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Effects of Verapamil and Tetracaine on Acetylcholine-and Oxytocin-induced Uterine Contraction Pattern

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ABSTRACT

The effects of verapamil and tetracaine on acetylcholine-and oxytocin-induced contraction of uterus from estrogen-treated rat were examined. Isometric tensions were recorded on the Physiograph and stored in TriGem 20XT computer as digitized data for off-line analysis of the components, which described the contraction patterns: trought tension (T), peak tension (P), contraction frequency (F), and duration (D).

In the acetylcholine-induced contraction, verapamil (0.25 μM) significantly decreased P and D. In contrast, tetracaine (42μM) decreased F, but increased D. In the low oxytocin-induced contraction, verapamil (0.25μM) decreased P and D, and tetracaine (42 μM) decreased F but increased D. In the high oxytocin-induced contraction, verapamil decreased P and D, but tetracaine decreased P without affecting on other components.

These results suggest that the analysis of effects of a certain inhibitor on the components of contraction allow to postulate its specific inhibitory mechanism of the smooth muscle contraction.

Key Words: Uterine contraction, Verapamil, Tetracaine, Acetylcholine, Oxytocin

INTRODUCTION

Smooth muscle contraction is closely related to levels of intracellular free calcium. Intracelluar calcium levels are regulated by transport via calcium-channel (Baker, 1972; Reuter, 1973; Puteney, 1978; Bolton, 1979; Kostyuk, 1980), calcium-pump (Schatzmann, 1975), Na^+, Ca^{2+}-exchange site (Blaustein, 1974; Soloff & Sweet, 1982; Grover *et al* ., 1983), and uptake and release from sarcoplasmic reticulum, and release from the binding site of cell membrane (Carafol & Crompton, 1978). There have been some reports on the effects of drugs with the specific inhibitory action on the stimulant-induced contraction to interpret the relation between smooth muscle contraction and intracellular free calcium concentration (Sybertz *et al*., 1983; Raeburn *et al*., 1986; Ratz & Flaim, 1983; Nakaki *et al*., 1985; Nielsen-Kudsk *et al*., 1986; Karaki *et al*., 1984). Since changes of contraction tension were evaluated in these experiments, in vitro drugs with different inhibitory mechanisms may produce the same decrease in tension without any other changes. Therefore, we considered that more information for control mechanism of intracellular calcium concentration may be obtained when effects of selective inhibitory agents on contraction frequency and duration as well as tension of contraction produced by stimulants are examined.

In this report, contraction produced by acetylcholine and oxytocin was divided into four components, and effects of verapamil and tetracaine on these components were observed to give more information about the mechanism controlling contraction pattern.

MATERIALS AND METHODS

Female Sprague-Dawley rats weighing 150 -250 g were injected subcutaneously with 0.1 mg estradiol valerate (Progynon depot, Schering Korea Ltd.) in soybean oil per rat. 2 days after the

injection the rats were anesthetized with ether and killed by cutting carotid artery and the uterine horns were removed. The tissues were placed in Tris-Tyrode's solution at 0℃ and excess fat and connective tissue were trimmed. The cervical side of uterine horn was cut open along its mesometrial borders and four 1 mm×5 mm longitudinal strips per horn were prepared and used for recording isometric tension change.

The strips were mounted separately in 80 ml organ baths containing Tris-Tyrode's solution of the following composition (mM): NaCl, 136.9; KCl, 2.7; $MgCl_2$, 1.0; $CaCl_2$, 1.8; D-Glucose, 5.6; tris (hydroxymethyl) aminomethane, 8.3; pH adjusted with 1 N HCl to 7.4 at 37℃. The solution was aerated with 100% O_2 and kept at 37±1℃. Isometric contractions were recorded on a Physiograph model IV-P (Narco Biosystem, USA) and stored in TriGem 20 XT computer as digitized data with 1 sec sampling interval for off-line analysis of the components that describe the contraction patterns such as trough tension (T), peak tension (P), contraction frequency (F) and duration (D) (Fig. 1). The initial tension applied to the strip was adjusted to 0.5 g. After 30-60 minutes equilibration in the solution, the preparations were stimulatied with 6.9 μM acetylcholine or 6.25 IU/L oxytocin (standard). Each agoinst was applied cumulatively to the organ bath in concentrations from threshold to sub- or supra-maximal levels (cumulative concentration-response) in the presence or absence of inhibitors, verapamil or tetracaine contained in the solution. The response at each concentration was observed for 10 minutes. Each component of contraction at each concentration of stimulants was expressed as a fraction of its standard.

