

## Effect of Higenamine on $^3\text{H}$ -Norepinephrine Release in the Rabbit Pulmonary Artery<sup>1</sup>

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**Abstract**—It has been reported that higenamine increased the cardiac output and the heart rate and decreased the systemic vascular resistance and blood pressure by stimulating  $\beta$ -adrenoceptors. This study was undertaken to investigate the effects of higenamine on the pre- and post-synaptic adrenergic neurotransmission in the spiral strips of pulmonary arteries of New Zealand white rabbits. As the pre-synaptic response, the spontaneous and stimulation-induced release of  $^3\text{H}$ -norepinephrine ( $^3\text{H}$ -NE), and as the post-synaptic response, the contractile response were measured. The results were as follows:

1. Higenamine ( $3 \times 10^{-6}$ ,  $3 \times 10^{-5}$  and  $10^{-4}\text{M}$ ) increased both spontaneous and stimulation-induced  $^3\text{H}$ -NE release dose-dependently, by 20-90% and 20-220%, respectively but decreased the contractile response in a dose-dependent manner.
2. The effect of higenamine in increase of spontaneous and stimulation-induced  $^3\text{H}$ -NE release was partially inhibited by the treatment of propranolol ( $3 \times 10^{-6}\text{M}$ ), but the effect in contractile response was completely blocked. Isoproterenol ( $1.3 \times 10^{-8}$ ,  $1.3 \times 10^{-7}$  and  $1.3 \times 10^{-6}\text{M}$ ) had no effect on spontaneous and stimulation-induced  $^3\text{H}$ -NE release and contractile response.
3. The effect of higenamine was not inhibited by treatment with cocaine ( $3 \times 10^{-5}\text{M}$ ).
4. The  $\alpha_2$ -adrenoceptor antagonist, yohimbine ( $3 \times 10^{-8}$  and  $3 \times 10^{-7}\text{M}$ ) increased the stimulation-induced  $^3\text{H}$ -NE release but did not affect spontaneous release.
5. Papaverine ( $3 \times 10^{-6}$ ,  $3 \times 10^{-5}$  and  $10^{-4}\text{M}$ ) increased spontaneous and stimulation-induced  $^3\text{H}$ -NE release in a dose-dependent manner.

These results suggest that higenamine increases neurotransmitter release by the mechanism of presynaptic action through the enhancement of cyclic AMP level in addition to stimulation of the presynaptic  $\beta$ -receptor and that the mechanism of post-synaptic action of higenamine lies in the stimulation of  $\beta$ -adrenoceptors.

**Key words:** *Higenamine,  $\beta$ -adrenoceptor, Presynaptic  $^3\text{H}$ -norepinephrine release, Vascular smooth muscle*

### INTRODUCTION

Higenamine is an active component of Aconiti

tuber which has long been used as a cardiac stimulant, a diuretic or an analgesic in Chinese medicine. Park *et al.* (1984) have found that higenamine increases the rate of tension development and shortens both the time to peak tension and the total duration of contraction in a dose-dependent manner. These effects of higenamine were competitively blocked by proprano-

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lol, and the  $pA_2$  values of higenamine and epinephrine against propranolol were  $8.58 \pm 0.14$ ,  $7.50 \pm 0.82$ , respectively. they concluded that the positive inotropic action of higenamine was likely due to stimulation of cardiac adrenoceptors. It was also reported that higenamine increased the cardiac output and the heart rate, and decreased the systemic vascular resistance and the blood pressure (Chang *et al.* 1981; Chang 1983; Kim *et al.* 1986). These pharmacologic effects of higenamine were blocked by propranolol and it was suggested that higenamine has stimulating action on adrenergic  $\beta_1$  and  $\beta_2$ -receptors (Kim *et al.* 1986). In the studies about dissociation constants on  $\beta$ -receptors, it was proved that higenamine had the same affinity as isoproterenol (Feng *et al.* 1982). The results that higenamine exerted relaxant effect on the contraction of rabbit thoracic aorta by norepinephrine, and that these effects were largely inhibited by  $\beta$ -antagonist, propranolol, suggested that the relaxant effect of higenamine on the vascular smooth muscle may involve postsynaptic  $\beta$ -adrenoceptors (not published).

