CHAPTER 4

RESULTS

1. Effect of PA on uterine contraction

Cumulative concentrations of PA (10^{-6}-10^{-3} gm/ml) caused no changes in resting tension of the uterus. The representative tracing of PA induced uterine contraction was demonstrated in Figure 19 and the log concentration-response relationship was shown in Figure 20.

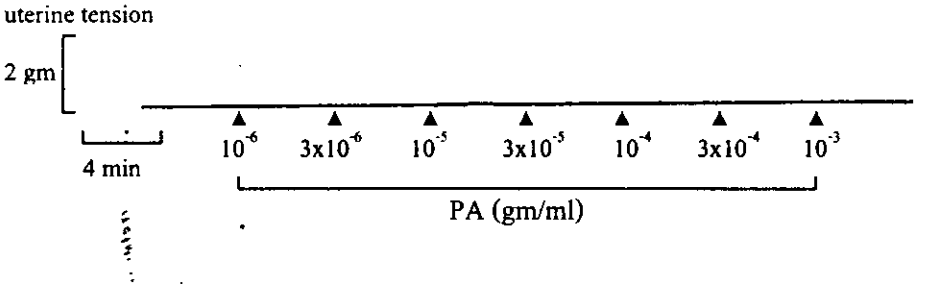


Figure 19: The representative tracing of contractile response of isolated rat uterus to the cumulative concentration of PA

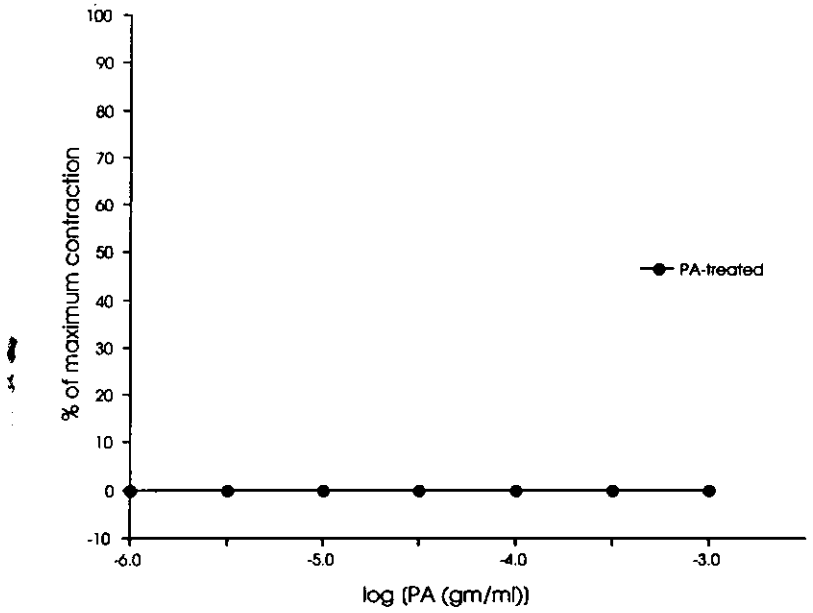


Figure 20. The concentration-response relationship of the cumulative concentration of PA (10^{-6} – 10^{-3} gm/ml) in isolated rat uterus. Each data point represented as the mean ± S.E. of four experiments.

2. Effect of PA on uterine relaxation

2.1 Effect of PA on uterine contraction induced by depolarizing solution

2.1.1 Determination of optimal KCl concentration in depolarizing solution

In this study, the optimal KCl concentration in depolarizing solution was determined. The representative tracing of the uterine contraction induced by various concentration of KCl was demonstrated in Figure 21. The uterine contraction and KCl concentration relationship was shown in Figure 22. The replacement of Jalon-Ringer solution with depolarizing solution caused a rapid phasic contraction, then relaxed and reached a prolong plateau contraction (tonic phase) in the uterine muscle. Various concentrations of KCl (36.3, 46.3,

56.3, 66.3 and 76.3 mM) containing in depolarizing solution were treated and all concentrations of KCl rapidly caused a phasic contraction. However, after phasic contraction the depolarizing solution containing 36.3 mM of KCl rapidly caused a large relaxation and then contracted again with a minor fluctuation in contraction. For depolarizing solution containing 76.3 mM of KCl, the contraction of the uterus in a tonic phase appeared to decline slowly with time. In contrast, KCl at the concentration of 46.3, 56.3 and 66.3 mM caused a prolonged plateau contraction in a tonic phase which remained stable for a long period of time. The tonic contraction induced by depolarizing solution containing 56.3 KCl remained unchange for at least 2 hours or in some cases declined very slowly with no more than 10% of the initial contraction within 2 hours. The concentration of KCl at 56.3 mM was then chosen for the preparation of depolarizing solution in the following studies. The representative tracings of the uterine contraction induced by depolarizing with 56.3 mM KCl were demonstrated in Figure 22, 48, 49, 51 and 52, respectively.

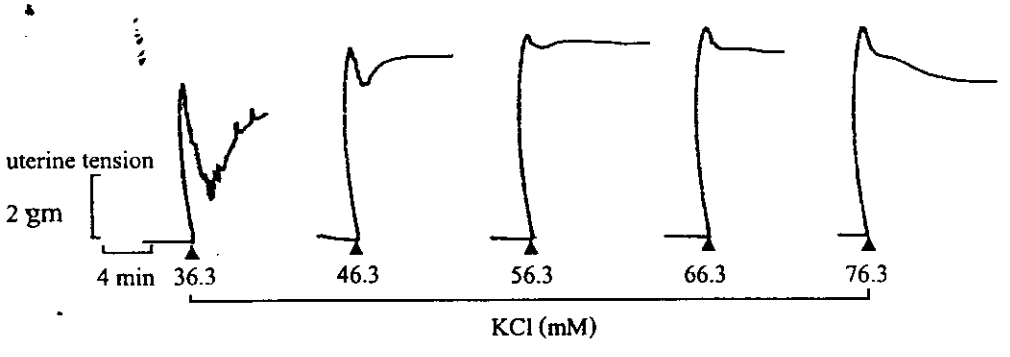


Figure 21. The representative tracing of the uterine contraction induced by depolarizing solutions with various concentrations of KCl in isolated rat uterus.

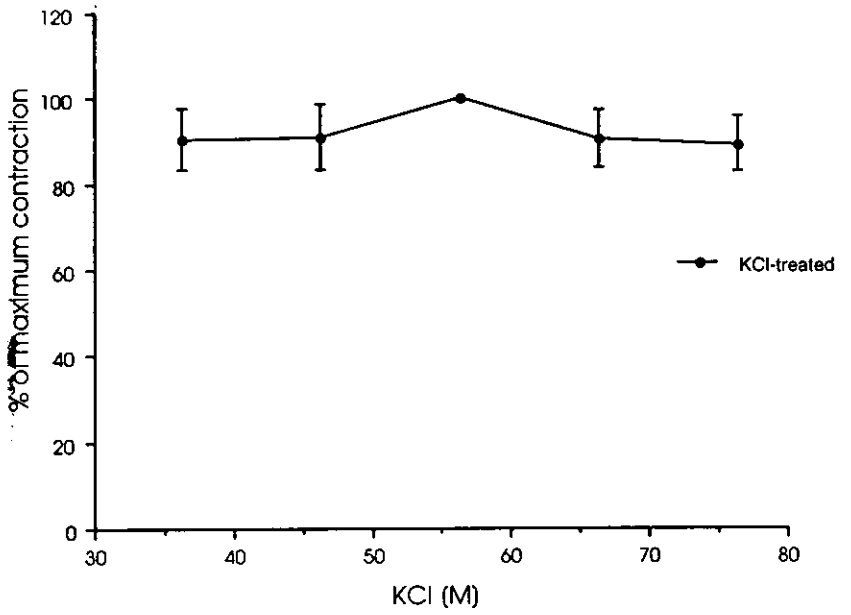


Figure 22. The concentration-response relationship of depolarizing solutions containing various concentrations of KCl induced contraction within 10 minutes after exposure to depolarizing solution in isolated rat uterus. Each value was expressed as a percentage of the maximal contraction in response to depolarizing solution with 56.3 mM of KCl. Each data point represented the mean ± S.E. of seven experiments.

2.1.2 Effect of PA on uterine contraction induced by depolarizing solution

The representative tracing of the relaxing effects of PA on uterine contraction induced by depolarizing solution (KCl 56.3 mM) were illustrated in Figure 23. The addition of the vehicle of PA had no effects on uterine contraction induced by depolarizing solution. In contrast, the addition of PA (10^{-6}-10^{-3} gm/ml) inhibited this contraction in a concentration-dependent manner. A significant inhibition on uterine contraction occurred with PA at the concentration of 10^{-5} – 10^{-3} gm/ml while PA at the concentration of 10^{-3} gm/ml

completely abolished the contraction. The IC_{50} of PA was 8.37 ± 0.58 x 10^{-5}gm/ml. The cumulative inhibitory concentration-response curve of PA on uterine contraction induced by depolarizing solution was illustrated in Figure 24, IC_{50} value was demonstrated in Table 4.

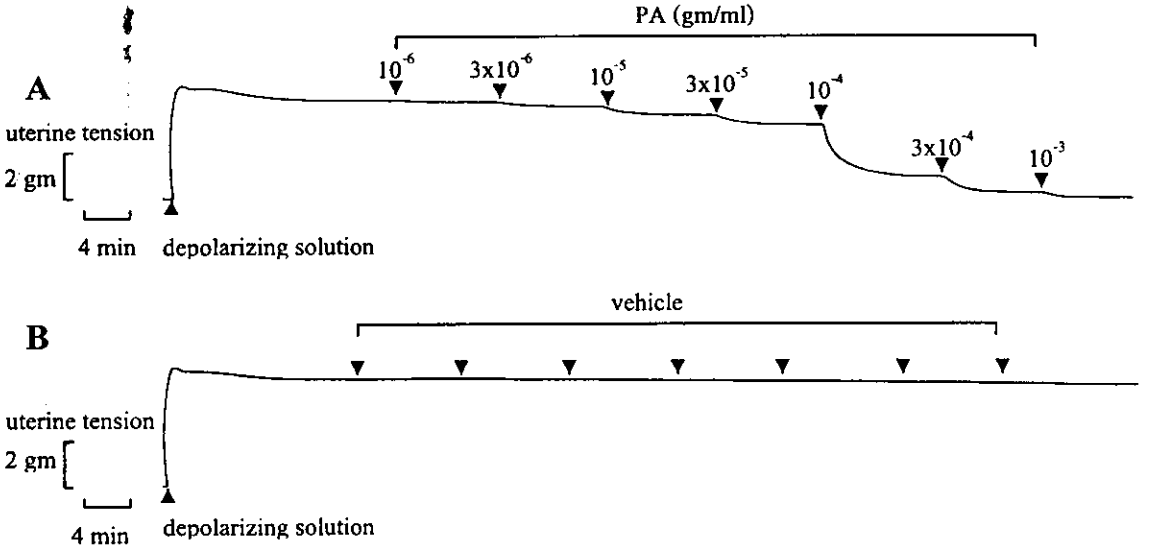


Figure 23. The representative tracing of the relaxing effect of cumulative concentrations of PA in isolated rat uterus precontracted with depolarizing solution (KCl 56.3 mM); A = PA-treated, B = time control.

