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The Effect of Higenamine upon the Interval-Strength Relationship in Isolated Rabbit Heart

Ki Churl Chang

Dept. of Pharmacology, College of Medicine, Gyeong Sang National University

=국문요약=

적출 가토 심장에 있어서 수축빈도—수축력 상관 관계에 대한 Higenamine의 강심 효과

경상대학교 의과대학 약리학교실

장 기 철

현재 사용되고 있는 강심제로는 디지탈리스 배당체가 유일한 약물이지만 그 안전역이 대단히 좁아서 보다 안전한 강심제의 개발이 요구되고 있다.

최근 강심작용이 현저할 뿐 아니라 안전역이 비교적 넓을 것으로 인정되는 higenamine이 개발되어 그 작용기전을 밝히려는 시도가 많이 있었다. 본 연구에서는 higenamine의 강심작용 기전을 규명하기 위한 연구의 일환으로 수축빈도—수축력 상관관계(interval-strength relationship)에 있어서 1) Rested-state 수축력 2) Positive inotropic effect of activation(PIEA) 3) Negative inotropic effect of activation(NIEA)에 대한 higenamine의 영향을 Ca^{++} 및 epinephrine과 비교 분석하였다.

실험결과 칼슘은 PIEA를 증가시키기 보다는 오히려 NIEA의 소멸을 촉진함으로써 강심작용을 나타내며 epinephrine 및 higenamine은 PIEA를 증가시키고 NIEA에는 거의 영향을 미치지 않았다.

INTRODUCTION

It is of interest to study as to whether what kinds of natural plants have pharmacological effects and is also attractive to investigate the mechanism(s) of action if they have.

Among them aconite tuber is being actively investigated to find out the mechanism of action. Aconite tuber, the root of Aconiti Radix, which belongs to one of Ranunculaceae family, has long been used as a heart stimulant, diuretic agent, and painkiller in chinese medicine. But the active component of it was not known until 1979.

Kosuge et al.(1979) isolated a compound from Aconite root by limited combination of gel filtration through Sephadex LH-20 and counter current distribution and named it as higenamine.

Subsequently, Chang et al.(1982) confirmed the marked positive inotropic effect of higenamine using rabbit heart and supposed that its potency might 1,000 times greater than that of the butanol fraction of Aconite tuber.

Even though great advances having been made for grasping the mechanism(s) of action

by which cardiotonic agents exert their pharmacological effect, there still remains somewhat controversial in the exact mechanism(s) of action of them.

Since Bowditch(1871) had pioneered the influence of interval between contractions on their strength in experiments with electrically stimulated preparations of the frog heart, there have been made many attempts to unravel the inotropic action machanism of cardiotonic agents with the hope that the interval-strength relationship might give some clues.

Blink et al.(1961) proposed the hypothesis that the interval-strength relationship arises from the interaction of three factors(see below), and coined that contractions recorded after an intervals of rest which is so long that their strength is independent of previous activity as "rested-state contraction", the effect of increasing in the force which occurs with reduction in the interval between contractions was termed as the "positive inotropic effect of activation or PIEA", and the effect giving rise to the diminution of the strength of contraction as "negative inotropic effect of activation or NIEA".

According to their hypothesis that drugs having cardiotonic effect might influence one of these three bring about the increment of strength of contraction.

Higenamine showed the positive inotropic effect by influencing the movement of calcium ion through sarcolemma(Chang et al., 1982) and shortened not only the time of total duration of contraction but time to peak tension (in press).

Hence, the purpose of this study was to analyze the effect of higenamine upon the interval-strength relationship in terms of the hypothesis described above, and effects of epinephrine, calcium on the interval-strength relationship were also discussed.

METHODS AND MATERIAL

1) Preparation of isolated heart muscle

Rabbits of either sex ranging in weight 1.5 ~3 kg were killed by a blow on the head. The thorax was opened and the heart was removed rapidly and transfered to the Krebs' solution equilibrated with 95% O_2 −5% CO_2 at room temperature, where left atrium was swiftly excised.

The atrium was mounted in an organ bath containing 10 ml of continuously oxigenated Krebs' solution of composition in mM(pH 7.4); NaCl 119.8, KCl 4.6, $CaCl_2 \cdot 2H_2O$ 2.5, $MgCl_2$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and d-glucose 10. The solution was kept at a temperature 37°C during the whole experiment by circulating water bath. One end of the tissue was tied to platinum electrode and the other was ligated to stainless clamp connected to isometric force transducer.