In this study, to investigate the effect of higenamine on the adrenergic neurotransmission, the release of  $^3H$ -NE from the sympathetic nerve terminal as the pre-synaptic response, and the contractile response of vascular smooth muscles, as the post-synaptic response, were examined.

#### MATERIALS AND METHODS

The healthy male New Zealand White rabbits, weighing 2-3 kg were sacrificed by a blow on the head, the thorax was opened and the main pulmonary artery was excised. The spiral strip made of pulmonary artery was transferred to Krebs' solution (NaCl 119.8 mM, KCl 4.6 mM,  $MgCl_2$  1.2 mM,  $CaCl_2$  2.5 mM,  $KH_2PO_4$  1.2 mM, glucose 11.1 mM, EDTA 2Na 1.5 mg/l and ascorbic acid 100 mg/l) equilibrated with 95%  $O_2$  and 5%  $CO_2$  at  $37^\circ C$  for 30 min.  $^3H$ -NE ( $3 \times 10^{-7} \mu M$ ,  $5 \mu Ci/ml$ , New England Nuclear Corp., specific activity 14.5 Ci/mmol) was uptaken into the spiral strips for 2 hr, and was washed out with norepinephrine-free Krebs' solution for 5 min. The spiral strip was mounted in an organ bath of perfusion apparatus in parallel to the platinum plate electrodes and was connected to an isometric force transducer with the tension of 2

g. The organ bath was perfused continuously with oxygenated (95%  $O_2$ -5%  $CO_2$ ) Krebs' solution ( $37^\circ C$ ) at a rate of 1.25 ml/min. After 1.5 hr, the spontaneous release of radioactive norepinephrine became constant, electrical stimuli of 2 ms, square wave pulses were applied for 60 s with the frequencies of 2, 5 and 20 Hz by Grass S 48 stimulator. The current voltage was modified to obtain the maximum contraction with the transmural field stimulation by the platinum electrodes. The contractile response developed was recorded by an isometric force transducer connected to a Device-recorder polygraph. Simultaneously, the perfused effluents of 1.25 ml were collected into vials every 1 min, from 1 min before the stimulation to 5 min after the stimulation and radioactivities of released  $^3H$ -NE measured by the liquid scintillation spectrometer (Nuclear Enterprises Ltd. NE 8310/1/2). Drugs were added to the medium 20 min before the nerve stimulation.

**The drugs used were:** higenamine (supplied from Dong-A Pharmaceutical Research Institute), l-isoproterenol HCl(Sigma), dl-propranolol HCl(Sigma), cocaine HCl(Sigma), yohimbine HCl(Sigma), papaverine HCl(Sigma), tetrodotoxin(Sigma).

#### RESULTS

After 1.5 hr perfusion, the spontaneous  $^3H$ -NE release without stimulation remained constant. With electrical stimulations of 2, 5, 20 and 50 Hz frequency,  $^3H$ -NE release in the perfusate was maximal between 1 min and 2 min after electrical stimulation. the release of  $^3H$ -NE and contractile response of vascular smooth muscles were potentiated proportionally to stimulation frequency (Fig. 1). Because the stimulation frequency of 5 Hz seemed to be ideal for determining the change of  $^3H$ -NE release evoked by stimulation, hereafter the stimulation frequency was set at 5 Hz.

In order to prove whether this electrical transmural stimulation of 5 Hz induced selectively the release of  $^3H$ -NE from presynaptic nerve terminal, tetrodotoxin was added to the medium in a concentration of  $2 \times 10^{-7} M$  for 20 min before electrical stimulation. It was found that stimulation-induced  $^3H$ -NE release and contractile response were inhibited almost completely by tetrodotoxin (Data not shown).