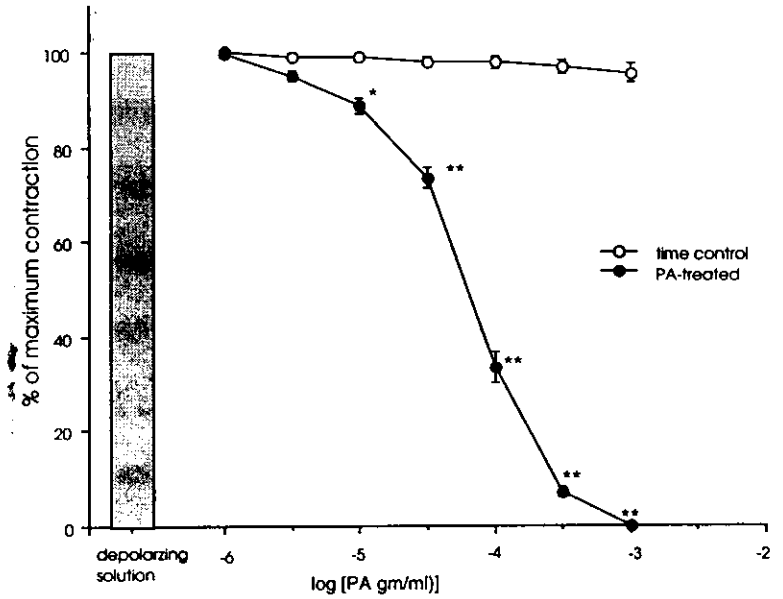


Figure 24. The concentration-response relationship of PA-induced uterine relaxation in isolated rat uterus precontracted with depolarizing solution. Each value is expressed as a percentage of the initial maximal contraction in response to depolarizing solution. Each data point represents the mean ± S.E. of ten experiments. (* = $p < 0.05$, ** = $p < 0.01$ as compared to the control)

2.2 Effect of PA or verapamil on uterine contraction induced by $CaCl_2$ in K^+-depolarized uterus

2.2.1 Determination of optimal concentration of verapamil induced uterine relaxation precontracted by $CaCl_2$

In this study, the optimal concentration of verapamil for the inhibition of uterine contraction induced by $CaCl_2$ was determined. The addition of $CaCl_2$ (10^{-3} M) rapidly caused a phasic contraction, when the maximum contractions was reached, cumulative concentrations of verapamil (10^{-9} – 10^{-4} M) or its vehicle were then added. The addition of vehicle of verapamil had no effects on uterine contraction induced by $CaCl_2$. In contrast, the addition of

verapamil (10^{-9} – 10^{-4} M) inhibited this contraction in a concentration-dependent manner. A significant of inhibition on uterine contraction first appeared with verapamil at concentration 10^{-9} M ($p < 0.05$) while, at the concentration 10^{-4} M of verapamil completely abolished this contraction. The representative tracing and the log concentration-response relationship of verapamil induced uterine relaxation were demonstrated in Figure 25 and 26 respectively. Verapamil at the concentration of 10^{-9}, 10^{-8} and 10^{-7} M produced uterine relaxation to approximately 20-90% of maximum of contraction were then chosen to use in the next experiment.

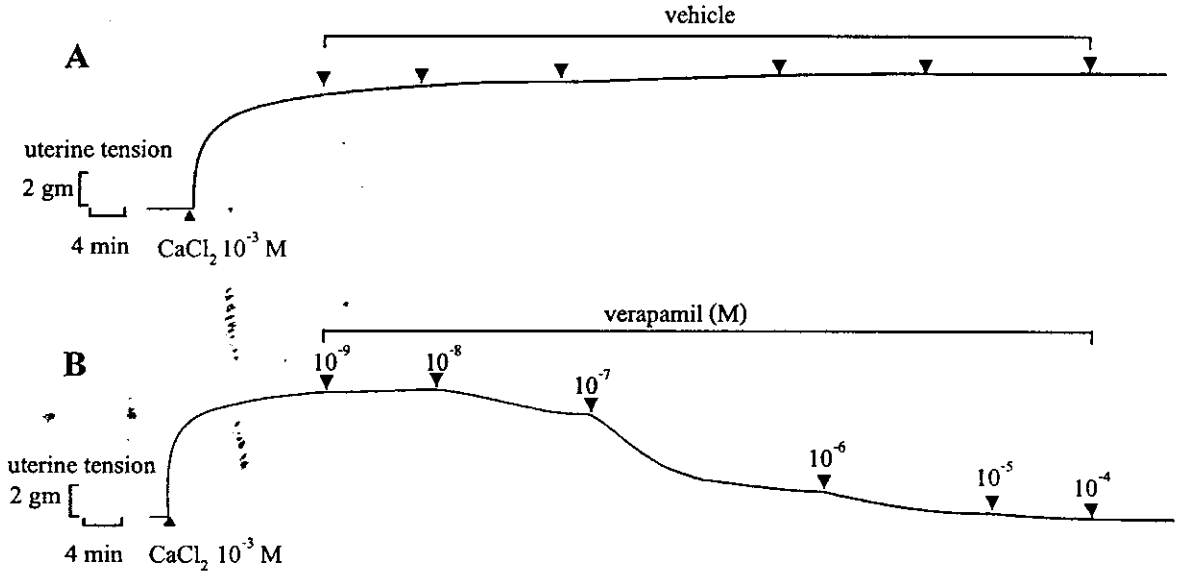


Figure 25. The representative tracing of the relaxing effect of the cumulative concentrations of verapamil in isolated rat uterus precontracted with $CaCl_2$ (10^{-3} M); A = time control, B = verapamil-treated.

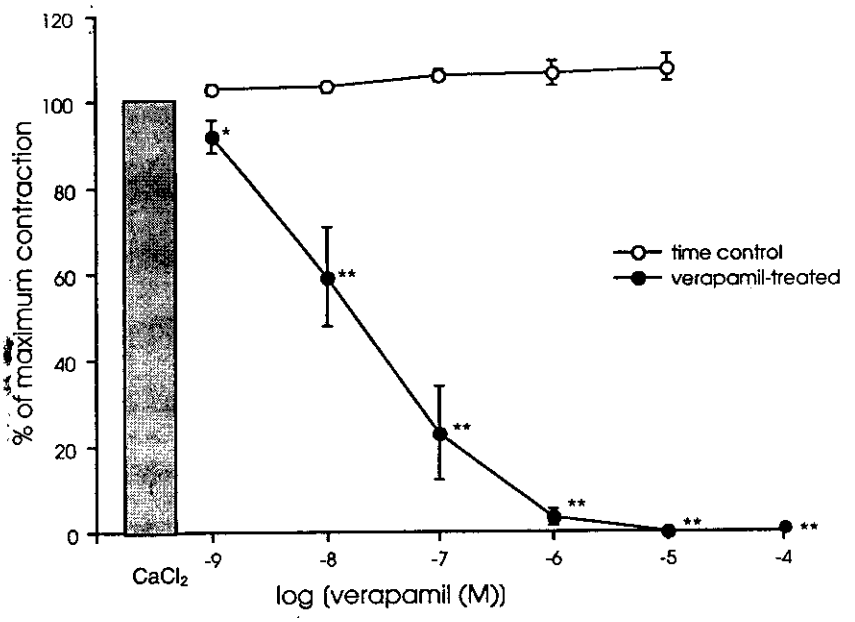


Figure 26. The concentration-response curves of verapamil-induced relaxation in isolated rat uterus precontracted with $CaCl_2$ (10^{-3} M). Each value is expressed as a percentage of the maximal contraction in response to $CaCl_2$. Each data point represents as the mean ± S.E. of ten experiments. (* = $p < 0.05$, ** = $p < 0.01$ as compared to the control)

2.2.2 Effect of PA or verapamil on uterine contraction induced by $CaCl_2$ in K^+-depolarized uterus

The representative tracing of the effect of PA and its vehicle, verapamil and its vehicle on uterine contraction induced by $CaCl_2$ (10^{-5}-$3x10^{-2}$ M) were demonstrated in Figure 25 and 26 respectively. In Ca^{2+}-free high KCl (60 mM) solution containing 0.02 mM of EDTA, the cumulative concentrations of $CaCl_2$ (10^{-5}-3 x 10^{-2} M) produced uterine contraction in a concentration dependent manner (Figure 27). In the presence of various concentrations of PA (10^{-5}, $3x10^{-5}$ and 10^{-4} gm/ml), the uterine contraction induced by $CaCl_2$ (10^{-5}– 3 x 10^{-2} M) was significantly reduced ($p < 0.05$ for 10^{-5} gm/ml, $p < 0.01$

for $3x10^{-5}$ gm/ml and $p < 0.01$ for 10^{-4} gm/ml, ANOVA). The log concentration-response relationship of $CaCl_2$ was shifted to the right, the maximum contraction was also depressed. EC_{50} of $CaCl_2$ alone was 4.74 ± 0.68 $x10^{-4}$ M, but the value was changed to $1.38 \pm 0.17 \times 10^{-3}$ ($p < 0.05$), $2.60 \pm 0.26 \times 10^{-3}$ ($p < 0.01$) and $7.50 \pm 0.44 \times 10^{-3}$ M ($p < 0.01$) in the presence of PA at the concentration of 10^{-5}, $3x10^{-5}$ or 10^{-4} gm/ml, respectively. Similarly, verapamil also inhibited the uterine contraction induced by $CaCl_2$ in the concentration-dependent manner. The pretreated uterine horn with verapamil (10^{-9}, 10^{-8} and 10^{-7} M) significantly reduced the contraction induced by $CaCl_2$ ($p < 0.05$ for 10^{-9} M, $p < 0.05$ for 10^{-8} M and $p < 0.05$ for 10^{-7} M, ANOVA). The log concentration-response curves of $CaCl_2$ (10^{-5}-$3x10^{-2}$ M) were shifted to the right when compared to the effect of $CaCl_2$ alone, while the maximum contraction was also depressed. EC_{50} value of $CaCl_2$ alone was $5.59 \pm 1.78 \times 10^{-4}$ M, but the value was also significantly suppressed to $5.83 \pm 0.15 \times 10^{-3}$ ($p < 0.05$), $2.51 \pm 0.11 \times 10^{-3}$ ($p < 0.01$) and $1.20 \pm 0.26 \times 10^{-3}$ M ($p < 0.01$) when the uterus was pretreated with verapamil 10^{-9}, 10^{-9} or 10^{-7} M, respectively. The log concentration-response curves of $CaCl_2$ in the presence or absence PA or verapamil were illustrated in Figure 27, EC_{50} were presented in Table 2 and 3.

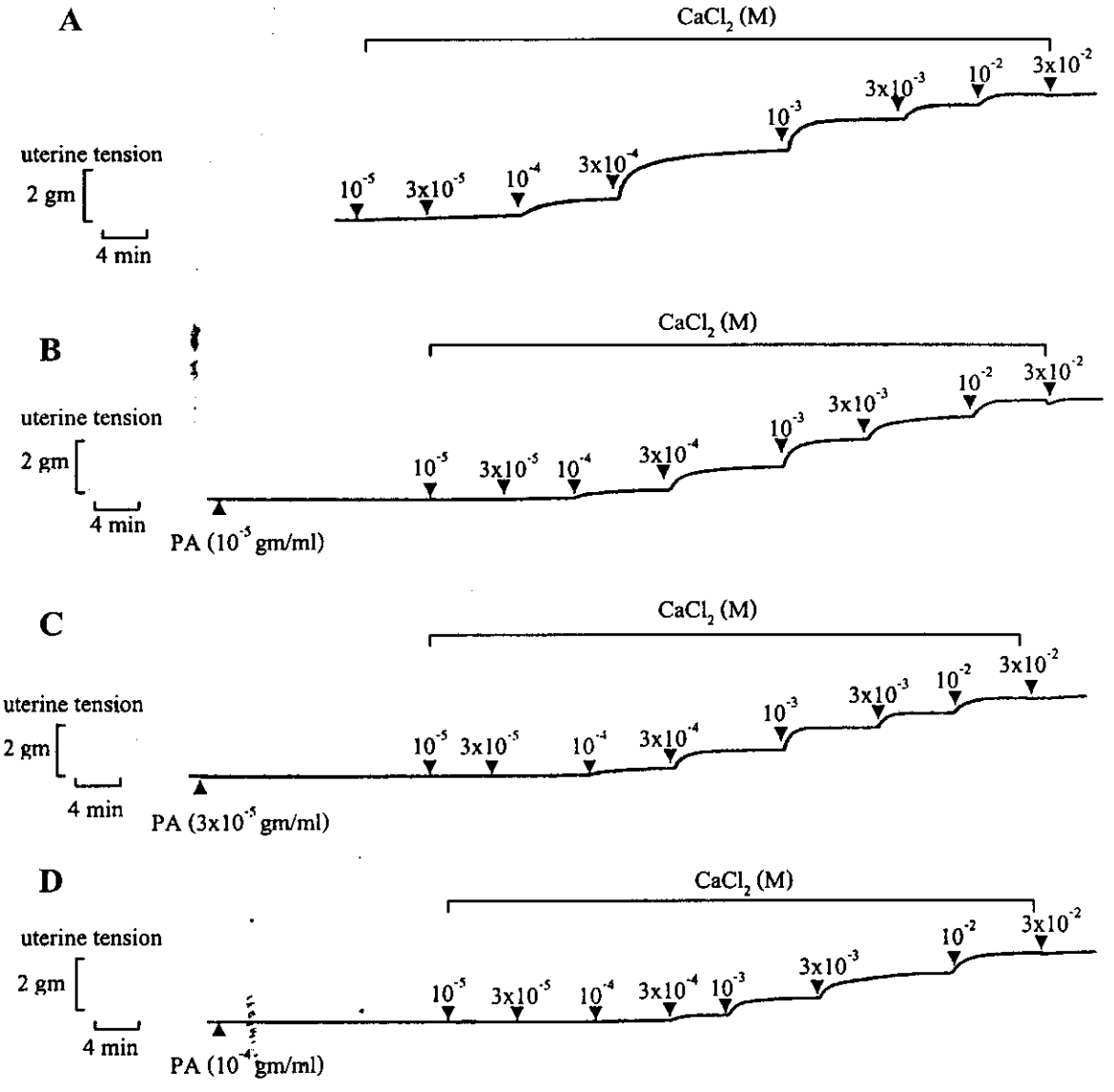


Figure 27. The representative tracings of the contractile response of isolated rat uterus to cumulative concentrations of $CaCl_2$ in Ca^{2+}-free high KCl solution. A = $CaCl_2$ alone, B, C or D preincubated with PA 10^{-5}, 3 x 10^{-5}, and 10^{-4} gm/ml respectively for 20 minutes.