2) Method

The isolated heart muscle was driven electrically by square wave impulse through platinum electrode paralleled to the muscle for field stimulation (Grass model s6c). The impulse was of 5 msecond duration and the current was slightly greater than threshold voltage, the preparations used were quiescent unless stimulated. Contractions were recorded on a physiograph.

In each preparation the developed tension was allowed to reach a steady-state at 12 different frequencies of contraction and from these data a steady-state interval force curve was plotted.(Fig. 1 and 2). NIEA decay curves were determined by placing test contractions at many diffrent intervals after rested-

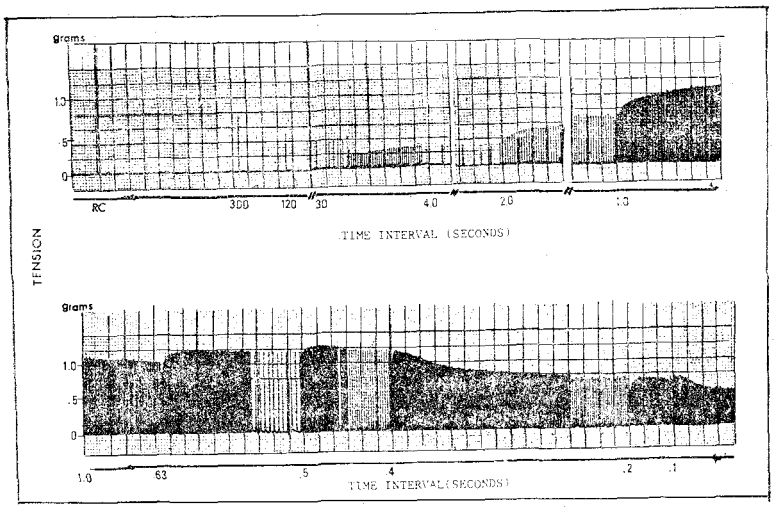

Fig. 1. Interval-strength relationship in heart muscle. Rabbit, left atrium, 37°C. Tracing: tension developed by contractions. Numbers under tracings indicate intervals between beats in seconds: RC indicates rested-state contraction.

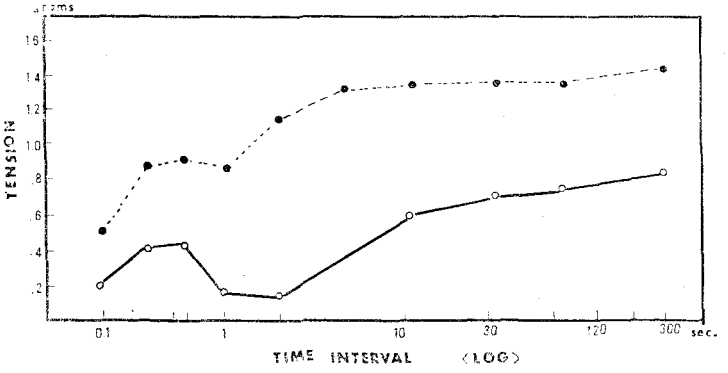

Fig. 2. Effects of calcium on the interval-strength curve of rabbit auricle.
●·····● calcium 5mM, ○———○ calcium 2.5 mM

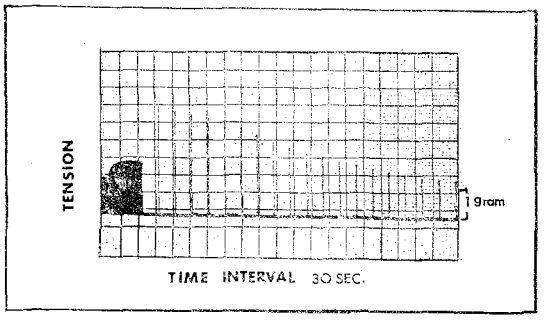

Fig. 3. An example of PIEA measurement.

state contractions. The time course of the disappearance of the NIEA produced by a single contraction was thus established.

The amount of the NIEA produced by a single beat cannot be determined accuratedly since the rate of decay is initially high and changes rapidly. PIEA decay curves (Fig. 3) were determined by a) allowing the PIEA to be accumulated during short-interval stimulations, b) switching to a long interval at which only a negligible amount of PIEA was present under steady-state conditions, c) subtracting the steady-state force at the long interval from the force of each beat during the transition phase, d) plotting the difference against the time elapsed since the end of the rapid

stimulation.

These determinations were made while the preparations were suspended in drug-free solution and after the full effect of epinephrine (2×10^{-7}M), higenamine(10^{-6}M, 5×10^{-6}M) had been reached.