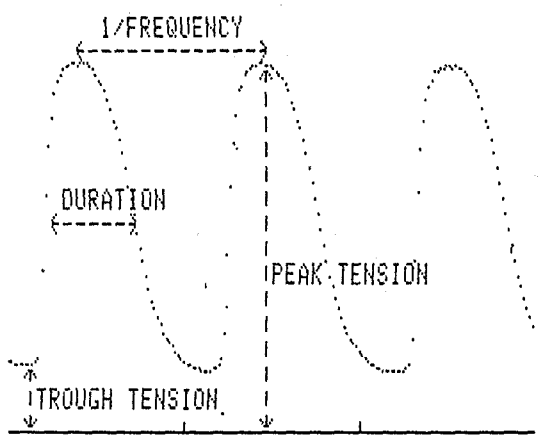

Fig. 1. A recording of uterine contraction and its four components.

The data obtained were expressed as mean±s.e.m. Student's t-test was used for statistical evaluation of the data.

The drugs used were acetylcholine chloride (Sigma, USA), oxytocin (OXCITON inj, Yu Han Ltd, Korea), verapamil hydrochloride (Sigma, USA), and tetracaine hydrochloride (T-CAIN, Dae-Han Pharm. Co., Korea). All drugs were dissolved in Tris-Tyrode's solution. Drug concentrations described in this paper are expressed as final concentration in the organ bath. The volume of drug added were less than 0.3 ml

RESULT

The effect of verapamil and tetracaine on the trough tension (T) of the uterine contraction induced by acetylcholine (ACH) and oxytcin (OXT)

As the concentration of verapamil increased from 2.5×10^{-8} to 2.5×10^{-6} M, the T of the ACH-induced contraction did not change. The T of the OXT-induced contraction had no changed in the presence of verapamil at a concentration of 2.5×10^{-8}, 2.5×10^{-7} M, but significantly decreased at a concentration of 2.5×10^{-6} M ($p<0.05$). In the presence of tetracaine with above concentrations, the T of the ACH-and OXT-induced contraction had no significant change (Fig. 2.).

The effect of verapamil and tetracaine on the peak tension (P) of the ACH-and OXT-induced contraction

As the concentration of verapamil was increased from 2.5×10^{-8} M to 2.5×10^{-6} M, the P of the ACH-and OXT-induced contraction gradually decreased ($p<0.05$). When the concentration of tetracaine increased from 4.2×10^{-7} M to 4.2×10^{-5} M, the P of the ACH-induced contraction changed in significantly. In the OXT-induced contraction, tetracaine at a concentration of 4.2×10^{-7} M and 4.2×10^{-6} M did not significantly change P, but the P of the high OXT-induced contraction (0.63 IU/L-19 IU/L) decreased 23 to 29% in the presence of 4.2×10^{-5} M tetracaine ($p<0.05$) (Fig. 3).