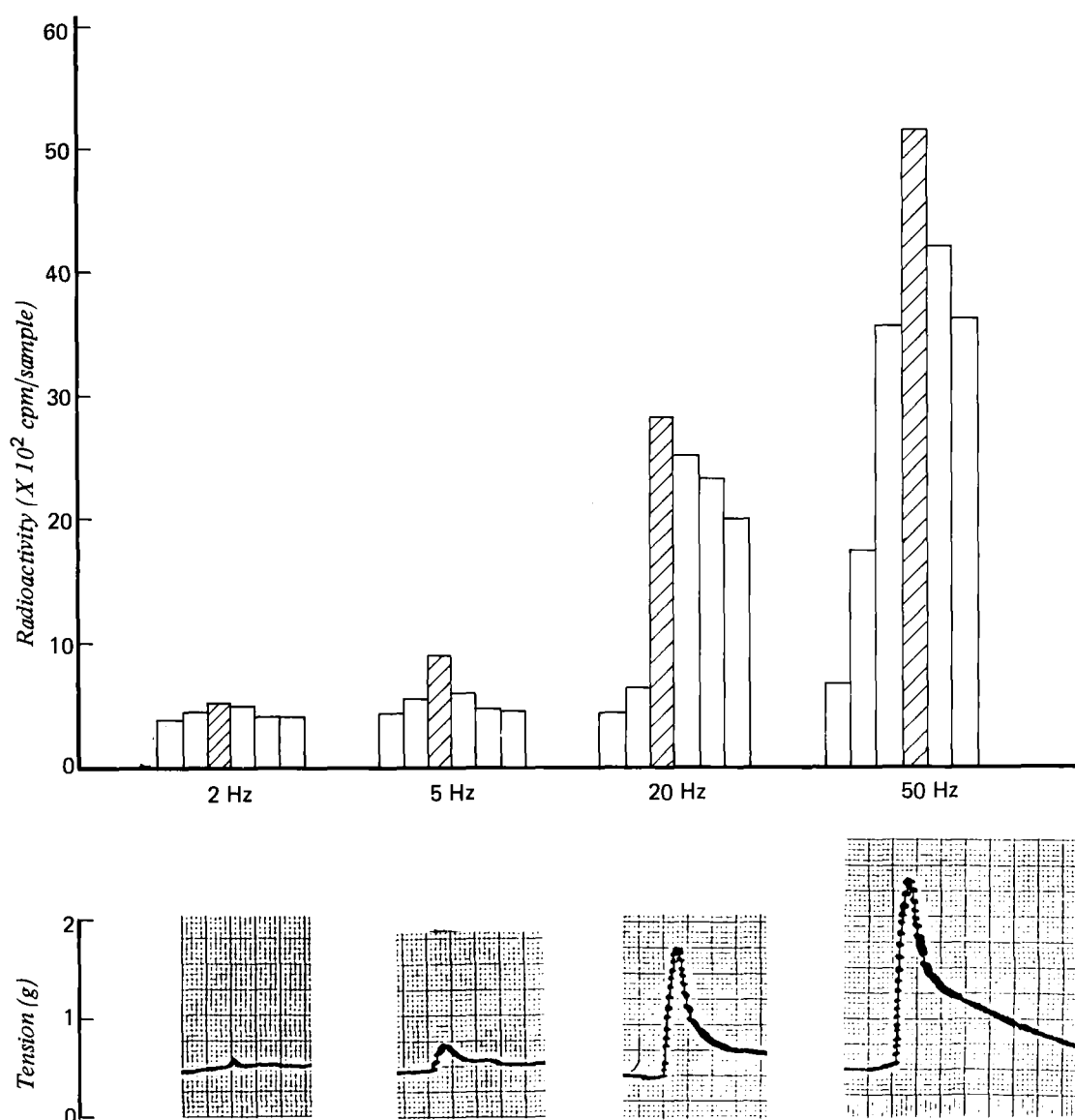


Fig. 1. Effect of transmurial field stimulation on release of <sup>3</sup>H-norepinephrine and contractile response in the perfused rabbit pulmonary artery. Arteries were preloaded with <sup>3</sup>H-NE ( $3 \times 10^{-7}$ M,  $5 \mu\text{Ci/ml}$ ) for 2 hours and perfused with norepinephrine-free medium. The perfusates were collected every 1 min and the radioactivities of released <sup>3</sup>H-NE were measured. The first open bars represent the spontaneous release and hatched bars represent the peak radioactivity of stimulation-induced release.