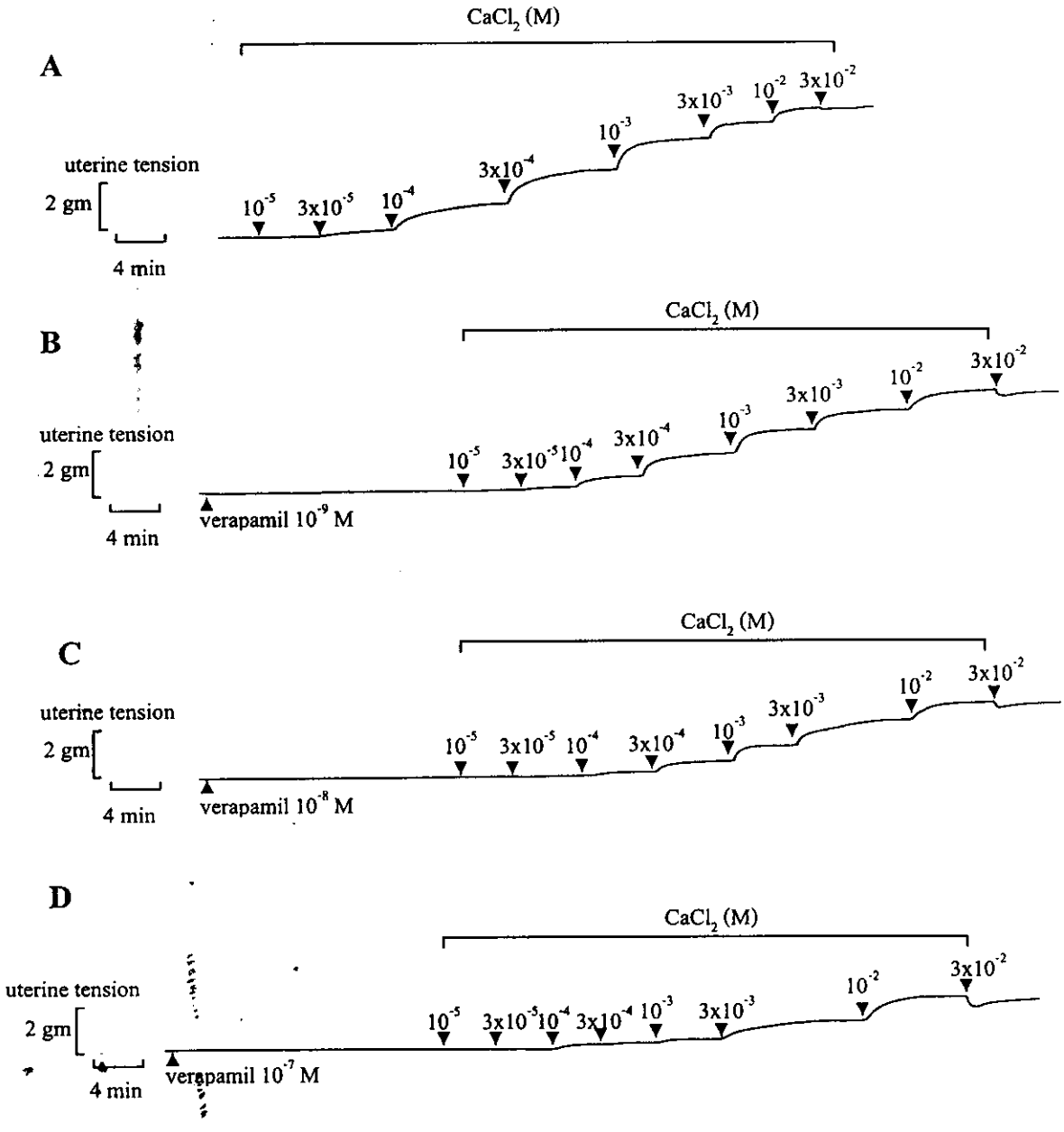


Figure 28. The representative tracings of the contractile response of isolated rat uterus cumulative concentrations of $CaCl_2$ in Ca^{2+}-free high KCl solution. A = $CaCl_2$ alone, B, C or D = preincubated with verapamil at 10^{-9}, 10^{-8}, and 10^{-7} M respectively for 20 minutes.

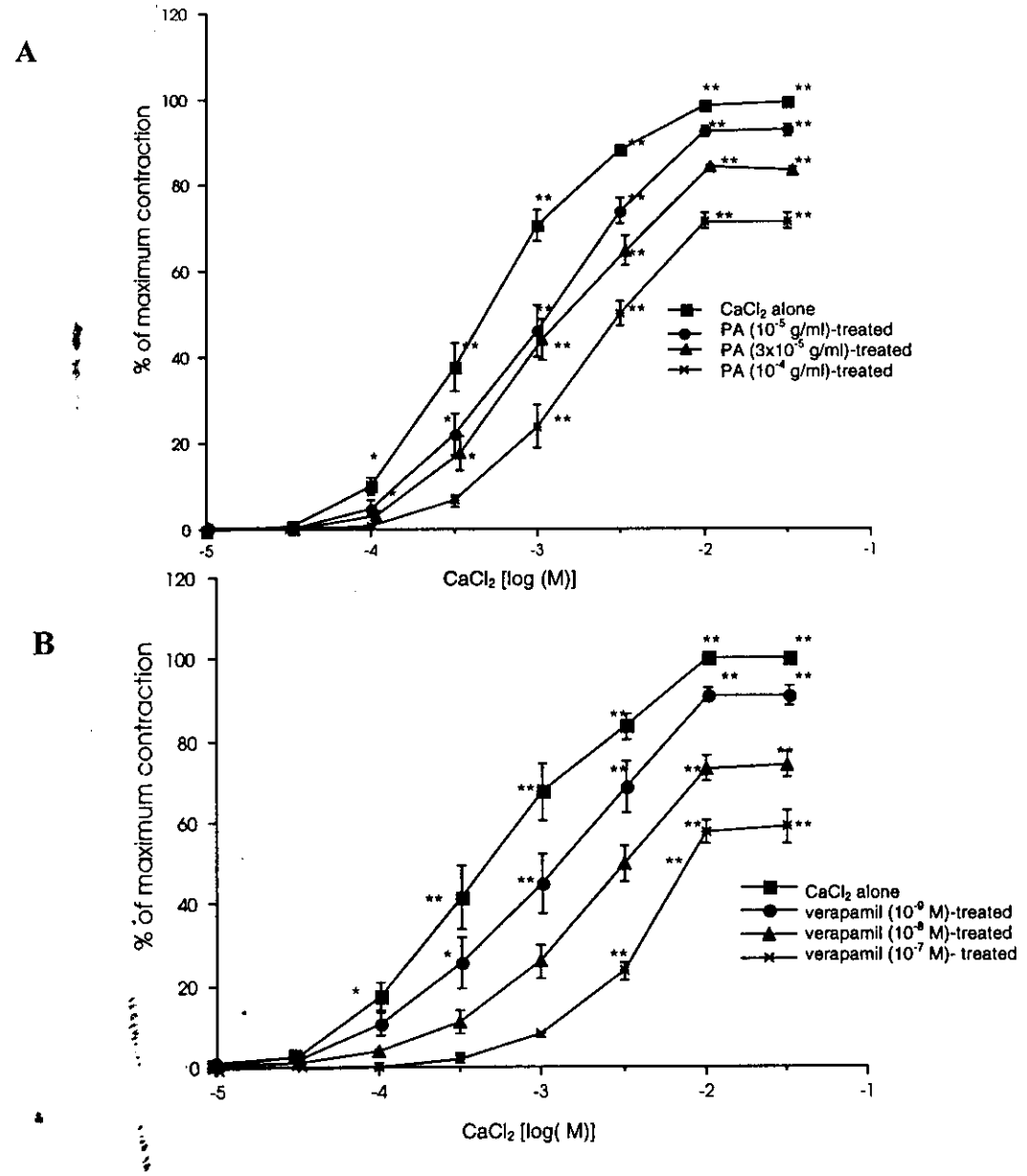


Figure 29. The concentration-response relationship of $CaCl_2$ (10^{-5}-3 x 10^{-2} M)-induced contraction in isolated rat uterus in the presence or absence of PA (A) or verapamil (B). Each value is expressed as a percentage of the maximal contraction in response to $3x10^{-2}$ M $CaCl_2$. Each data point represents the mean ± S.E. of ten experiments. (* = $p < 0.05$, ** = $p < 0.01$ when compare to the effect of $CaCl_2$ alone)

Table 2 Comparison of the EC_{50} values of $CaCl_2$-induced contraction in the presence or absence of PA in isolated rat uterus.

EC_{50} (Mean ± S.E.)			
$CaCl_2$ alone	PA-treated (gm/ml)		
	10^{-5}	$3x10^{-5}$	10^{-4}
$4.74 \pm 0.68x10^{-4}$	$1.38 \pm 0.17x10^{-3*}$	$2.60 \pm 0.26x10^{-3**}$	$7.50 \pm 0.44x10^{-3**}$

(*$p < 0.05$, ** $p < 0.01$ when compared to $CaCl_2$ alone

Table 3 Comparison of the EC_{50} values of $CaCl_2$-induced contraction in the presence or absence of verapamil in isolated rat uterus.

EC_{50} (Mean ± S.E.)			
$CaCl_2$ alone	verapamil-treated (M)		
	10^{-9}	10^{-8}	10^{-7}
$5.59 \pm 1.78x10^{-4}$	$1.20 \pm 0.26x10^{-3*}$	$2.51 \pm 0.11x10^{-3**}$	$5.83 \pm 0.15x10^{-3**}$

(*$p < 0.05$, ** $p < 0.01$ when compared to $CaCl_2$ alone)

2.2 Effect of PA on the uterine contraction induced by uterine stimulants

2.2.1 Determination of optimal concentration of oxytocin, $PGF_{2\alpha}$ and acetylcholine.

The effects of oxytocin, $PGF_{2\alpha}$ and acetylcholine on uterine contraction were determined. Cumulatively addition of oxytocin (0.01–10.00 mU/ml), $PGF_{2\alpha}$ (10^{-9}–10^{-4} M) or acetylcholine (10^{-7}–10^{-4} M) caused uterine contraction in a concentration dependent manner. The representative tracing ofoxytocin, $PGF_{2\alpha}$ or acetylcholine induced uterine contraction were demonstrated in

Figure 30, 32 and 34, respectively and the log concentration-response relationships were shown in Figure 31, 33 and 34, respectively. The concentration 1 mU/ml of oxytocin, 10^{-5} M of $PGF_{2\alpha}$ or 3 x 10^{-5} M of acetylcholine which produced approximately 70-80% of maximum contraction was then chosen for the induction of uterine contraction in the next experiments. Furthermore, this concentration of oxytocin or $PGF_{2\alpha}$ also produced regular amplitude of contraction for at least 2hours which was long enough to complete a series of experiment. However, in some experiments, the frequency of contraction induced by oxytocin (1mU/ml) or $PGF_{2\alpha}$ (10^{-5} M) declined very slowly with, at the end of experiment, no more than 10% of the initial frequency. The representative tracings of the frequency of contraction induced by oxytocin or $PGF_{2\alpha}$ and the time control were demonstrated in Figure 36 and 39.

It is noted that the effect of acetylcholine on the rhythmic contraction of the uterus was unstable as could be seen by a dramatically gradual decrease in both force and frequency of contraction from time control. This would make and erratic interpretation whether the effect was due to PA or verapamil on the common effect of acetylcholine when cumulative concentration of PA or verapamil was used. To overcome this problem, the experimental design was improved by preincubating the uterus with a single concentration of either PA or verapamil before acetylcholine ($3x10^{-5}$ M) was added. The force of uterine contraction was measured by a maximum of first peak of contraction.

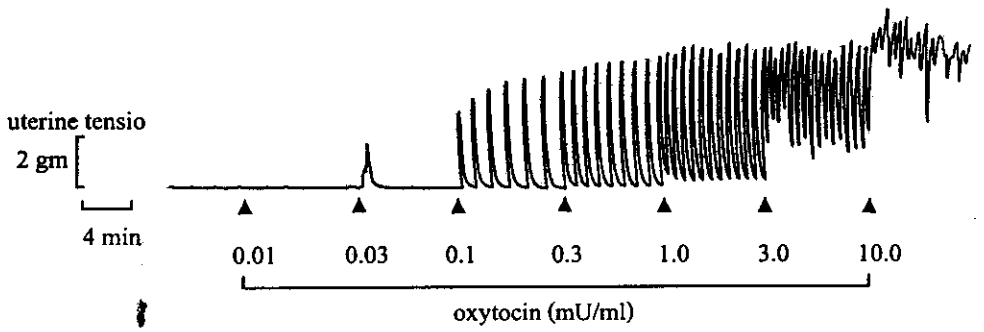


Figure 30. The representative tracing of the contractile response of isolated rat uterus to cumulative concentrations of oxytocin.

