursts of spike discharges (Mashima and Yoshida, 1965; Mashima *et al.* 1966; Hukuhara and Fukuda, 1968; Golenhofen and Loh, 1970). If stretched too much, taenia coli continued to produce spike discharges resulting in large contraction. The spike potentials of taenia coli have been proved experimentally to be Ca spikes generated by Ca-entry from outside the cell (Brading *et al.* 1969).

The frequency of spontaneous contractions was supposed most likely to be determined by periodic fluctuation of electrogenic Na pump (Connor *et al.* 1974). The amplitude was related to the extent of Na efflux determined by ATP supply and to the activity of electrogenic Na pump related to Ca (Prosser 1978).

In this experiment, higenamine made a dose-dependent decrease of frequency and amplitude of the spontaneous contraction of taenia coli, which could be interpreted to be due to the decrease of the burst frequency and the spike frequency, respectively. And it depolarized membrane potential (Fig. 19). All these effects to the spontaneous contraction were blocked mostly by treatment with propranolol. From the above results higenamine was supposed to have an effect on the slow wave and the spike potential through β-adrenoceptors. However, the phenomenon of membrane depolarization could not be explained well by this experiment alone and required more investigation.

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=국 문 초 록=

Higenamine이 기니피그 대동맥 및 결장뉴 평활근의 수축성에 미치는 영향

서울대학교 의과대학 소아과학교실 및 생리학교실*

석상복 · 고광욱 · 이상진* · 황상익* · 김기환*

한의학에서 극약에 속하는 부자에서 강심작용을 일으키는 핵심물질로서 알려져 있고 합성 가능한 higenamine의 혈관 및 결장뉴 평활근의 수축성에 대한 직접적인 효과를 규명하고자, 기니피그 흉부대동맥과 결장뉴를 이용하여 이들에 대한 higenamine의 작용효과와 그 기전을 비교 · 분석하였다.

대동맥 절편의 수축은 norepinephrine, K-Tyrode's 및 전장자극으로 유발시켰으며, 자발적 수축을 하는 결장뉴의 활동전압(가시전압)과 수축곡선을 동시에 기록하기 위하여 전통적인 흡입전극법이나 단일 슈크로즈 간극법을 사용하였다. 모든 실험은 35℃에서 100% O_2와 평형을 이루고 있는 tris-완충 Tyrode's 용액에서 시행하여 다음과 같은 결과를 얻었다.

1) Norepinephrine 유발 수축에 대하여 higenamine은 농도 의존적으로 수축을 억제시켰다.

2) Norepinephrine 유발 수축곡선 중 세포내 Ca^{2+} 동원으로 생기는 성분이 higenamine에 의하여 심하게 억제되었다.

3) K-경축성분 중 potential-sensitive Ca^{2+}-통로를 통한 Ca^{2+} 유입 증가로 발생되는 수축성분은 higenamine에 의하여 억제되지 않았다.

4) 전장자극으로 유발시킨 대동맥 평활근의 위상성 수축은 higenamine 투여로 기초장력과 능동장력의 크기가 모두 감소하였으나 propranolol 투여로 회복되지 않았다.

5) Higenamine은 결장뉴의 전기활동 중 가시전압 무리 빈도와 한 무리내의 가시전압 빈도를 농도 의존적으로 억제시켜 자발적 수축의 빈도와 크기를 감소시켰다. 이와 같은 higenamine의 작용은 propranolol에 의하여 차단되었다.

6) Higenamine은 결장뉴의 Ca^{2+} 가시전압 발생을 억제하였고 막전압을 탈분극시켰다.

이상의 실험결과들을 근거로

1) 기니피그 대동맥 평활근의 norepinephrine 유발 수축에 대한 higenamine의 수축억제작용은 β-수용체를 통하여 receptor-operated Ca^{2+}-통로를 통한 Ca^{2+} 유입억제 및 세포내 Ca^{2+} 동원억제 기전을 통하여 나타났고

2) 대동맥 평활근의 potential-sensitive Ca^{2+} 통로를 통한 Ca^{2+} 이동에는 higenamine이 영향을 못 미치며

3) 결장뉴의 자발적 수축억제작용도 β-수용체를 통하여 가시전압 무리 빈도와 가시전압 빈도를 감소시켜 발생된 것으로 결론을 내릴 수 있었다.