The spontaneous <sup>3</sup>H-NE release was not significantly changed in the control group by the four consecutive stimulation with 30 min interval. However, when higenamine was applied in the concentration of  $3 \times 10^{-6}$ ,  $3 \times 10^{-5}$  and  $10^{-4}$ M, the spontaneous release was increased by 21, 51 and 91% respectively, compared with the first stimulation period. With higenamine in the concentrations of  $3 \times 10^{-6}$ ,  $3 \times 10^{-5}$  and  $10^{-4}$ M, the stimulation-induced releases were increased significantly by 21, 176 and 219% respectively, compared with the first stimulation period. The contractile responses were

also significantly inhibited by higenamine, dose-dependently (Table 1).

When  $\beta$ -blocker propranolol ( $3 \times 10^{-6}$ ,  $3 \times 10^{-5}$  and  $10^{-4}$ M) was applied for 20 min before electrical stimulation, the effect of higenamine in increase of spontaneous and stimulation-induced <sup>3</sup>H-NE release was not completely inhibited while the effect of higenamine on contractile response was completely blocked (Fig. 2).

When a major  $\beta$ -agonist, l-isoproterenol ( $1.3 \times 10^{-8}$ ,  $1.3 \times 10^{-7}$  and  $1.3 \times 10^{-6}$ M) was applied for 20 min before electrical stimulation, spontaneous and stimulation-induced <sup>3</sup>H-NE re-