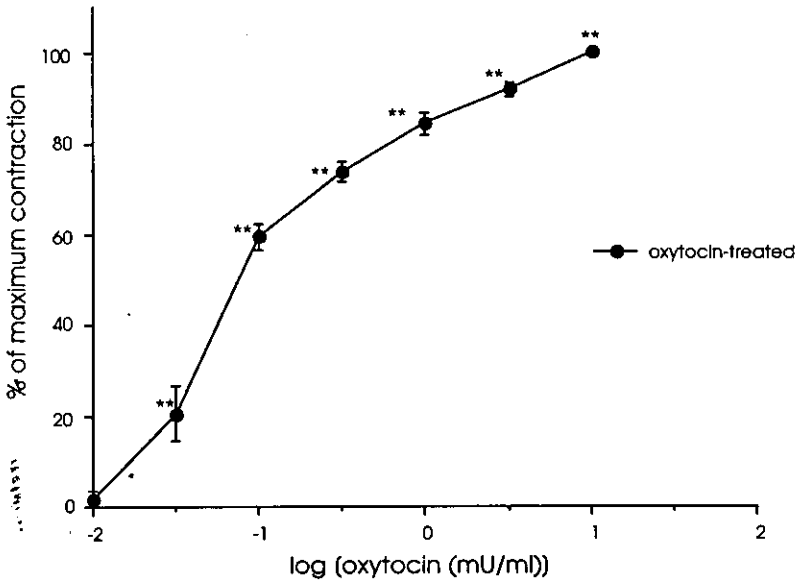


Figure 31. The concentration-response relationship of cumulative concentrations of oxytocin (0.01-10.0 mU/ml) in isolated rat uterus. Each value is expressed as a percentage of the maximal contraction in response to oxytocin. Each data point is the mean ± S.E. of ten experiments. (* = $p < 0.05$, ** = $p < 0.01$ as compared to the control)

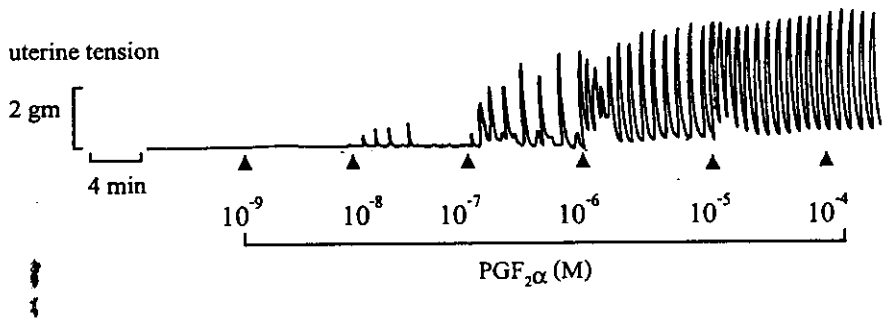


Figure 32; The representative tracing of the contractile response of isolated rat uterus to cumulative concentrations of $PGF_{2\alpha}$.

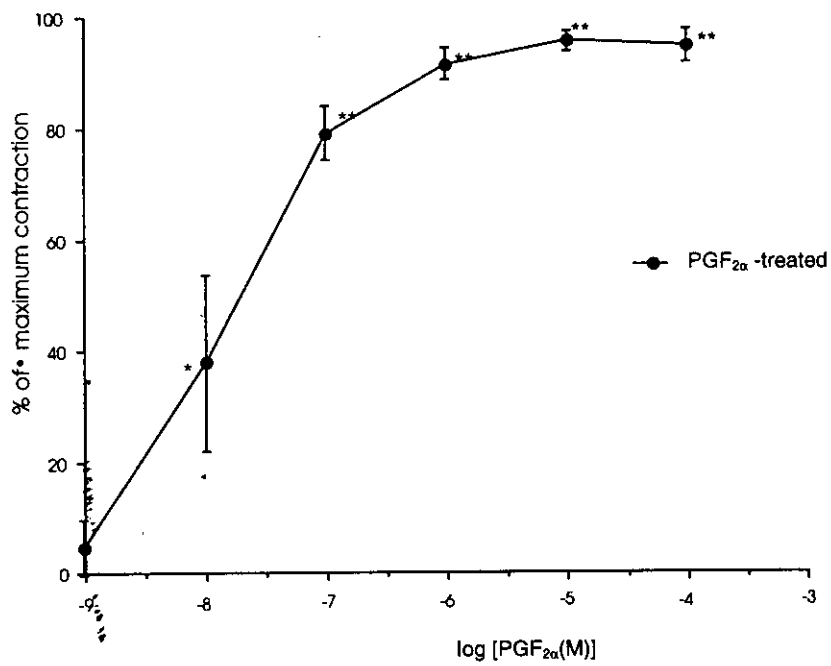


Figure 33. The concentration-response relationship of cumulative concentrations of $PGF_{2\alpha}$ in isolated rat uterus. Each value is expressed as a percentage of the maximal contraction in response to $PGF_{2\alpha}$ and each data point is the mean ± S.E. of seven experiments. (* = $p < 0.05$, ** = $p < 0.01$ as compared to the control)

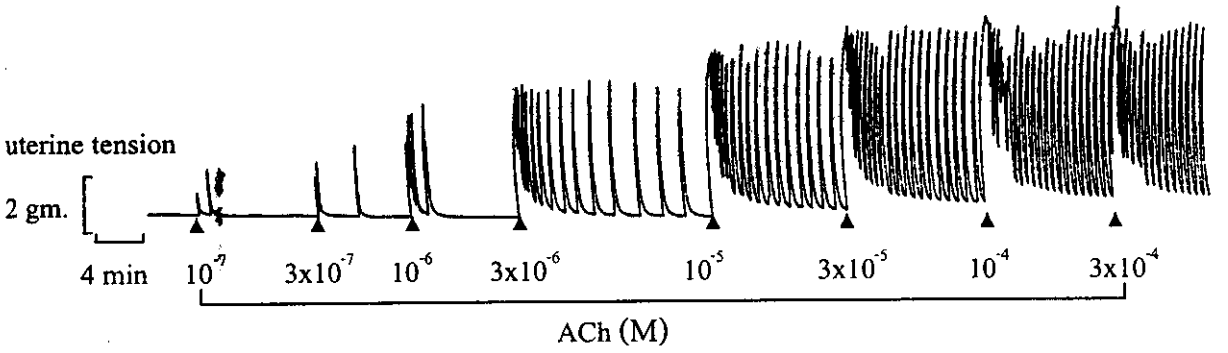


Figure 34. The representative tracing of the contractile response of isolated rat uterus to cumulative concentrations of ACh.

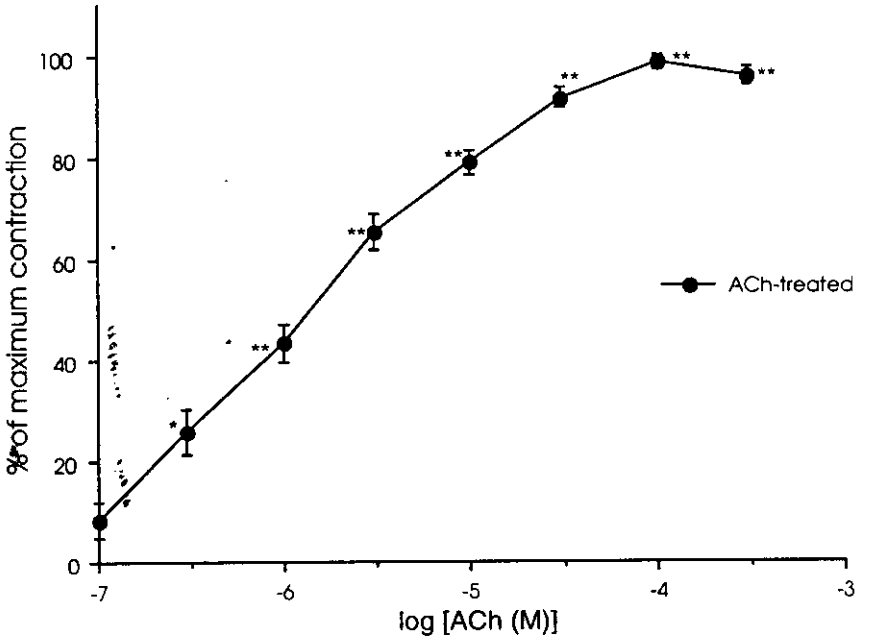


Figure 35. The concentration-response relationship of cumulative concentrations of ACh in isolated rat uterus. Each value is expressed as a percentage of the maximal contraction in response to ACh. Each data point is the mean ± S.E. of ten experiments. (* = $p < 0.05$, ** = $p < 0.01$ as compared to the control)

2.2.1 Effect of PA and verapamil on uterine contraction induced by oxytocin

The representative tracings of the relaxing effects of PA and its vehicle, or verapamil and its vehicle on uterine contraction induced by oxytocin (1mU/ml) were demonstrated in Figure 36. In the time control uterus,oxytocin 1 mU/ml stimulated the uterus to contract rhythmically which the amplitude of contraction was stable throughout the experiment. However, in some experiments, the frequency of contraction declined slowly with time. The addition of the vehicle of either PA or verapamil had no effects on uterine contraction induced by oxytocin (1 mU/ml). In contrast, the addition of cumulative concentration of PA (10^{-6}-10^{-3} gm/ml) or verapamil (10^{-8}-10^{-4} M) significantly inhibited both frequency ($p < 0.01$, ANOVA) and amplitude of contraction ($p < 0.01$, ANOVA). The frequency of contraction was more sensitive to PA than the amplitude of contraction, as observed by the first concentration of PA which significantly affected to the force and frequency of contraction. PA at the concentration of $3x10^{-6}$ gm/ml significantly reduced the frequency of contraction ($p < 0.05$), while the first concentration of PA that significantly inhibited the force of contraction was $3x10^{-5}$ gm/ml ($P < 0.05$). The comparison between the first concentration of verapamil which significantly affected to the frequency of contraction (10^{-7} M) and amplitude of contraction (10^{-6} M) has shown that the frequency of contraction was more sensitive to verapamil than the amplitude of contraction. The cumulative inhibitory concentration-response relationship of PA andverapamil on uterine

contraction induced by oxytocin were illustrated in Figure 37 and 38, IC_{50} value was presented in Table 4 and 5.

