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Effect of Higenamine on Pulmonary Aorta of Rabbit*

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ABSTRACT

Higenamine, which is one of active component of Aconiti tuber, has been known to have positive inotropic effect through adrenergic beta-receptor. The effect of higenamine on norepinepharine or potassium induced contraction of pulmonary aorta in rabbit were studies.

1. The contraction of aortic strips induced by norepinephrine was suppressec by pre-or post-treatment of higenamine dose dependently and those effects of higenamine were prevented by propranolol.

The pA_2 value of higenamine against propranolol calculated as 8.25.

2. The effect of higenamine was not affected by phentolamine.

3. Isoprotrenol has shown 10 times stronger vasodiatory effect on norepinephrine induced cntracture than that of higenamine but high concentration (3.3×10^{-6}M) of isoproterenol produce intrinsic activity.

4. Vasodilatory effect of higenamine or isoproterenol was not observed in potassium induced contacture of pulmonary aortic strips.

These results strongly suggest that higenamine dilated the pulmonary vascular smooth muscle through stimulation of adrenergic beta-receptor.

Key Words: Aortic strip, norepinephrine, isoproterenol, potassium, higenamine, phentolamine, adrenergic receptor

INTRODUCTION

Aconiti tuber has been known as a cardiotonic agent in oriental medicine for a long time. Higenamine was isolated from Aconti tuber as an active component by Kosuge and Yokota (1976) and was chemically synthesized by using 4-hydroxy-3-methoxy benzaldehyde as a starting material (Chang *et al.*, 1984).

The effect of higenamine on heart has been extensively studied but its effect on vascular smooth muscle was not studied well. Chang *et al.*, (1981) observed the positive inotropic effecct of higenamine in the isolated electrically driven left atrium of rabbit and the influence of extracellular calcium, lanthanum and verapamil on positive inotropic effect of higenamine. From those results, they concluded that one of possible mechanism of positive inotropic action of higenamine should be acceleration of calcium influx through sarcolemma. The increase in slow inward calcium current and contractile force of papillary muscle was reported by Kwon *et al.*, (1981). Park and his colleague (1984) reported that the positive inotropic effect of higenamine is likely due to stimulation of adrenergic beta-receptor because the effect of higenamine on isolated atrium in rabbit was competitively blocked by propranolol. The adrenergic beta-receptor agonist effect of higenamine also observed in vivo hemodynamic study in rabbit (1986).

In this study, the effect of higenamine on adrenergic receptor in vascular smooth muscle was conducted with spiral strips of pulmonary

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aorta in rabbit.

MATERIALS AND METHODS

The pulmonary aorta was excised from New Zealand White male rabbit.

The spiral strips (3×30 mm) of vascular smooth muscle were prepared in the Kreb's solution (NaCl 119.8, KCl 4.6, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.1 mM, pH 7.4) gased wityh 95% O_2 and 5% CO_2 at 37°C and then suspended in organ bath contatining same Kreb's solution with 2g preload. After 60 mins stabilization, contracture was induced by adding norepinephrien (10^{-7}, 10^{-6} M) and isotonic or isometric contraction was measured by polygraph. The effect of higenamine on norepinephrine induced contracture was determined by treatment of higenamine before or after induction of norepinephrine contracture. The effects of adrenergic blocking agents (propranolol or phentolamine) on higenmaine action were studied by addition of blockades 15 mins before treatment of higenamine. The effect of higenamine on contracuture induced by 25 mM potassium was also observed. Higenamine used in this study was kindly synthesized by Research Intitute of Don A Pharmaceutical Co. Ltd.

RESULTS

The effect of higenamine on norepinephrine induced contracture

The aortic strips contracted by norepinephrine were dilated by pre- and post-treatment of higenamine dose dependently in concentration from 3×10^{-7} to 3.3×10^{-5} M (Fig. 1, 2, 3).

The effect of propranoloe and action of higenamine

The vasodilatory action of higenamine in norepinephrine induced contracture was prevented by pre-treatment of propranolol ($3\times10^{-8}\sim10^{-6}$ M) (Fig. 1, 3). And dose response curves of higenamine were parallel shift to the right by propranlol (Fig. 4). Its pA_2 value was calculated as 8.25 from Schild plot.

The effect of phentolamine on higenamine action

Pre-treatment of phentolamine (10^{-8} M) did not affect the vasodilatory action of higenamine in norepinephrine induced vasoconstriction (Fig. 2).