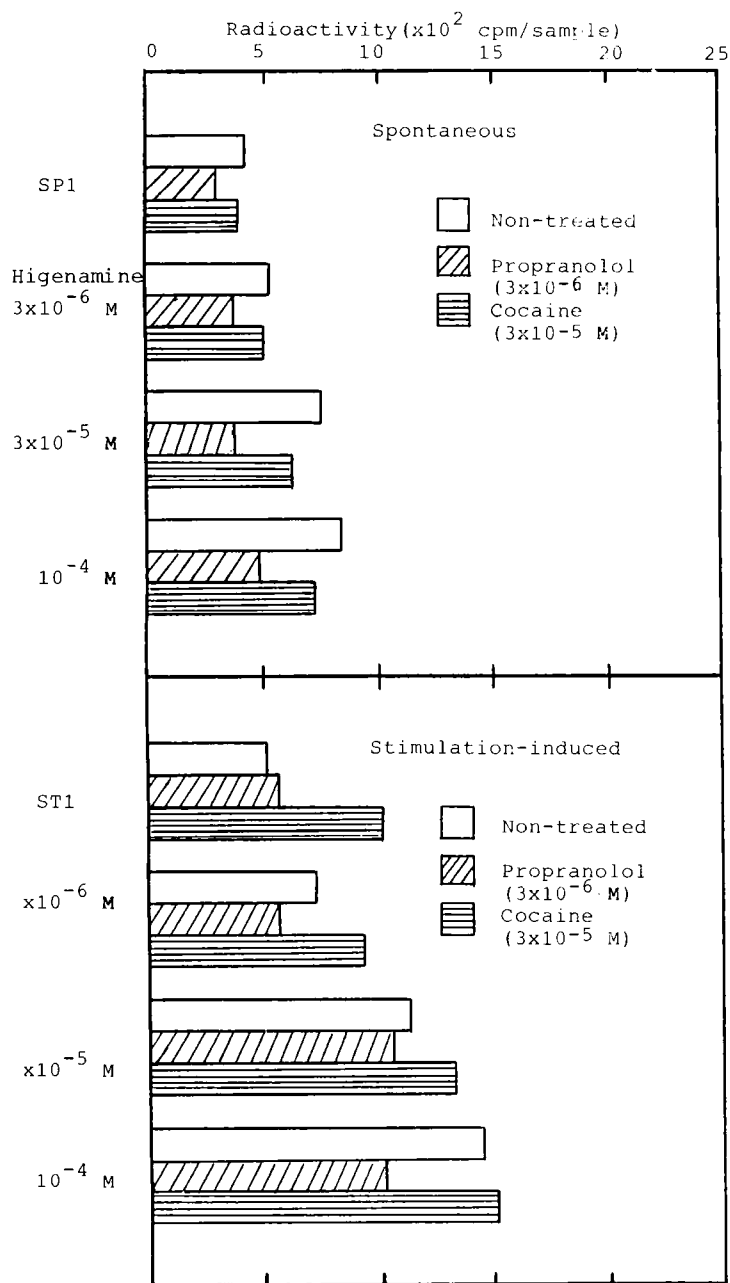


Fig. 2. Effect of higenamine in the presence of propranolol or cocaine on <sup>3</sup>H-NE release from the rabbit pulmonary artery. Higenamine was perfused after propranolol or cocaine pretreatment. Stimulation-induced release was calculated by subtraction of the spontaneous release from the peak value of stimulation-induced release. SP1: spontaneous release without higenamine, ST1: stimulation-induced release without higenamine.

lease and contractile response showed no change compared with that of control group (Fig. 3).

When cocaine ( $3 \times 10^{-5}$ M) and higenamine were simultaneously applied, there were no change in the higenamine effect in increase of spontaneous and stimulation-induced <sup>3</sup>H-NE re-

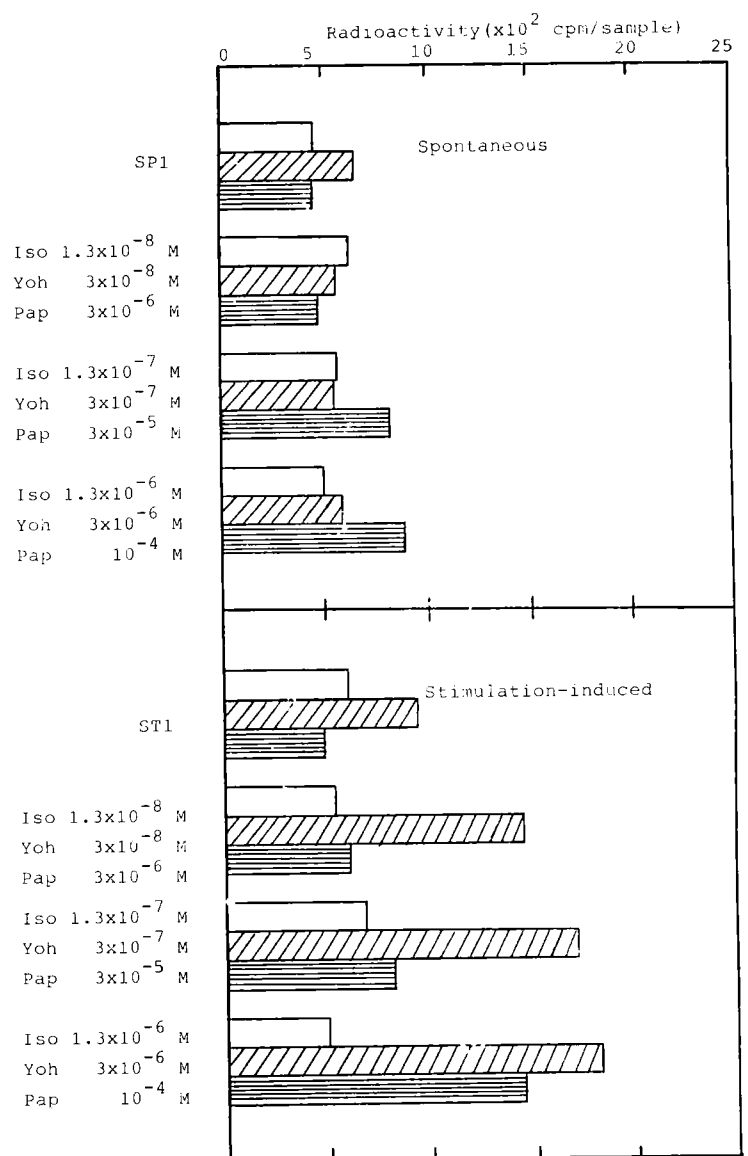


Fig. 3. Effect of isoproterenol, yohimbine and papaverine on H-NE release from rabbit pulmonary artery.