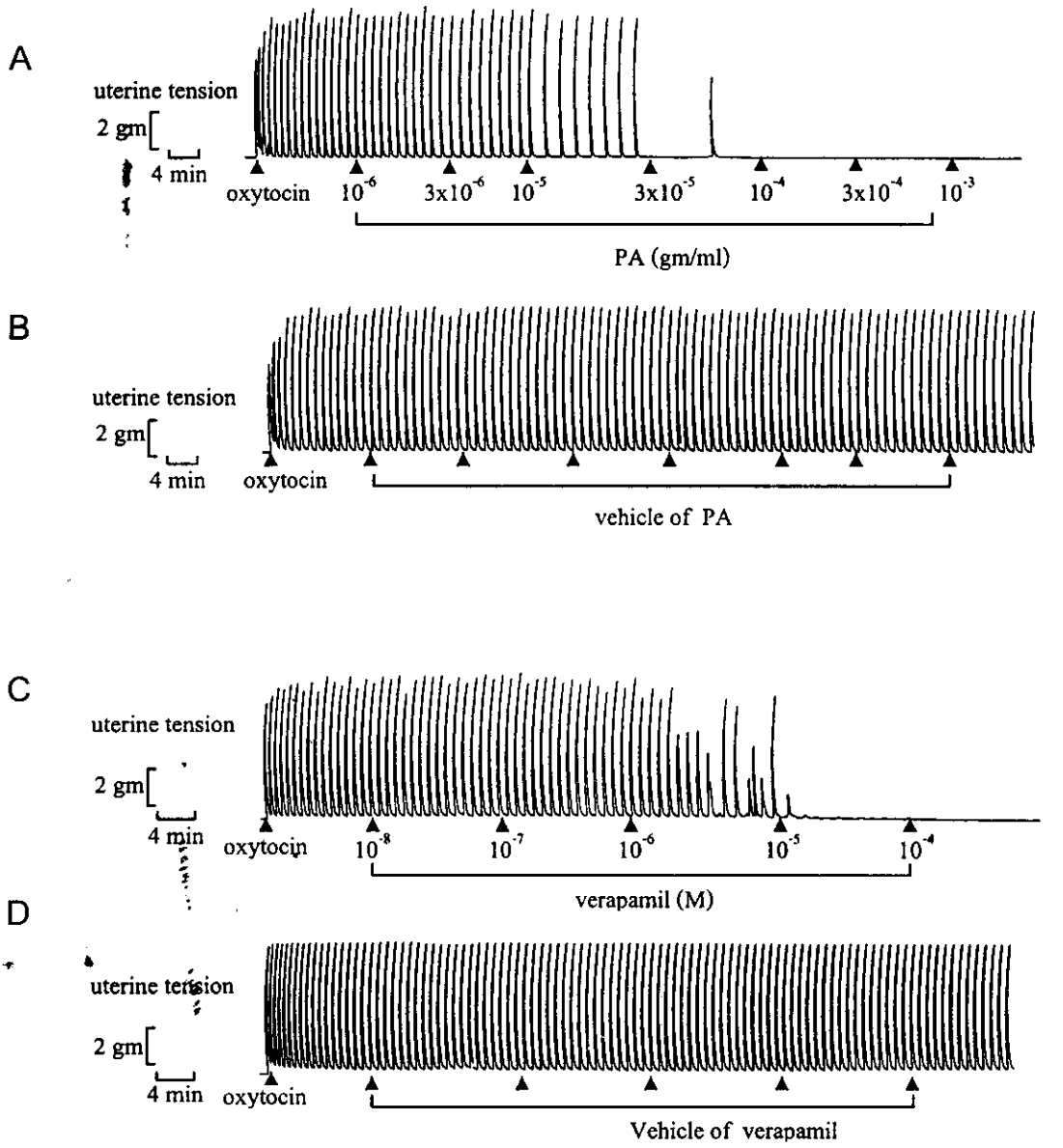


Figure 36. The representative tracings of the relaxing effect of the cumulative concentrations of PA (A) and its time control (B), verapamil (C) and its time control (D) on uterine contraction induced by oxytocin (1mU/ml) in isolated rat uterus.

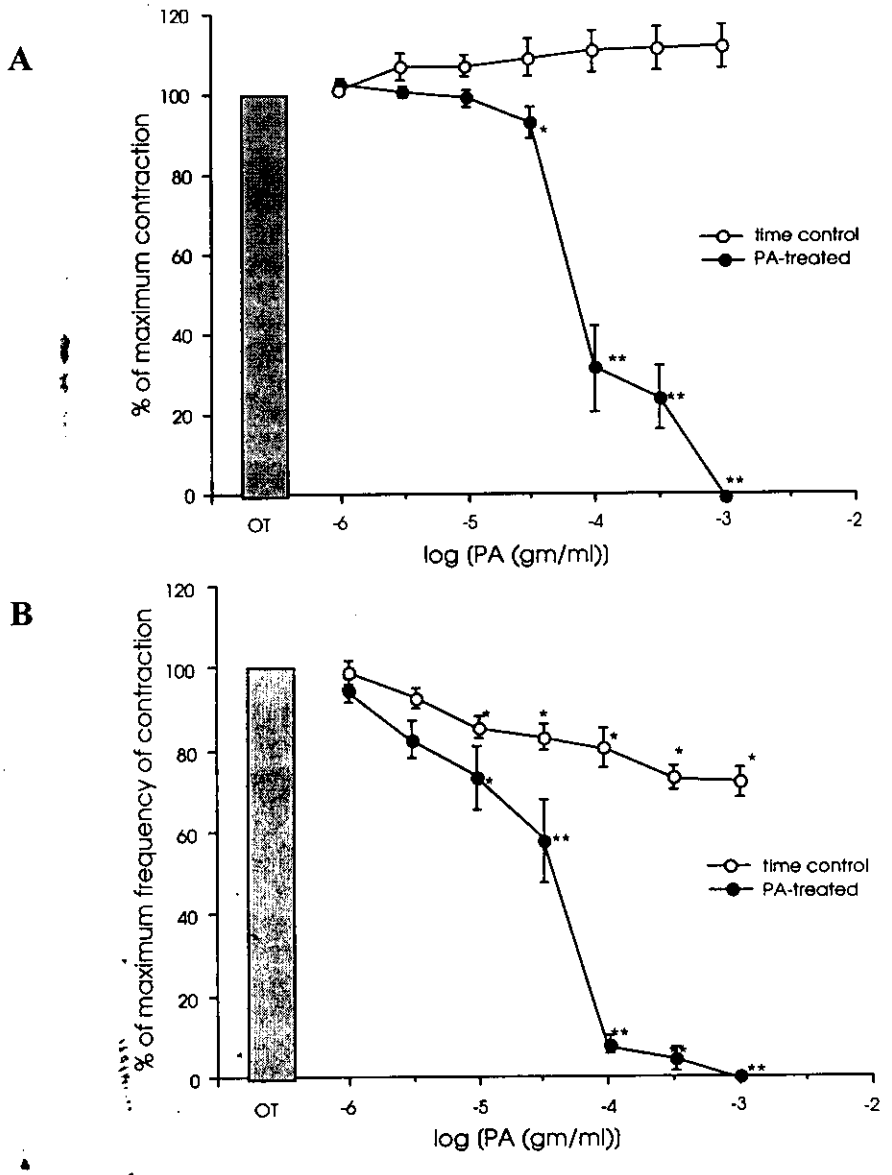


Figure 37. The concentration-response relationship of PA and its relaxing effect in isolated rat uterus precontracted with oxytocin (1mU/ml). (A) The effect of PA on force of contraction and (B) the effect of PA on frequency of contraction. Each value is expressed as a percentage of the initial maximal contraction in response to oxytocin. Each data point is the mean ± S.E. of ten experiments. (* $P < 0.05$, ** $P < 0.01$ as compared to the control)

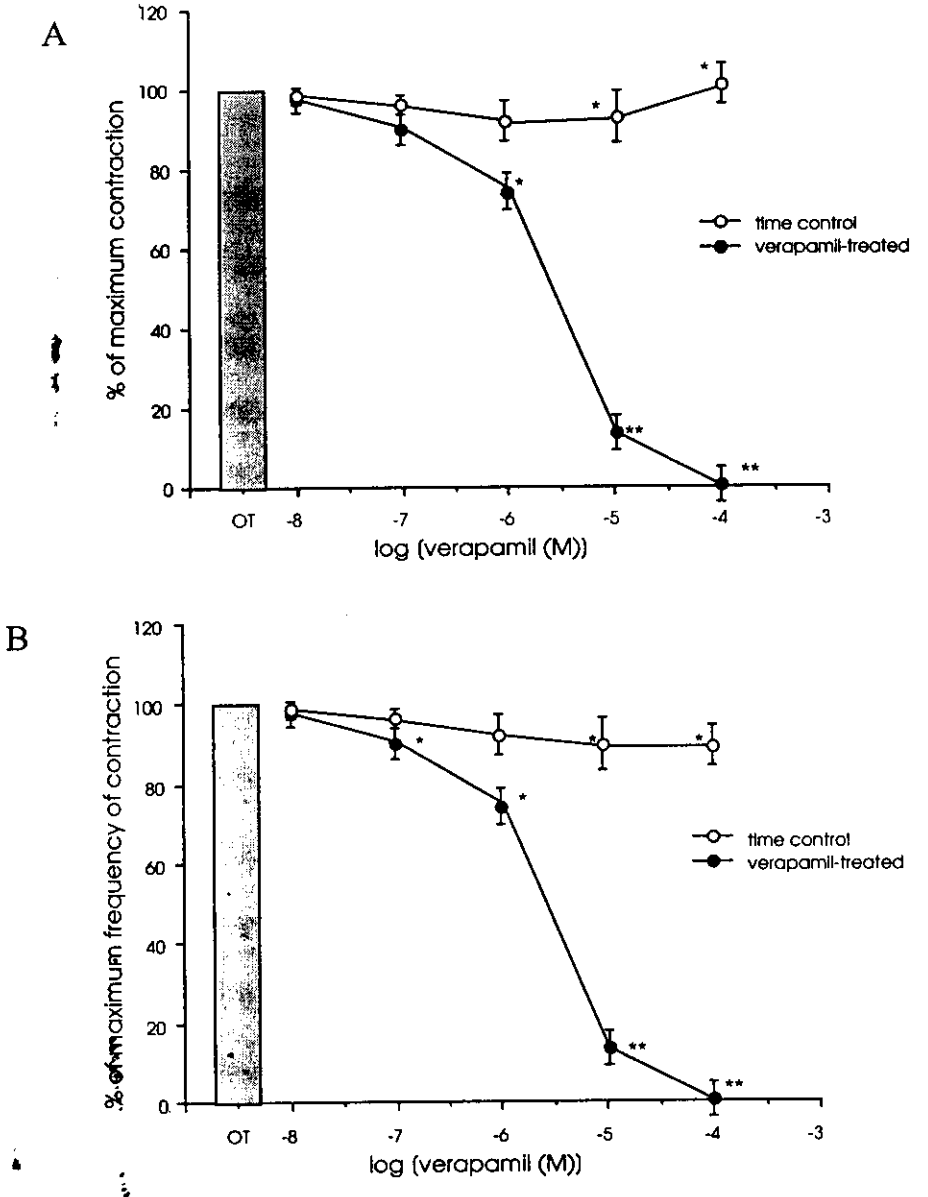


Figure 38. The concentration-response relationship of verapamil and its relaxing effect in isolated rat uterus precontracted with oxytocin (1 mU/ml). (A) The effect of verapamil on force of contraction and (B) the effect of verapamil on frequency of contraction. Each value is expressed as a percentage of the initial force of contraction or frequency of contraction in response to oxytocin (1mU/ml). Each data point is the mean ± S.E. of ten experiments. (* $P < 0.05$, ** $P < 0.01$ as compared to the control)

2.3.3 Effect of PA on the uterine contraction induced by $PGE_{2\alpha}$

The representative tracings of the relaxing effect of PA and its vehicle on uterine contraction induced by 10^{-5} M $PGF_{2\alpha}$ were demonstrated in Figure 39. The addition 10^{-5} M of $PGF_{2\alpha}$ produced a stable of force of contraction throughout the experiment as observed in the time control treatment. However, the frequency of contraction declined slowly with time but not more than 20% of the initial frequency was observed at the end of experiment. The addition of cumulative concentration of the vehicle of PA had no effects on the force of contraction induced by $PGF_{2\alpha}$ (10^{-5} M). In contrast, the addition of cumulative concentration of PA (10^{-6}-10^{-3} gm/ml) significantly inhibited both the frequency ($p < 0.05$) and the amplitude ($p < 0.05$) of the contraction. The comparison between the first concentration of PA which significantly affected to the frequency of contraction (3x 10^{-5} gm/ml, $p < 0.05$) and force of contraction (3 x 10^{-6} gm/ml $p < 0.05$) has shown that the frequency of contraction was more sensitive to PA than the force of contraction. The cumulative inhibitory concentration-response relationship of PA on uterine contraction induced by $PGF_{2\alpha}$ (10^{-5} M) was illustrated in Figure 40, IC_{50} value was presented in Table 4.