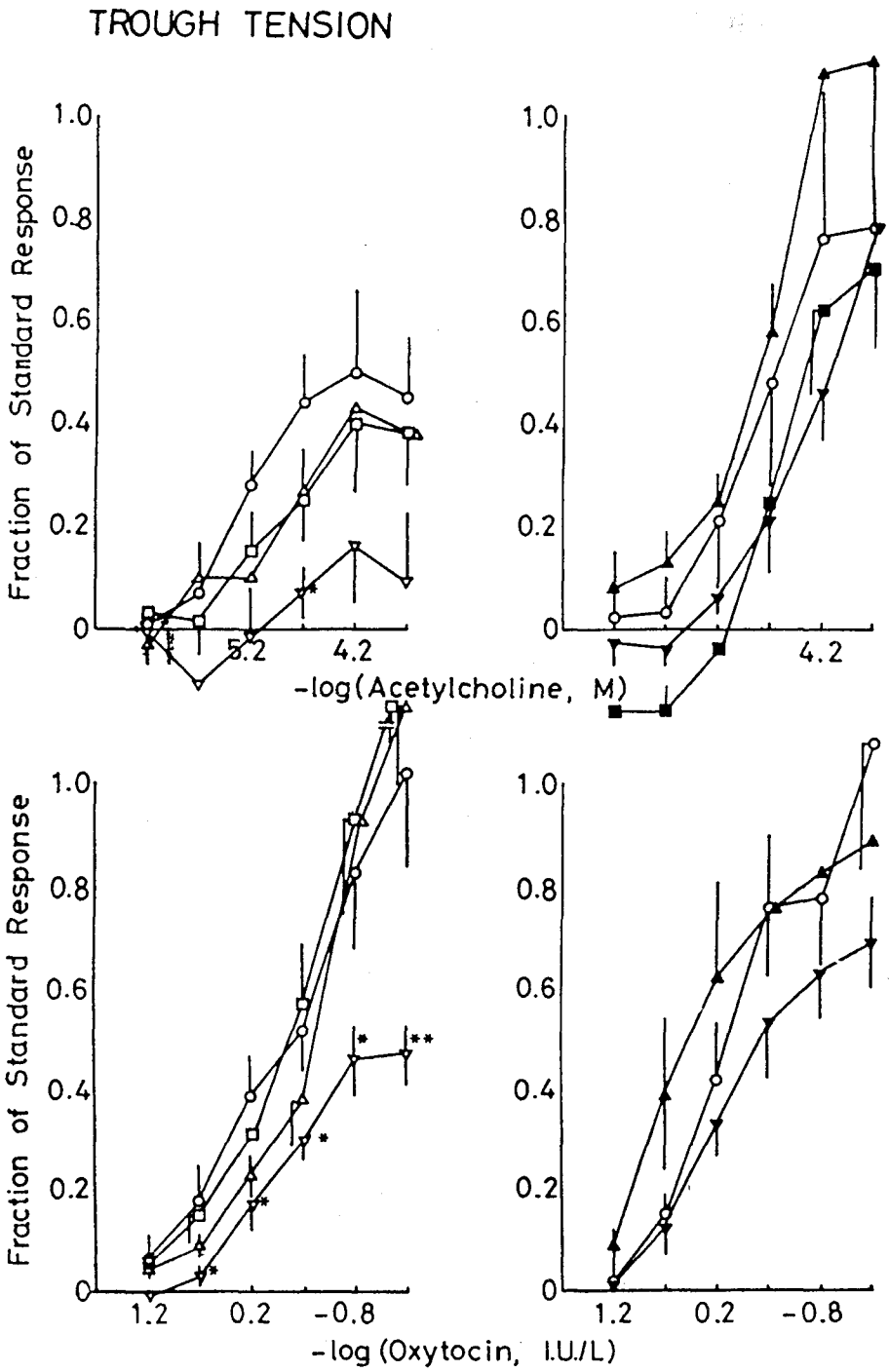

Fig. 2. The effect of verapamil (left panel) and tetracaine (right panel) on the through tension of the uterine contraction induced by acetylcholine (upper panel) and oxytocin (lower panel). Each point is the mean ± S.E.M. of 5 cases. The symbols correspond to the followings ; ○ control ; □ 2.5×10^{-8} M ; △ 2.5×10^{-7} M ; ▽ 2.5×10^{-6} M verapamil ; ■ 4.2×10^{-7} M ; ▲ 4.2×10^{-6} M ; ▼ 4.2×10^{-5} M tetracaine.
* $P < 0.05$ and ** $P < 0.01$ compare to control.