3) Quantitative analysis of NIEA and PIEA

The decay of the PIEA with time is exponential, so it can be written as follows:

$$p=p_0e^{-t/k_p}$$

where p is the amount of the PIEA present at time t, p_0 is the amount present at time zero, and k_p is a constant which has the dimension of time and represents the time at which 1/e or 37% of p_0 remains.

Both p_0 and k_p can be determined graphically from experimental plots such as that of Fig. 4.

Under steady-state conditions p, the amount of PIEA produced per beat at the interval t, is equal to the amount of the PIEA which decays in the interval between beats.

Thus, $p=p_0-p_0e^{-t/k_p}$

p and k_p serve as quantitative indices of the rates of production and disappearance of the PIEA respectively.

RESULT

1) Effect of calcium on the interval-strength curve

polyphasic patterns were seen by calcium ion on the interval-force curve of rabbit atrial muscle as is in Fig. 2. Each point indicates the tension developed after a steady-state force of contraction had been reached at one of 12 different frequencies of regular beats.

Contractions after intervals of 300 are rested state contraction, which has the greatest force. Shortening of the interval in the range of 300 to 2 second, the steady state force of contraction reduced. In this range, the residual NIEA exceeded that of PIEA. As interval was further shortened, the residual PIEA exceeded that of NIEA and the steady state force of contraction rised a second but lower maximum than the rested state contraction.

When the concentration of calcium was duplicated, the rested state contraction increased markedly, and the disappearance of NIEA was significantly influenced, but scarcely affected the PIEA produced per beat as shown

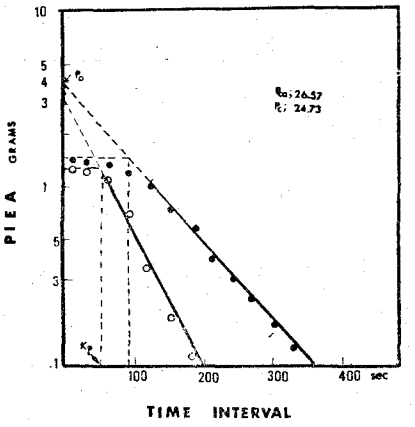

Fig. 4. Effect of calcium on the disappearance of PIEA with time.
○—○ calcium 2.5 mM
●·····● calcium 5.0 mM

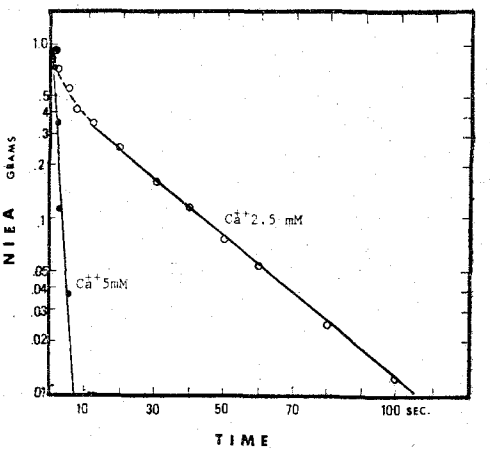

Fig. 5. Effects of calcium on the disappearance of the negative inotropic effect of activation (NIEA) with time.

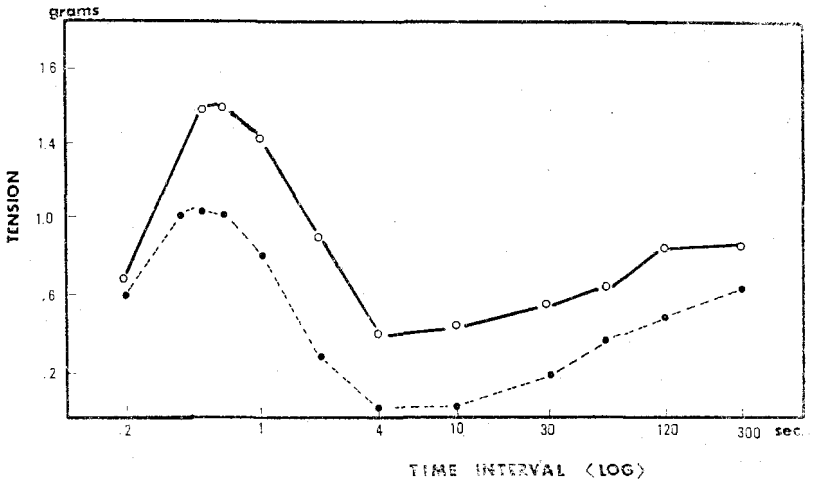

Fig. 6. Effects of epinephrine on the interval-strength curve of rabbit atrial muscle.
●·····● : control, ○——○ epinephrine 2×10^{-7}M