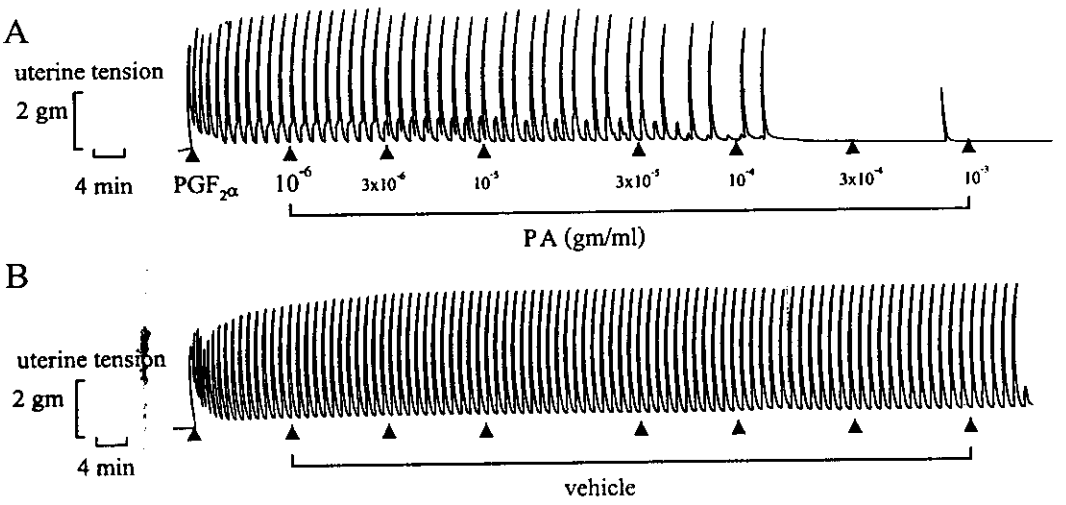
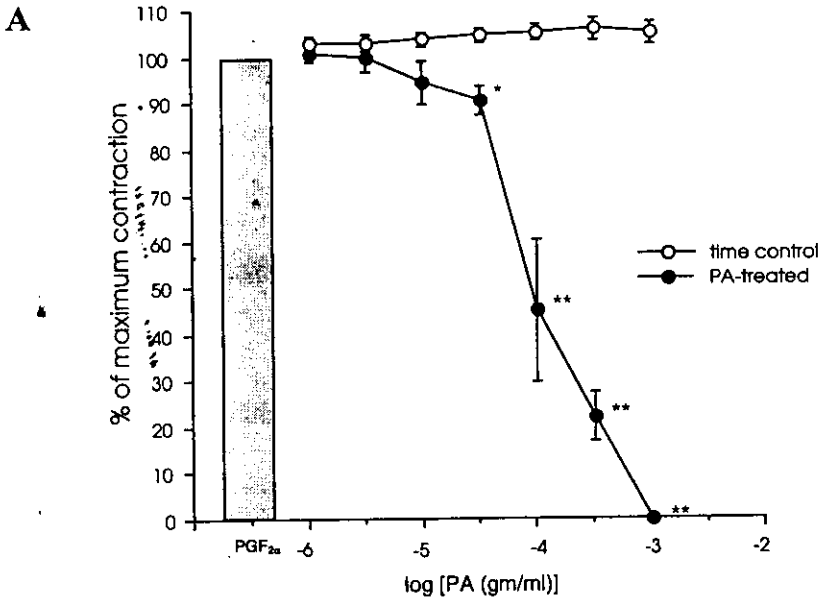


Figure 39. The representative tracings of the relaxing effect of cumulative concentrations of PA and its time control in isolated rat uterus precontracted with $PGF_{2\alpha}$(10^{-5} M); A = PA-treated, B = time control.



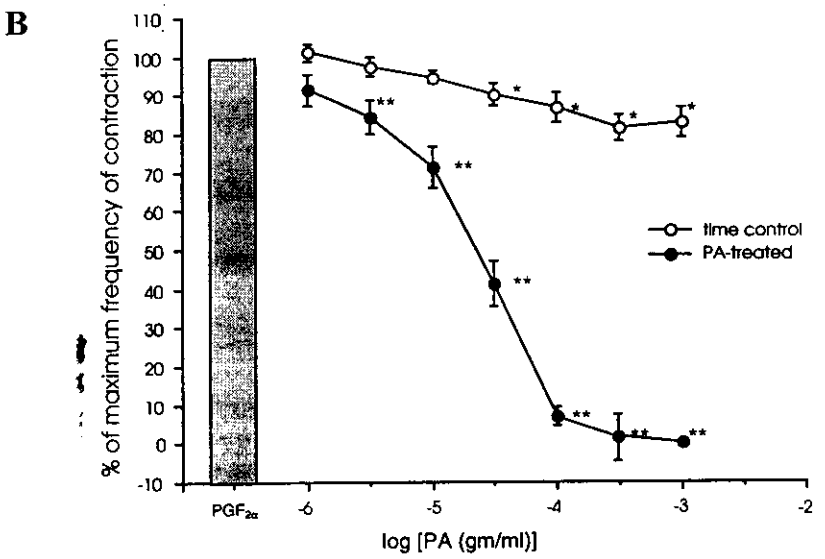


Figure 40. The concentration-response relationship of PA and its relaxing effect in isolated rat uterus precontracted with $PGF_{2\alpha}$ (10^{-5} M). (A) the effect of PA on force of contraction and (B) the effect of PA on frequency of contraction. Each value is expressed as a percentage of the initial maximal contraction or frequency of contraction in response to $PGF_{2\alpha}$. Each data point is the mean ± S.E. of seven experiments. (* $P < 0.05$, ** $P < 0.01$ as compared to the control)

2.3.4 Effects of PA and verapamil on uterine contraction induced by acetycholine

- Time-course relationship of PA or verapamil on uterine contraction induced by acetylcholine

The optimal incubation period of PA or verapamil on the inhibition of uterine contraction induced by acetylcholine was determined. Acetylcholine ($3x10^{-5}$ M) caused the uterus to contract to 4.04±0.55 gm. After preincubation of the uterus with PA (10^{-4} gm/ml) or verapamil (10^{-6} M) for various time period (5, 10, 15 or 20 minutes), this contraction was reduced significantly at

all time point study ($p < 0.05$) except at 5-minute incubation period with verapamil. This inhibitory effects of PA or verapamil were maximum with the preincubation time of 10 to 20 minutes with which there were no significant difference in the uterine tensions. The incubation time of PA orverapamil for 15 minutes was therefore selected to use in the next experiment. The representative tracings of the effect of PA andverapamil at various incubation time were illustrated in Figure 41 and 42, respectively, and the time-course relationship was demonstrated in the Figure 43.

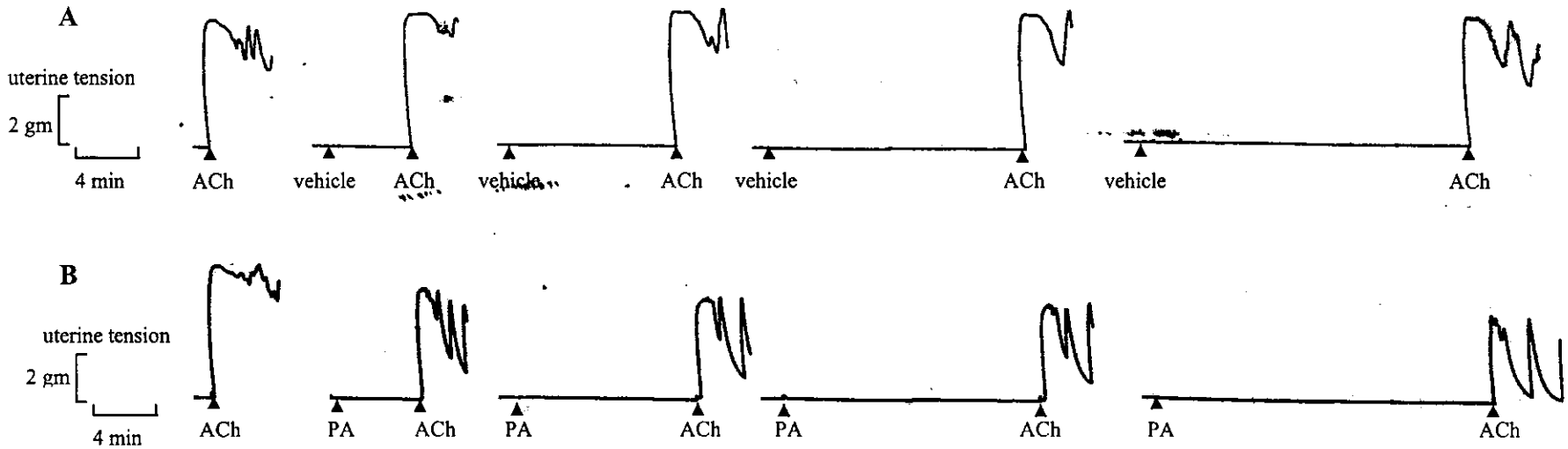


Figure 41. The representative tracings of the inhibitory effect of PA (10^{-4} gm/ml) at various incubation times (5, 10, 15 and 20 minutes) in isolated rat uterus precontracted with ACh ($3x10^{-3}$ M); A = time control, B = PA-treated

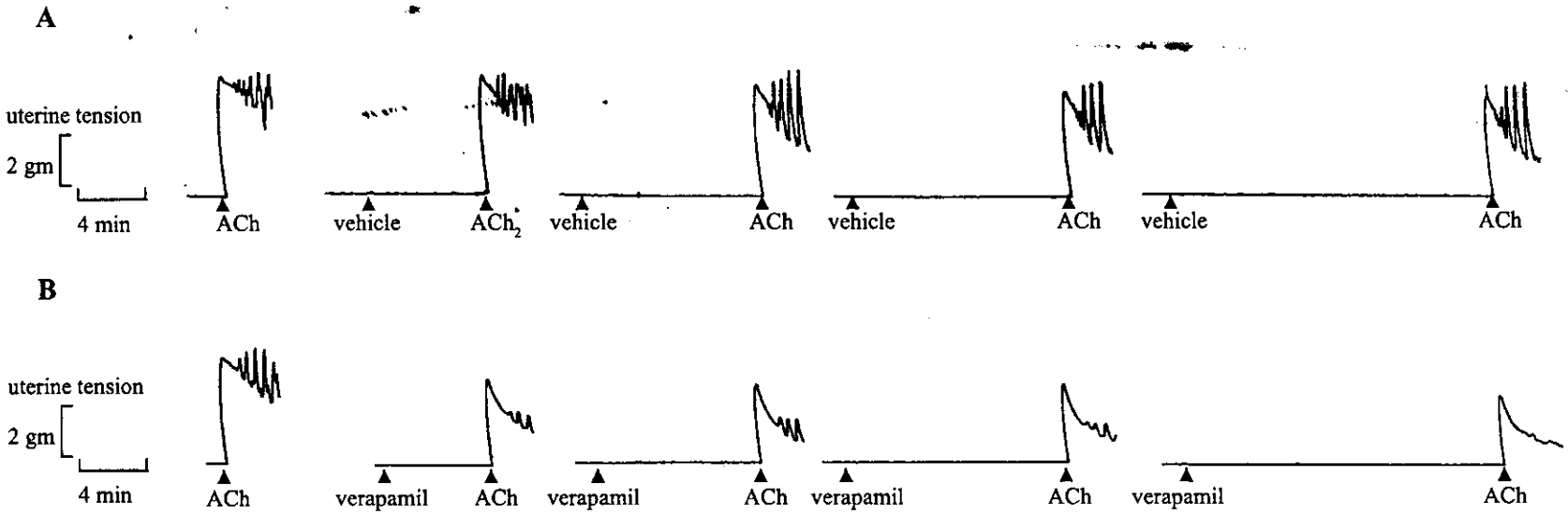


Figure 42. The representative tracings of the inhibitory effect of verapamil (10^{-7} M) at various preincubation times (5, 10, 15 and 20 minutes) on isolated rat uterus precontracted with ACh ($3x10^{-5}$ M); A = time control, B = verapamil-treated.

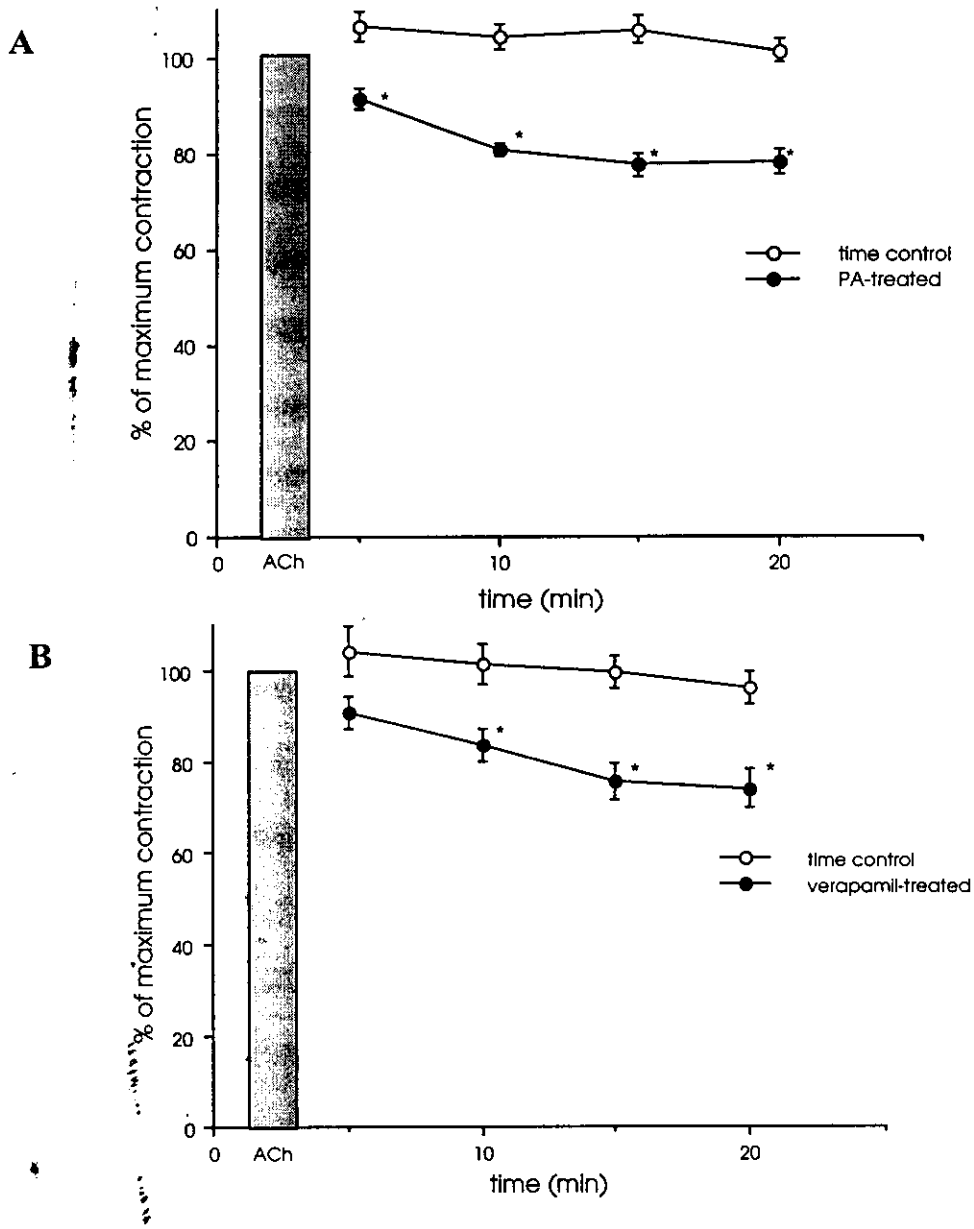


Figure 43. The time-course relationship of the inhibitory effect of PA (10^{-4} gm/ml) (A) and verapamil (10^{-7} M) (B) on isolated rat uterus precontracted with ACh ($3x10^{-5}$ M). The uterus were preincubated with PA or verapamil for a various time period (5, 10, 15 or 20 minutes). Each value is expressed as a percentage of the maximal contraction in response to ACh. Each data point is the mean ± S.E. of seven experiments. (* $p < 0.05$, ** $p < 0.01$ as compared to the control)