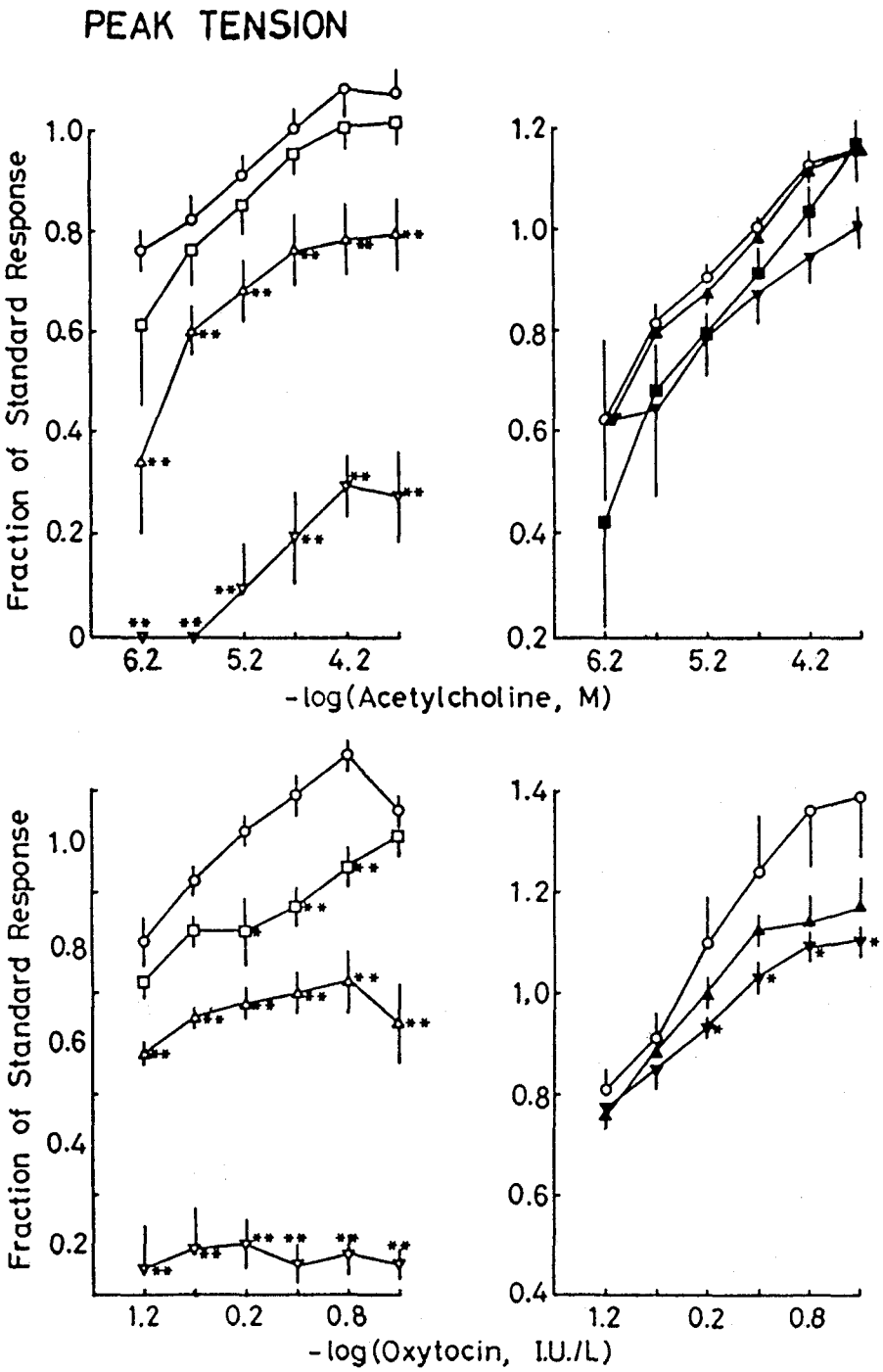

Fig. 3. The effect of verapamil (left panel) and tetracaine (right panel) on the peak tension of the uterine contraction induced by acetylcholine (upper panel) and oxytocin (lower panel). Each point is the mean ± S.E. M. of 5 cases. The symbols correspond to the followings ; ○ control ; □ 2.5×10^{-8} M ; △ 2.5×10^{-7} M ; ▽ 2.5×10^{-6} M ; verapamil ; ■ 4.2×10^{-7} M ; ▲ 4.2×10^{-6} M ; ▼ 4.2×10^{-5} M tetracaine.
* $P < 0.05$ and ** $P < 0.01$ compare to control.