Drugs were applied for twenty min during S2 to S4 with different concentration. Iso: Isoproterenol, Yoh: yohimbine, Pap: papaverine. SP1 and ST1: spontaneous and stimulation-induced release without drug treatment respectively.

lease and decrease of contractile response (Fig. 2).

Yohimbine ( $3 \times 10^{-8}$ ,  $3 \times 10^{-7}$  and  $3 \times 10^{-6}$ M) increased the stimulation-induced <sup>3</sup>H-NE release, dose-dependently, but did not affect the spontaneous release (Fig. 3). contractile response was potentiated in low concentrations, proportionally to the increase in <sup>3</sup>H-NE release, but was inhibited in high concentrations ( $3 \times 10^{-6}$ M).

**Table 1.** The influence of higenamine on spontaneous and stimulation-induced  $^3\text{H}$ -norepinephrine release from spiral strips of rabbit pulmonary arteries.

	Spontaneous				Stimulation-induced			
	S1	S2	S3	S4	S1	S2	S3	S4
Control	100	100.4 $\pm 2.1$ (6)	103.3 $\pm 5.8$ (6)	88.6 $\pm 6.3$ (6)	100	91.3 $\pm 2.9$ (6)	95.2 $\pm 3.2$ (6)	87.2 $\pm 4.5$ (6)
Higenamine	100	$3 \times 10^{-6}\text{M}$ 120.9** $\pm 4.2$ (4)	$3 \times 10^{-5}\text{M}$ 150.7** $\pm 12.3$ (4)	$19^{-4}\text{M}$ 191.3** $\pm 16.3$ (4)	100	$3 \pm 10^{-6}\text{M}$ 120.8* $\pm 11.0$ (4)	$3 \pm 10^{-5}\text{M}$ 276.2** $\pm 39.2$ (4)	$10^{-4}\text{M}$ 316.6** $\pm 23.9$ (4)

Note: S1 to S4 represent four stimulation periods at 30 min intervals (5Hz, 2m sec, for 60 sec). The results of  $^3\text{H}$ -NE release are expressed as a percent release to that in the first stimulation period (S1). Higenamine was infused during S2 to S4. The parentheses represent the number of experiments.

\* $P < 0.05$ , \*\* $P < 0.01$ , statistical difference between the control and the higenamine-treated arteries.

With papaverine, inhibitor of cyclic nucleotide phosphodiesterase, in the same concentrations as higenamine ( $3 \times 10^{-6}$ ,  $3 \times 10^{-5}$  and  $10^{-4}\text{M}$ ), spontaneous and stimulation-induced release of  $^3\text{H}$ -NE were increased by 20-230%, but the contractile response to the nerve stimulation was inhibited dose-dependently (Fig. 3).

### DISCUSSION

In this study, as the presynaptic response, the norepinephrine release from the sympathetic nerve terminal was estimated by assaying the radioactivities of  $^3\text{H}$ -NE in the perfused effluents. The radioactivities in the perfused effluent may not represent the total norepinephrine released from the synaptic nerve terminal, since a considerable amount of the released norepinephrine can be excluded from the effluent either by active reuptake into the synaptic nerve terminal (Iversen 1967) or by postsynaptic receptor binding and extraneural uptake. However, it is reasonable for studying the presynaptic response to analyze the relative changes in the radioactivities of perfusate after second, third and fourth stimuli versus the first because within the four stimulation periods with a 30 min interval, contractile response and  $^3\text{H}$ -NE release were increased proportionally to the stimulation frequency. Also the study could be performed with a drug in three different concentrations, because the radioactivities were

maintained over 80% of the initial radioactivities even after the fourth stimulation (Data not shown).