- Effects of PA or verapamil on uterine contraction induced by acetylcholine

The representative tracings of the relaxation effect of PA and its vehicle or verapamil and its vehicle on uterine contraction induced by acetylcholine ($3x10^{-5}$ M) were demonstrated in Figure 44 and 45, respectively. The cumulative inhibitory concentration-response relationship of PA or verapamil on uterine contraction induced by acetylcholine $3x10^{-5}$ M was illustrated in Figure 46. The addition of the vehicle of PA or verapamil had no effects on the uterine contraction induced by acetylchloine ($3x10^{-5}$ M). In contrast, the addition of PA 10^{-6}-10^{-3} gm/ml or 10^{-8}-10^{-4} M of verapamil significantly inhibited this contraction in a concentration-dependent manner with IC_{50} value of $5.03 \pm 0.43 \times 10^{-5}$ gm/ml and $2.48 \pm 0.25 \times 10^{-6}$ M respectively. The first concentration of PA with shown a significant inhibition on the contraction was $3x10^{-6}$ gm/ml and 10^{-3} gm/ml of PA completely inhibited the contraction. The first concentration of verapamil showed a significant reduction in the contraction induced by acetylcholine 10^{-7} M and at the concentration of 10^{-4} M, verapamil inhibited this contraction completely. IC_{50} values of PA and verapamil were shown in Table 4 and 5.

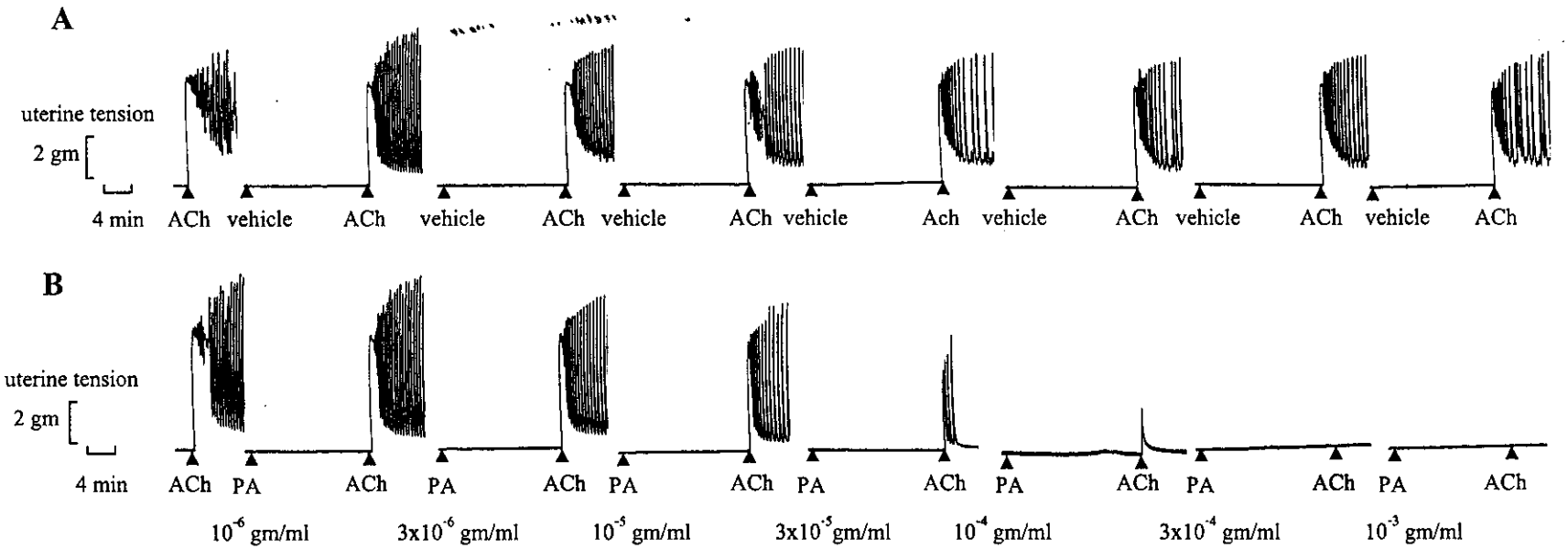


Figure 44. The representative tracings of the inhibitory effect of PA on isolated rat uterus. The uterus were preincubated with vehicle (A) or PA at various concentrations (B) for 15 min followed by acetylcholine ($3x10^{-5}$ M).

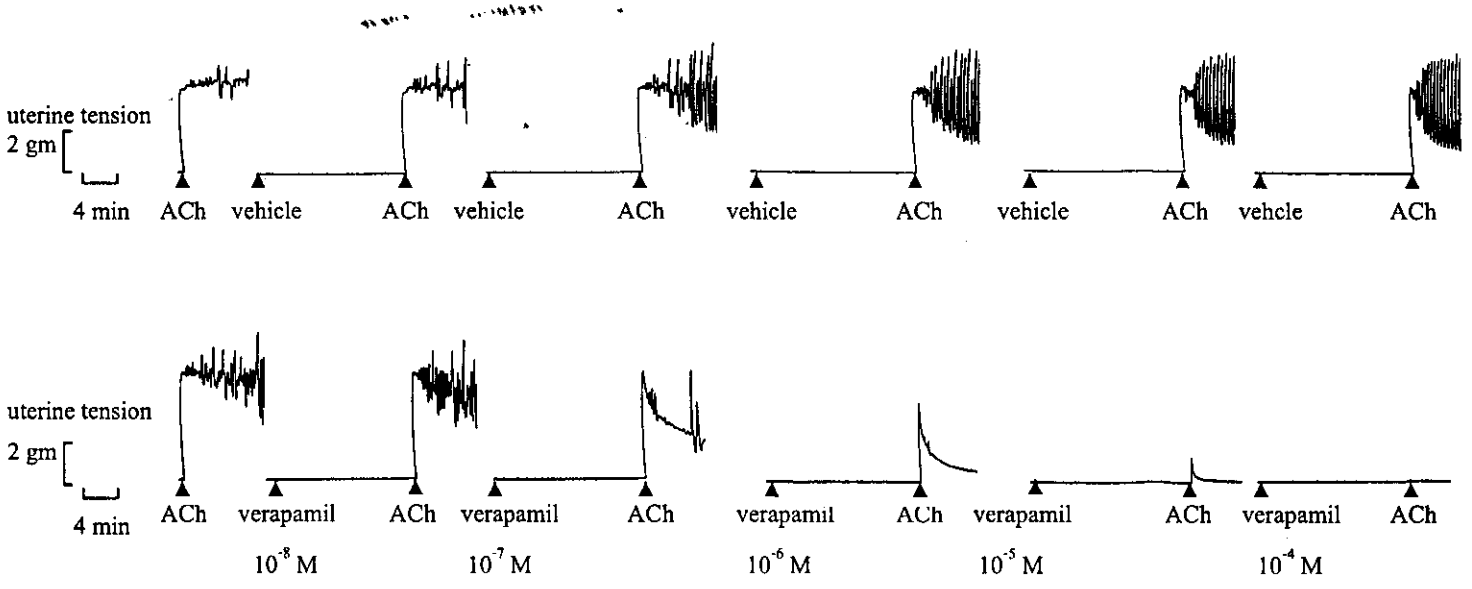


Figure 45. The representative tracings of the inhibitory effect of verapamil on isolated rat uterus. The uterus were preincubated with vehicle (A) or verapamil at various concentrations (B) for 15 min followed by acetylcholine ($3x10^{-5}$ M).

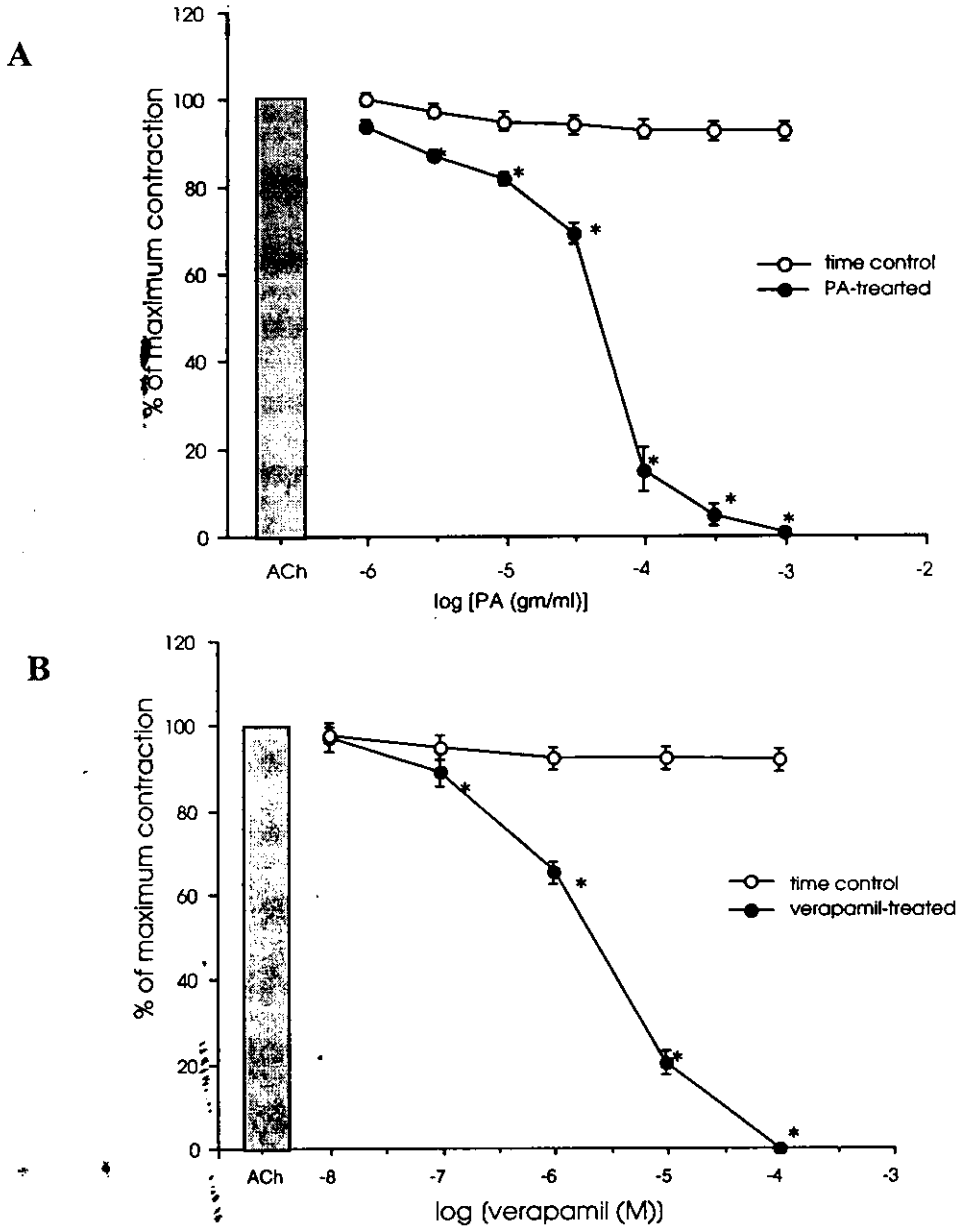


Figure 46. The concentration-response relationship of PA (A) and verapamil (B) on the inhibition of uterine contraction induced by acetylcholine ($3x10^{-5}$ M) in isolated rat uterus. Each value is expressed as a percentage of the contraction induced by acetycholine. Each data point represents the mean ± S.E. of ten experiments. (*$p < 0.05$, ** $p < 0.01$ as compared to the control)

2.4 Effect of PA and verapamil on uterine contraction induced by oxytocin in Ca^{2+}-free solution

Oxytocin at the concentration 10 mU/ml was chosen for the use in this experiment, according to Oishi, et al., (1991). At this concentration, oxytocin produced a rapid phasic contraction and a stable plateau of tonic contraction throughout the experiment (Figure 47).