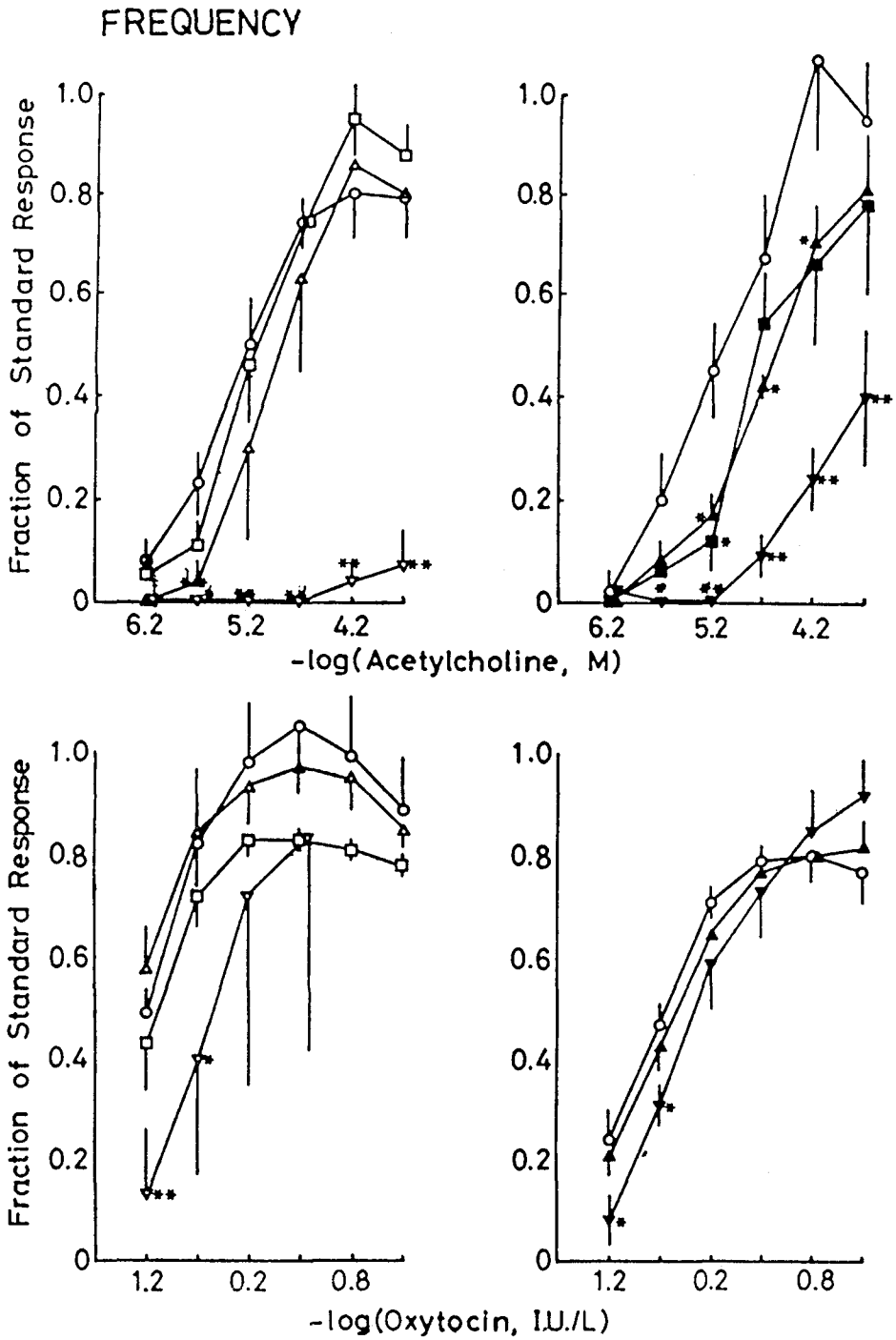

Fig. 4. The effect of verapamil (left panel) and tetracaine (right panel) on the frequency of the uterine contraction induced by acetylcholine (upper panel) and oxytocin (lower panel). Each point is the mean ± S.E.M. of 5 cases. The symbols correspond to the followings ; ○ control ; □ 2.5×10^{-8} M ; △ 2.5×10^{-7} M ; ▽ 2.5×10^{-6} M verapamil ; ■ 4.2×10^{-7} M ; ▲ 4.2×10^{-6} M ; ▼ 4.2×10^{-5} M tetracaine.
* $P < 0.05$ and ** $P < 0.01$ compare to control