When the various concentrations of higenamine was applied for 20 min before stimulation, higenamine significantly facilitated not only spontaneous  $^3\text{H}$ -NE release but also stimulation-induced release (Table 1). On the other hand, the contractile responses were decreased by higenamine treatment. The inhibitory effects of higenamine on contraction were blocked by  $\beta$ -blocker, propranolol. This finding suggests that the mechanisms of inhibitory action of higenamine on contractile response involves the post-synaptic  $\beta$ -receptor.

The facilitatory effects of higenamine on spontaneous and stimulation-induced  $^3\text{H}$ -NE were not completely inhibited by the treatment with  $\beta$ -blocker, propranolol, even with the sufficient concentration that blocked completely the inhibitory effect of higenamine on contraction (Fig. 2). Furthermore the  $\beta$ -agonist, isoproterenol, exerted no significant effect on  $^3\text{H}$ -NE release. These findings indicate that the major parts of higenamine effect on  $^3\text{H}$ -NE release are hardly mediated by  $\beta$ -receptor. Alternatively, since the enhancements of norepinephrine release by the positive feedback of  $\beta$ -agonist are modest and may not be significant under normal circumstances (Dixon *et al.* 1979), thus it could be postulated that the mechanism of ac-

tion of higenamine in increase of  $^3\text{H}$ -NE release may be due to inhibition of negative feedback in norepinephrine release by the blockade of pre synaptic  $\alpha$ -receptor. However, in the treatment with yohimbine which selectively blocks  $\alpha$ -receptor, no change appeared in spontaneous release of  $^3\text{H}$ -NE compared with increase in stimulation-induced release (Fig. 3). Concerning the structural similarity of higenamine with dopamine (Suh *et al.* 1984), it can be presumed that higenamine exerts the facilitatory effect on norepinephrine release by displacing it from the storage site of the sympathetic nerve terminal through the reuptake system. But the effects of higenamine were not inhibited by cocaine (Fig. 2), while cocaine completely inhibited dopamine induced spontaneous release of  $^3\text{H}$ -NE (not published).

Since papaverine, which also has structural similarity with higenamine, exerted facilitatory effect on spontaneous and stimulation-induced release of  $^3\text{H}$ -NE (Fig. 3), it was suggested that higenamine might involve increase of presynaptic cyclic AMP. Feng *et al.* (1981) and Gang & Liu (1985) suggested that higenamine potentiated the activity of adenylate cyclase.

Analyzing the effect of higenamine on increase in  $^3\text{H}$ -NE release and contractile response in rabbit pulmonary artery, it is concluded that higenamine increases neurotransmitter release in the adrenergic nerve system of rabbit pulmonary artery, possibly through enhancement of cyclic AMP level, in addition to stimulation of presynaptic  $\beta$ -adrenoceptors.