The effect of isoproterenol on norepinephrine induced vasoconstricution

The aortic strips contracted by norepinephrine were also dilated by isoproterenol and its potency

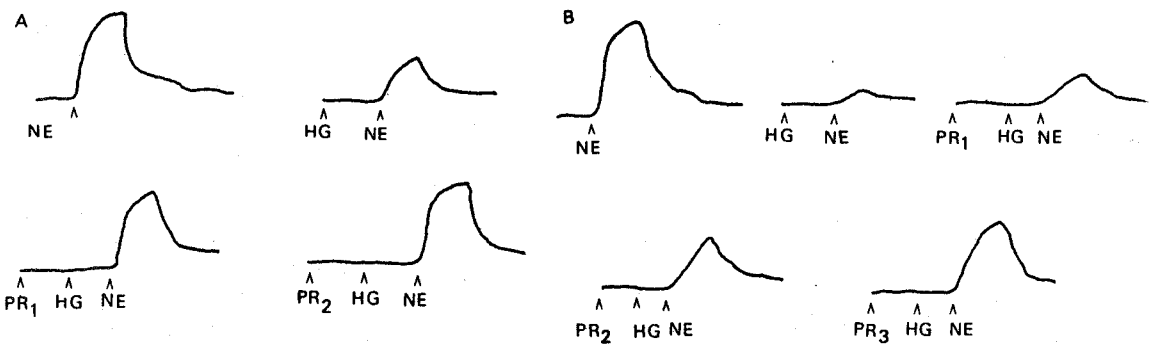

Fig. 1. Effects of higenamine pretreatment on norepinephrine induced contractile response of rabbit aorta. Panel A: Isotonic contraction NE: Norepinephrine 3×10^{-6} M, HG: Higenamine 3×10^{-6} M. PR_1: Propranolol 10^{-7} M, PR_2: Propranolol 10^{-6} M.
Panel B: Isometric contraction NE: Norepinephrine 10^{-7} M, HG: Higenamine 3×10^{-6} M, PR_1: Propranolol 10^{-8} M, PR_2: Propranolol 10^{-7} M. PR_3: Propranolol 10^{-6} M.

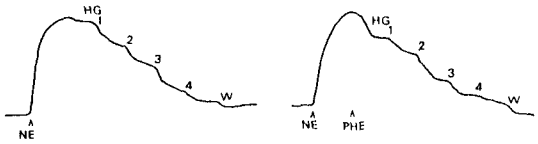

Fig. 2. Effects of phentolamine on the relaxation by higenamine in the norepinephrine induced contractile response of rabbit aorta. Five minutes after phentolamine pretreatment, hig-enamine was cumulatively applied.
HG_1: Higenamine 3×10^{-8} M, HG_2: Higenamine 3×10^{-7} M. HG_3: Higenamine 3×10^{-6} M, HG_4: Higenamine 3×10^{-5} M, NE: Norepinephrine 10^{-7} M, PHE: Phentolamine 10^{-6} M.

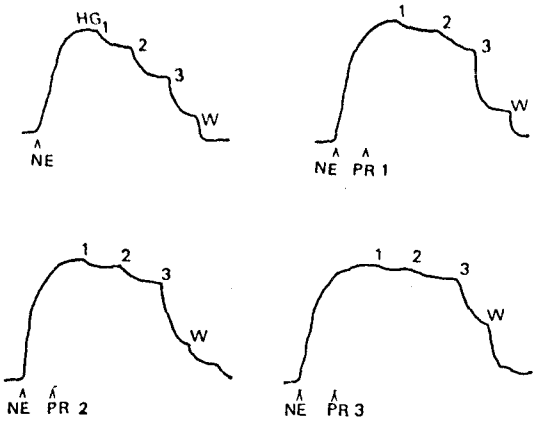

Fig. 3. Effects of propranolol on the relaxation by higenamine in the norepinephrine induced contractile response of rabbit aorta. Five minutes after propranolol pretreatment, higenamine was cumulatiely applied.
NE: Norepinephrine 10^{-7} M, PR_1: propranolol 10^{-8} M, PR_2: Propranolol 10^{-7} M. PR_3: Propranolol 10^{-6} M, HG_1: Higenamine 3×10^{-7} M, HG_2: Higenamine 3×10^{-6} M. HG_3: Higenamine 3×10^{-5} M, W: Wash

seems 10 times stronger than that of higenamine but isoproterenol showed intrinsic activity with high concentration (3.3×10^{-6} M) (Fig. 5).

The effects of higenamine and isoproterenol on potassium contracture

Either higenamine or isoproterenol did not af-

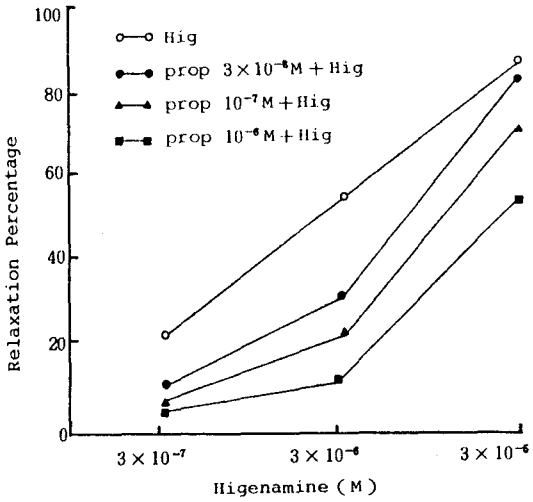

Fig. 4. Effects of propranolol on the dose-response curve of higenamine induced relaxation in rabbit aorta. Five minutes after propranolol pretreatment, higenamine was cumulatively applied.
The effect of higenamine was observed for 10 minutes in every concentration.