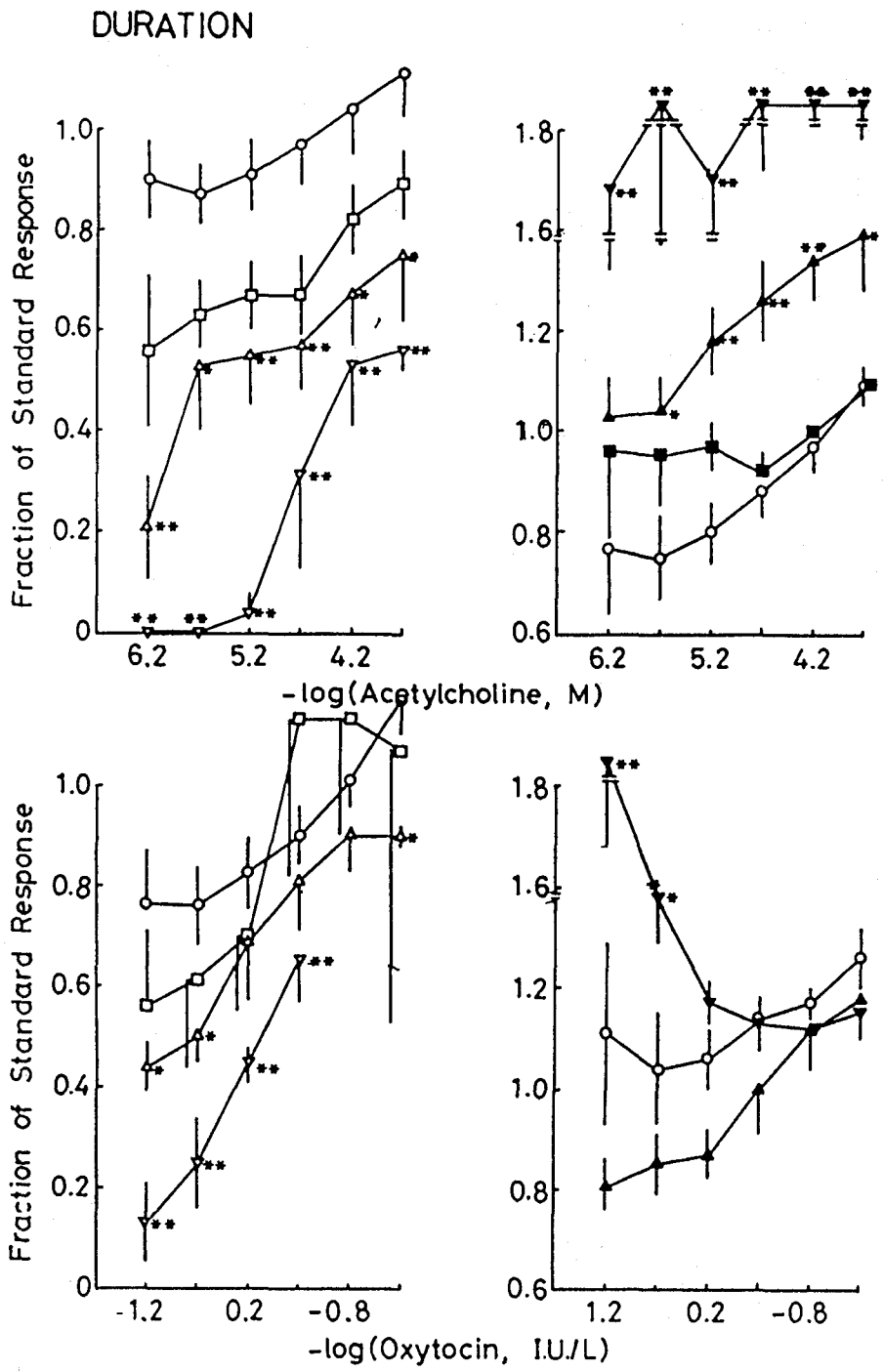

Fig. 5. The effect of verapamil (left panel) and tetracaine (right panel) on the duration of peak of the uterine contraction induced by acetylcholine (upper panel) and oxytocin (lower panel). Each point is the mean ± S.E.M. of 5 cases. The symbols correspond to the followings ; ○ control ; □ 2.5×10^{-8} M ; △ 2.5×10^{-7} M ; ▽ 2.5×10^{-6} M verapamil ; ■ 4.2×10^{-7} M ; ▲ 4.2×10^{-6} M; ▼ 4.2×10^{-5} M tetracaine.
* $P < 0.05$ and ** $P < 0.01$ compare to control

The effect of verapamil and tetracaine on the frequency (F) of the ACH-and OXT-induced contraction

The F of the ACH-and OXT-induced contraction did not change significantly in the presence of the 2.5×10^{-8} M and 2.5×10^{-7} M verapamil, but verapamil at a concentration of 2.5×10^{-6} M produced significant decrease in F of the ACH-induced contraction ($p<0.01$) and low OXT induced contraction (0.063 IU/L-0.19 IU/L) ($p<0.05$). As the concentration of verapamil increased from 4.2×10^{-7} M to 4.2×10^{-5} M, the F of the ACH-induced contraction gradually decreased ($p<0.05$). The F of the OXT-induced contraction changed little in the presence of tetracaine at a concentration of 4.2×10^{-7} M and 4.2×10^{-6} M, but tetracaine at a concentration of 4.2×10^{-5} M produced significant decrease in F of the low OXT-induced contraction ($p<0.05$) (Fig. 4).

The effect of verapamil and tetracaine on the duration (D) of the ACH-and OXT-induced contraction

As the concentration of verapamil increased from 2.5×10^{-8} to 2.5×10^{-6} M, the D of the ACH-and OXT-induced contraction decreased ($p<0.05$). In contrast, the D of the ACH and low OXT-induced contraction increased ($p<0.05$) and that of high OXT-induced contraction had no change as the concentration of tetracaine increased from 4.2×10^{-7} to 4.2×10^{-5} M (Fig. 5).

DISCUSSION

In acetylcholine-induced contraction, tetracaine increased D with no change in P whereas verapamil decreased P and D dose-dependently. These results appear to reflect that two antagonists have different effects on the process of calcium transport or metabolism involved in the smooth muscle contraction.