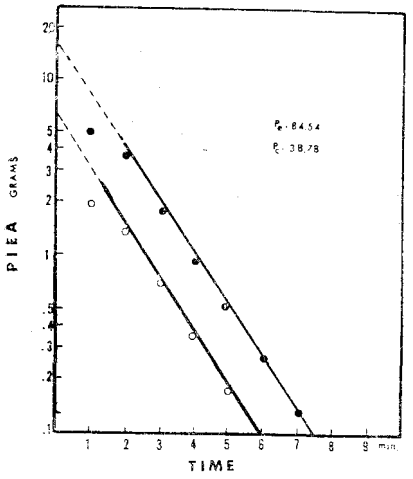

Fig. 7. Effect of epinephrine on the disappearance of the PIEA with time.
○——○ control
●·····● epinephrine 2×10^{-7}M

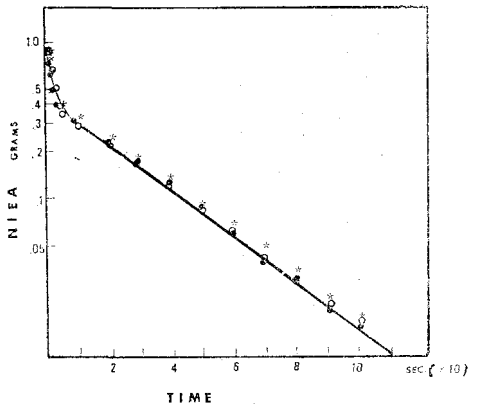

Fig. 8. Effect of epinephrine on the disappearance of the negative inotropic effect of activation(NIEA) with time.
○——○ control
●·····● epinephrine 2×10^{-7}M

in Fig. 4 and 5.

2) Effect of epinephrine on the interval-strength curve

Interval-strength curve by epinephrine was dipicted in Fig. 6, which also shows polyphasic pattern. Epinephrine slightly affected the rested-state contraction. In the range of 300 to 10 second, residual NIEA exceeded that of PIEA.

When interval was as shortened as 0.5 second, the steady state force of contraction rised higher than the rested state contraction.

Epinephrine increased the amount of the PIEA produced per beat from 38.78 to 84.54 mg, but didn't affect the disappearance of the NIEA as is in Fig. 7 and 8.

3) Effect of higenamine on the interval-strength curve

As shown in Fig. 9, higenamine effect on

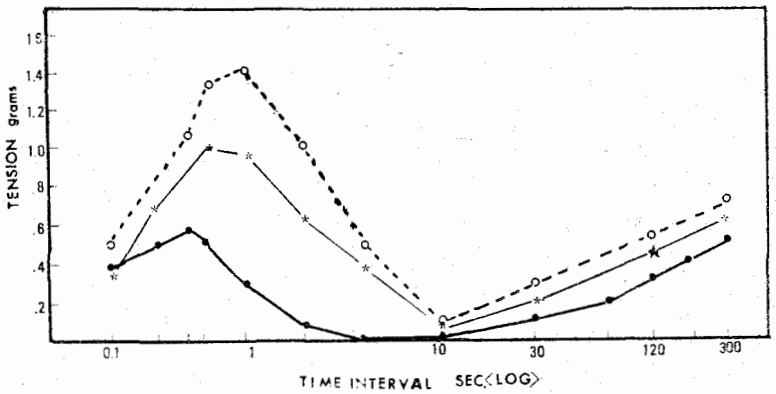

Fig. 9. Effect of higenamine on the interval-strength curve of rabbit atrial muscle.
●——● control, ☆——☆ higenamine 10^{-6}M, ○·····○ higenamine 5×10^{-6}M

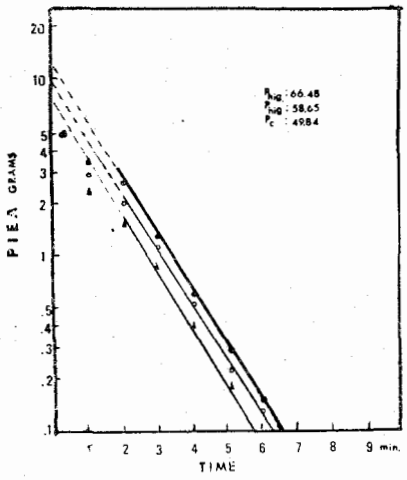

Fig. 10. Effect of Higenamine on the disappearance of PIEA with time.
▲——▲ control
○——○ Higenamine 10^{-6}M
●——● Higenamine 5×10^{-6}M