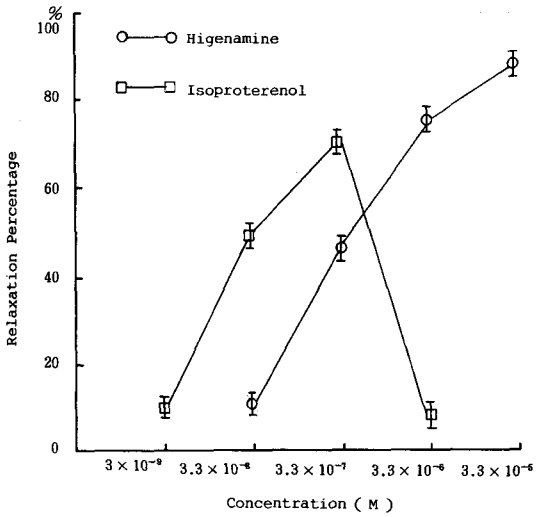

Fig. 5. Dose response curves of higenamine and isoproterenol on norepinephrine induced contractile response of rabbit aorta.
Shown, are mean values±S.E.M. n=3 per group.
The effects of higenamine and isoproterenol were observed for 10 min in every contration.

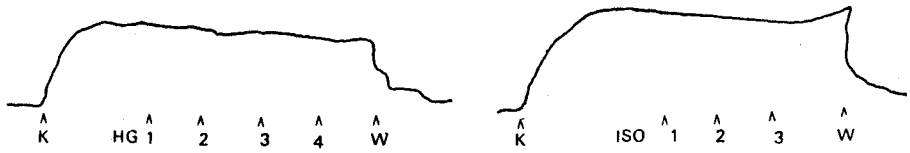

Fig. 6. Effects of higenamine and isoproterenol on contraction produced by elevated (potassium). For isotonic high potassium Krebs solution, 25 mM potassium was replaced by the same concentration of sodium.
K: Potassium 25 mM, HG_1: Higenamine 3×10^{-8} M, HG_2: Higenamine 3×10^{-7} M, HG_3: Higenamine 3×10^{-6} M, HG 4: Higenamine 3×10^{-5} M, ISO_1: Isopraterenol 3×10^{-8} M, ISO_2: Isoproterenol 3×10^{-7} M, ISO_3: Isoproterenol 3×10^{-6} M, W: Wash.

fect the vasoconstriction induced by 25 mM potassium (Fig. 6).

DISSCUSSION

The positive inotropic effect of higenamine was found to be potentiated by extracellular calcium and was prevented by lanthanum or verapamil (Chang *et al.*, 1981). The transmembrane potential study suggested that higneamine increase the slow inward calcium current through sarcolemma (Kwon *et al.*, 1981). These findings indicate that the mechanism of positive inotropic action of higenamine might be resemble to that of catecholamine.

The previous studies such as in isolated atrium (Park *et al.*, 1984) and in vivo hemodynamic study (Kim *et al.*, 1986) strongly suggest that the positive inotropic action of higenamine produced by stimulation adrenergic beta-receptor.

In this study, higenamine dilates the norepinephrine induced vasoconstriction and this vasodilatory effect of higenamine was competitively inhibited by propranolol. On the other hand higenamine did not affect the potassium contracture of vascular smooth muscle. Kwon (1981) also reported that higenamine did not affect the sodium current.

The findings indicate that higenamine has adrenergic beta-receptor agonist effect on vascular smooth muscle.

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=국문초록=

Higenamine이 토끼 폐동맥에 미치는 영향

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Higenamine은 부자의 활성 성분의 하나로서 아드레나린성 beta-수용체를 통하여 강심효과를 나타내는 것으로 알려졌다. 본 연구는 norepinephrine 또는 potassium에 의한 토끼 폐동맥의 수축에 미치는 Higenamine의 영향을 관찰한 것이다.

1. Norepinephrine으로 수축을 이르킨 폐동맥편은 higenamine의 전처치 또는 후처치로 용량의 존적으로 이완되었다. 이같은 higenamine의 효과는 propranolol 전처치로 억제되었다. Higenamine의 propranolol에 대한 pA_2 값은 8.25였다.
2. Higenamine의 폐동맥편에 대한 효과는 phentolamine 전처치로 억제되지 않았다.
3. Isoproterenol도 norepinephrine에 의한 폐동맥편 수축을 이완시켰으며 효력은 higenamine보다 10배 컸다. 그러나 고농도 (3.3×10^{-6})의 isoproterenol은 내인성 활성을 보였다.
4. Higenamine과 isoproterenol은 potassium으로 유도된 폐동맥편 수축에 대하여 이완효과를 나타내지 않았다.

이상의 결과로 미루어 higenamine은 혈관평활근에 대하여도 아드레나린성 beta-수용체를 통하여 이완효과를 나타낼 것으로 생각된다.