Verapamil, a calcium entry blocker, can inhibit contraction by blocking voltage-operated calcium channel (Fleckenstein, 1977; Putney, 1978). It blocks slow inward channel (Triggle, 1981). Slow inward current through smooth muscle cell membrane is related to two calcium channels (Sturek & Hermsmeyer, 1986). Therefore, these reports seem to support that P and D are related to intracellular calcium concentration regulated by calcium influx through voltage-operated channel.

Tetracaine causes depolarization and delays repolarization via blocking potassium channel (Bolton, 1979). It inhibits the release of calcium from storage site of sarcoplasmic reticulum (Chiarandini *et al.*, 1970). Local anesthetics block oscillatory release of calcium from sarcoplasmic reticulum initiated by calcium influx from extracellular site (Forssman, 1974). With these reports, our results of increase in D and no change in P suggest that tetracaine delay the rate of calcium flux through the membrane transport site but not affect the final maximal concentration of intracellular calcium produced by acetylcholine.

In the oxytocin-induced contraction, both verapamil and tetracaine had different effects on the P, as was observed in the acetylcholine-induced contraction: P was dose-dependently decreased by verapamil but not significantly decreased by tetracaine. These suggest that P is related to the calcium influx via voltage-operated channel, and not to the rate of calcium flux.

D was influenced differently by both verapamil and tetracaine depending on the concentration of oxytocin: In low oxytocin-induced contraction, verapamil decreased D whereas tetracaine increased D. These are similar to those observed in the acetylcholine-induced contraction. In high oxytocin-induced contraction, however, D was significantly changed by neither verapamil nor tetracaine. These indicate that the mechanism of contraction produced by oxytocin is different depending on the concentration of oxytocin.