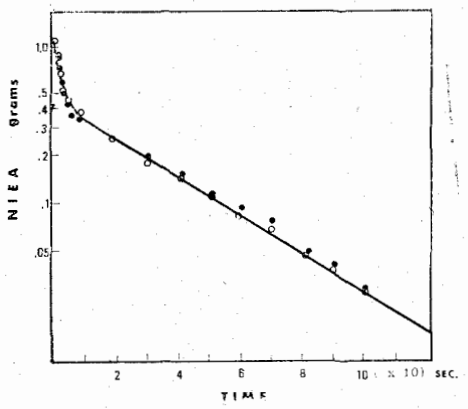

Fig. 11. Effect of higenamine on the disappearance of the negative inotropic effect of activation(NIEA) with time.
○——○ control
●——● higenamine 10^{-6}M,
☆——☆ higenamine 5×10^{-6}M

the interval-strength curve, was quite similar that of epinephrine.

Higenamine, dose-dependently, increased the amount of PIEA produced each beat, that is,; P value of control is 49.84 mg, when higenamine was introduced in concentration of 10^{-6} M, the amount of PIEA produced each beat was increased up to 58.65 mg, and 66.48 mg was obtained for 5×10^{-6}M concentration of higenamine.

On the other hand, the disappearance of NIEA was seldomly affected as in Figure 11.

DISCUSSION

In mammalian heart muscle, both NIEA and PIEA are generated whenever there is a propagated action potential. There are some species differences in showing the inotropic action of cardiotonic agents on the interval-strength relationships(Furchgott et al., 1958).

Blink et al.(1961) suggested that increment of contractility ensues when the accumulation

of PIEA exceeds that of NIEA at any given moment and vice versa. Atrial muscle differs from ventriuclar muscle in that the duration of action potential and of the active state are not affected by changes in the interval. Therefore, changes in the force of contraction with changes in the interval can be attributed entirely to the algebraic sum of residual NIEA and PIEA.

In the present study, calcium ion increased the rested-state contraction markedly but almost didn't affect PIEA. Instead, NIEA was rapid disappeared by calcium ion as in Fig. 4 and 5. This means that calcium ion exerts its own positive inotropic action not by in creasing PIEA but by decreasing NIEA, which correlated with those of Lim et al. earlier reported in 1977.

Epinephrine, on the other hand, increased the amount of PIEA produced each beat exceedingly from 38.78 mg to 84.54 mg, but didn't affect the disappearance of NIEA. Lim et al. (1977) reported that the positive inotropic effect of Aconite tuber butanol fraction at various contraction frequency might be due to increase of the PIEA produced by each beat.

Higenamine, dose-dependently, increased the amount of PIEA but not affected PIEA as shown in Fig. 10 and 11. More over, higenamine showed the positive inotropic action by accompanying of shortening the time required to peak tension as well as total duration of contraction time that was the mechanical characteristic features of catecholamines and the pA_2 value against propranolol was quite similar to that of epinephrine (in press). These findings imply that both epinephrine and higenamine might influence on the interval-strength relationship through same mechanism, even though the causes giving rise to PIEA and NIEA have not been fully elucidated.

There are many suggestions that both PIEA and NIEA reflect changes in excitation-contraction coupling (Koch-Weser et al., 1962) and the availability of calcium ions for the contraction. (Sands et al., 1970).

As described in introductory part, this study was confined only to analyze the effect of higenamine on the interval-strength relationship in terms of the hypothesis suggested by Blink et al., it is difficult to say what mechanisms involved in increasing the PIEA by higenamine. But there still remain lots of things to be unraveled the precise causes that evoke the PIEA and NIEA on the heart muscle.

SUMMARY

The effect of higenamine upon the interval-strength relationship was kinetically analyzed, and compared them with epinephrine and calcium ion.

The followings are result obtained:

1) Polyphasic patterns were seen by all agents applied on the interval-force curve of rabbit atrial muscle.

2) Higenamine, unlike calcium ion, increased the amount of PIEA produced per beat dose-dependently and scarcely affected the disappearance of NIEA.

3) Higenamine appeared to similar pattern with epinephrine in augmenting the PIEA, not affecting the NIEA.

4) Calcium ion slightly influenced the PIEA, rather hastened the disappearance of NIEA.

From these result the positive inotropic action of higenamine was attributed solely to increment of PIEA.

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