## REFERENCES

- Brun JH, Gibbons WR. The release of noradrenaline from sympathetic fibers in relation to calcium concentrations. *J. Physiol(Lond.)*. 1965, 181:214-223
- Burnstock G, Crowe R, Wong HK. Comparative Pharmacological and histochemical evidence for purinergic inhibitory innervation of the portal vein of the rabbit, but not guinea pig. *Br. J. Pharmacol.* 1979, 65:377-388
- Chang KC. The effect of Higenamine upon the interval strength relationship in isolated rabbit heart. *Korean J. Pharmacol.* 1983, 19(2):9-16
- Chang KC, Kim JK, Park CW, Kim MS. Studies on the mechanism of positive inotropic action of Higenamine. *Korean J. Pharmacol.* 1981, 17(2):59-68
- Dixon WR, Mosimann WF, Weiner N. The role of pre-synaptic feedback mechanisms in regulation of norepinephrine release by nerve stimulation. *J. Pharmacol. Exp. Ther.* 1979, 209:196-204
- Feng YP, Jia HJ, Zang CY, Zeng GY. Effect of dl-demethyl coclaurine on beta adrenergic receptors and adenylate cyclase in turkey erythrocyte membrane. *Acta Pharmaceutica Sinica.* 1982, 17:641-646
- Feng YP, Zhang Y, Zhan HS, Zeng GY. Effect of dl-demethyl coclaurine on plasma cAMP in mice. *Acta Pharmacologica Sinica.* 1981, 2:114-117
- Gang WZ, Liu GZ. Advances in natural products in China. *T.I.P.S.*, 1985, 6:423-426
- Iversen II. The uptake and storage of noradrenaline in sympathetic nerves. *Cambridge University Press, London.* 1977
- Kim NS, Hong CY, Park CW, Lim JK. An experimental study on the adrenergic effect of higenamine in rabbit cardiovascular system. *Korean Circulation J.* 1986, 16:1-18
- Misu Y, Kaiho M, Ogawa K, Kubo T. Adrenergic transmission failure via the blockade of presynaptic beta receptors in guinea-pig pulmonary arteries. *J. Pharmacol. Exp. Ther.* 1981, 218:242-247
- Park CW, Chang KC, Lim JK. Effects of higenamine on isolated heart adrenoceptor of rabbit. *Arch. Inter. Pharmacodyn. Ther.* 1984, 267:279-288
- Starke K, Endo T, Taube HD. Relative pre- and post-synaptic potencies of  $\beta$ -adrenoceptor agonists on the rabbit pulmonary artery. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 1975, 291:55-78
- Su C, Bevan JA. The release of  $^3\text{H}$ -norepinephrine in arterial strips studied by the technique of superfusion and transmural stimulation. *J. Pharmacol. Exp. Ther.* 1970, 172:62-68
- Suh YH, Park HY, Lim JK, Park CW. Effects of higenamine and its derivatives on the activity of rat brain mitochondrial monoamine oxidase. *Korean J. Pharmacol.* 1984, 20:73-80
- Vanhoutte PM, Collis MG, Janssens WJ, Verbeuren TJ. Calcium dependence of prejunctional inhibitory effects of adenosine and acetylcholine on adrenergic neurotransmission in canine saphenous veins. *Eur. J. Pharmacol.* 1981, 72:189-198

= 국문초록 =

## 토끼 폐동맥에서 Higenamine의 <sup>3</sup>H-Norepinephrine 유리에 대한 효과

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Higenamine( $C_{16}H_{17}NO_3 \cdot HCl$ , dl-1-(4-hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline hydrochloride)은 한방에서 강심, 이뇨 및 진통등의 목적으로 사용되어온 부자의 활성성분으로서, 심근 수축력 증가, 심박동수 증가, 전신말초저항 감소 및 혈압 강하를 나타내는데 이들작용은  $\beta$ -아드레날린 수용체를 경유할 가능성이 시사되었다. 본 연구에서는 Higenamine의 교감신경전달에 미치는 영향을 규명하기 위하여, 웅성의 토끼(New Zealand White)의 폐동맥을 절제하여 나선형 절편을 만들어 방사성 norepinephrine(<sup>3</sup>H-norepinephrine)을 흡수시킨 다음 관류장치에 조직을 현수하고 백금판에 의한 빈도 5Hz, 기간 2m sec의 방형과자극을 60초동안 시행하였다. 접합전부반응의 검토로서는 관류액 방사활성의 측정을 통하여 <sup>3</sup>H-norepinephrine(이하 <sup>3</sup>H-NE라고 생략함)의 자발적 및 자극에 의한 유리를 관찰하였고, 접합후부 반응의 검토로서는 등거리성 근수축변환기와 polygraph를 사용하여 조직표본의 수축반응을 관찰하여 다음과 같은 결론을 얻었다.

1. Higenamine은 토끼 폐동맥에서 교감신경전달에서의 신경전달물질 유리를 증가시킨다.
2. Higenamine의 작용기전이 접합전부에서는 대부분의 효과가 cyclic AMP증가에 의한 가능성이 크다.
3. Higenamine의 작용기전이 접합후부에서는 대부분의 효과가  $\beta$ -수용체를 경유하여 나타난다.