In the presence of verapamil, both acetylcholine-and oxytocin-induced contractions changed with similar tendency. In contrast, in the presence of tetracaine, changes in low oxytocin-induced contraction were similar to but those in high oxytocin-induced contraction were different from those in the acetylcholine-induced contraction. These results led us to take into consideration of action of both stimulants and inhibitors simultaneously.

Oxytocin produces contraction by inducing calcium influx through voltage-operated channel (Barnes & Senior, 1985) and calcium release from cellular storage site (Batra, 1986). The action mechanism of acetylcholine to produce contraction is similar to that of oxytocin (Izumi, 1985). However, while action of acetylcholine depends mainly on the extracellular calcium or on a calcium pool loosely bound to the cellular mem-

brane, the response to oxytocin seems to be dependent on the release of both tightly and loosely bound calcium (Calixto & Antonio, 1986). Acetylcholine acts on muscarinic receptor, and oxytocin acts on oxytocin receptor and on prostaglandin synthesis (Vane & Williams, 1973); Dubin *et al.*, 1979; Fuchs *et al.*, 1981).

Similar tendency of decrease in P and D, in the presence of verapamil, in both acetylcholine-and oxytocin-induced contractions suggest that voltage-operated channel blocking action of verapamil produces similar effect on contraction produced by acetylcholine and oxytocin.

In the presence of tetracaine, it produced similar tendency to decrease in F and increase in D, under the conditions of acetylcholine and low oxytocin-induced contraction. These results suggest that both acetylcholine and low concentration of oxytocin have similar mechanism of uterine contraction. But effect of tetracaine on the high oxytocin-induced contraction was different from those on the acetylcholine-induced contraction. This difference suggests that high concentration of oxytocin has a different mechanism of contraction of uterine smooth muscle from the mechanism of acetylcholine-induced contraction, or that inhibitory action of tetracaine produce minor effect on the stimulant action of oxytocin.

In conclusion, the analysis of effects of a certain inhibitor on the components of contraction may allow not only to postulate its specific inhibitory mechanism of the smooth muscle contraction but also to classify stimulants according to their mechanism of contraction.

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= 국문초록 =

Acetylcholine 및 Oxytocin에 의하여 야기되는 렛드 자궁수축에 미치는 Verapamil 및 Tetracaine의 영향

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Acetylcholine 및 oxytocin에 의하여 야기되는 흰쥐의 적출자궁수축을 Ca^{2+} 길항제의 일종인 verapamil 및 tetracaine 존재하에서 actylcholine 및 oxytocin에 의한 자궁수축곡선을 4개의 요소 (trough tension, T: peak tension, P; contraction frequency, F; duration, D)로 나누어 비교분석하여 다음과 같은 결과를 얻었다.

Verapamil (0.25 μM)은 자발수축을 억제시켰으나 tetracaine (42 μM)은 자발수축을 억제시키지 못하였다. 이들 길항제의 존재하에서 acetylcholine 및 oxytocin에 의하여 야기되는 자궁수축의 각 구성요소에 변화를 관찰하였다. 즉 acetylcholine에 의한 수축에서 verapamil은 P와 D를 감소시켰고 tetracaine은 F를 감소시키고 D를 증가시켰다. oxytocin에 의한 수축에서 verapamil은 P와 D를 감소시켰으나 tetracaine은 oxytocin 농도에 따라 차이가 있었는데, 저농도의 oxytocin에 의한 수축에서는 F를 감소시키고 D를 증가시켰으나 고농도의 oxytocin에 의한 수축에서는 F와 D에는 영향을 주지 않고 P만 감소시켰다.

이상의 결과로 미루어 acetylcholine 및 oxytocin에 의하여 야기되는 수축곡선은 시각적으로 큰 차이가 없었으나 작용기전이 다른 Ca^{2+} 길항제에 의하여 acetylcholine 및 oxytocin의 수축의 구성요소에 다르게 영향을 미칠 수 있었다는 것은 수축곡선의 구성요소의 변화를 면밀히 검토하면 자궁수축제의 수축작용기전이 다름을 예측할 수 있을 뿐만 아니라 수축억제제에 의한 억제기전의 차이점도 예측할 수 있을 것으로 생각되어 진다