The representative tracings of the relaxing effect of PA and verapamil on uterine contraction and the concentration-response relationship of PA or verapamil and uterine contraction induced by oxytocin 10 mU/ml in Ca^{2+}-free solution was illustrated in Figure 47. The addition of vehicle of PA or verapamil had no effects on uterine contraction induced by oxytocin in Ca^{2+}-free solution. In contrast, the addition of PA appeared to inhibit this contraction. A significant inhibition by PA was first appeared at the concentration of 10^{-5} gm/ml and, at concentration of 10^{-3} gm/ml, PA was able to inhibit the contraction completely. IC_{50} value was $1.21 \pm 0.18 \times 10^{-4}$ gm/ml. Verapamil (10^{-8}-10^{-6} M) had no effect on the uterine contraction. However, at the concentration 10^{-5}-10^{-4} M, verapamil inhibited the contraction to about 60% of contraction. The concentration-response relationship of PA or verapamil and uterine contraction was shown in Figure 48 and the IC_{50} value was shown in Table 4.

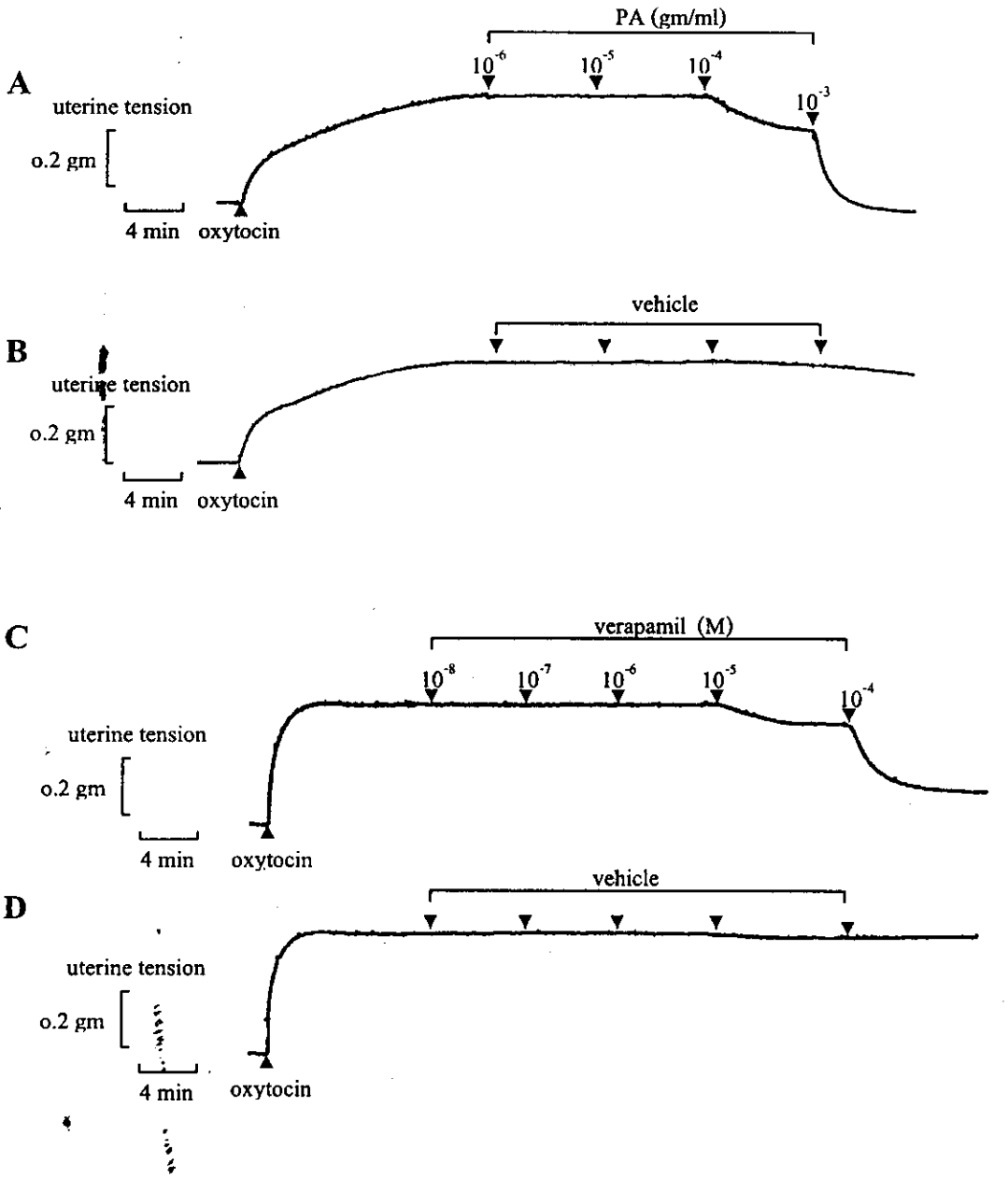


Figure 47. The representative tracings of PA (A) and its time control (B), verapamil (C) and its time control (D) induced relaxation in isolated rat uterus precontracted with oxytocin (10 mU/ml) in Ca^{2+}-free solution.

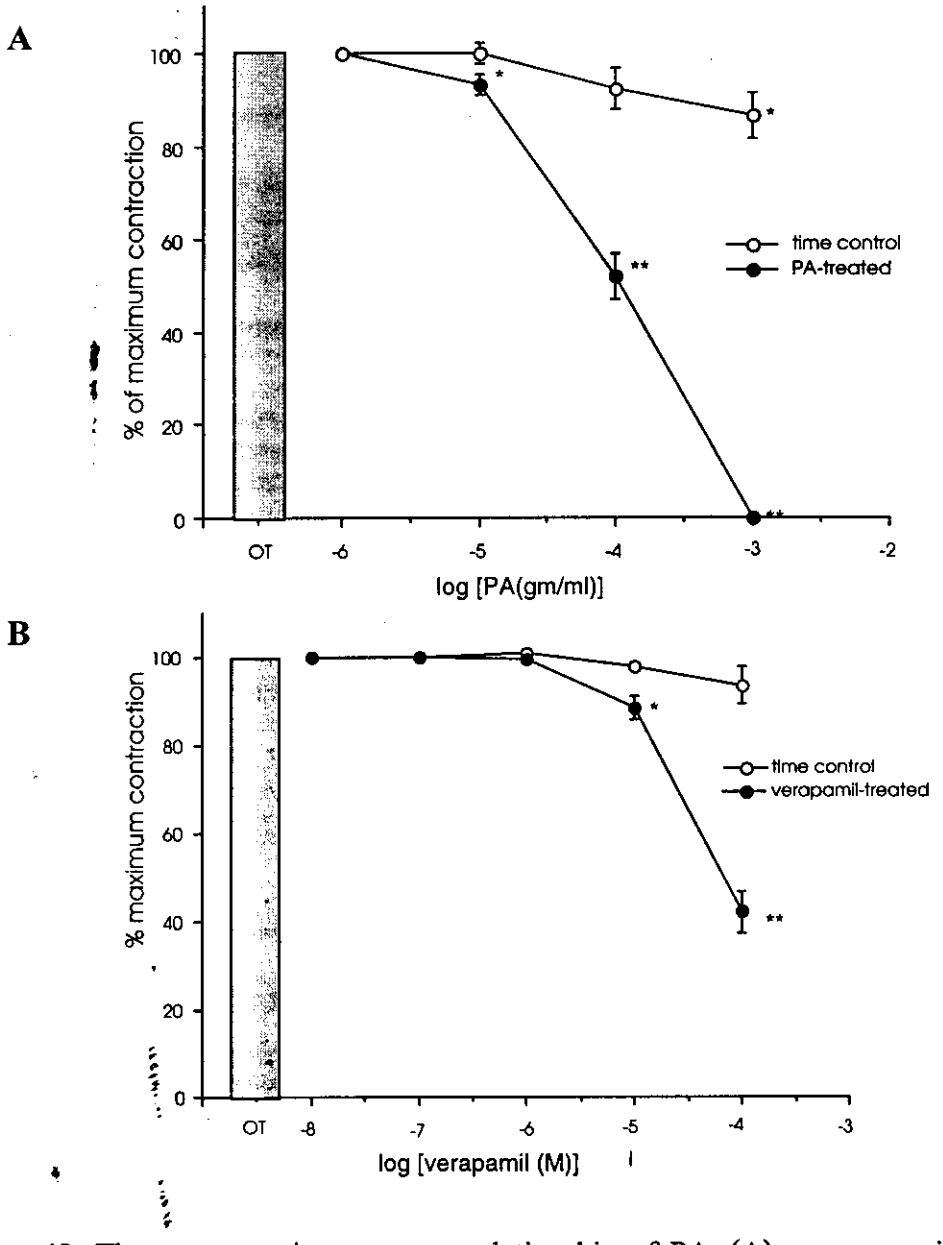


Figure 48. The concentration-response relationship of PA (A) or verapamil (B) and uterine contraction on isolated rat uterus precontracted with oxytocin 10 mU/ml in Ca^{2+}-free solution. Each value is expressed as a percentage of the initial maximal contraction in response to oxytocin. Each data point represents the mean ± S.E. of seven experiments. (* $p < 0.05$, ** $p < 0.01$ as compared to the control)

Table 4 Comparison of the IC_{50} values of PA on frequency and amplitude of contraction in isolated rat uterus precontracted with uterine stimulants.

Stimulants drug	IC_{50} of PA (gm/ml) (Mean ± S.E.)	
	Force of contraction	Frequency of contraction
Oxytocin	$7.39 \pm 1.35 \times 10^{-5}$	$6.81 \pm 1.64 \times 10^{-5}$
$PGF_{2\alpha}$	$8.27 \pm 1.80 \times 10^{-5}$	$3.51 \pm 1.81 \times 10^{-5}$
acetylcholine	$6.07 \pm 1.43 \times 10^{-5}$	-
Depolarizing solution (KCl 56.3 mM)	$8.37 \pm 0.58 \times 10^{-5}$	-
Oxytocin (Ca^{2+} free solution)	$1.21 \pm 0.18 \times 10^{-4}$	-

Table 5 Comparison of the IC_{50} values of verapamil on frequency and amplitude of contraction in isolated rat uterus precontracted with uterine stimulants.

Stimulants drug	IC_{50} of verpamil(M) (Mean ± S.E.)	
	Force of contraction	Frequency of contraction
Oxytocin	$2.24 \pm 1.27 \times 10^{-6}$	$3.52 \pm 0.74 \times 10^{-6}$
Acetylcholine	$2.48 \pm 0.25 \times 10^{-6}$	-
Oxytocin (Ca^{2+} free solution)	$7.50 \pm 1.09 \times 10^{-5}$	-

2.5 Effect of PA and isoproterenol on uterine contraction induced by depolarizing solution

2.5.1 Determination of optimal concentration of propranolol

Cumulative increase in concentration of isoproterenol produced relaxing effect on uterine contraction precontracted with depolarizing solution, EC_{50} value of isoproterenol was $6.62 \pm 1.25 \times 10^{-9}$. In the presence of 10^{-8}, 10^{-7} and 10^{-6} M of propranolol, the inhibition was inhibited, as observed by a shift of the log concentration-response curve of isoproterenol to the right. EC_{50} value of isproterenol in the presence of 10^{-8}, 10^{-7} and 10^{-6} M of propranolol were $5.21 \pm 0.76 \times 10^{-7}$, $7.60 \pm 0.89 \times 10^{-6}$ and $7.09 \pm 1.10 \times 10^{-5}$, respectively (Table 6). The representative tracings of the blocking activity of propranolol on uterine relaxing effect of isoproterenol at various concentrations were demonstrated in Figure 49 and the log concentration-response relationship was illustrated in Figure 51. The concentration of propranolol at 10^{-7} M was then chosen to use in the next experiment.

2.5.2 Time-course relationship of propranolol on the inhibition of uterine relaxing effect of isoproterenol

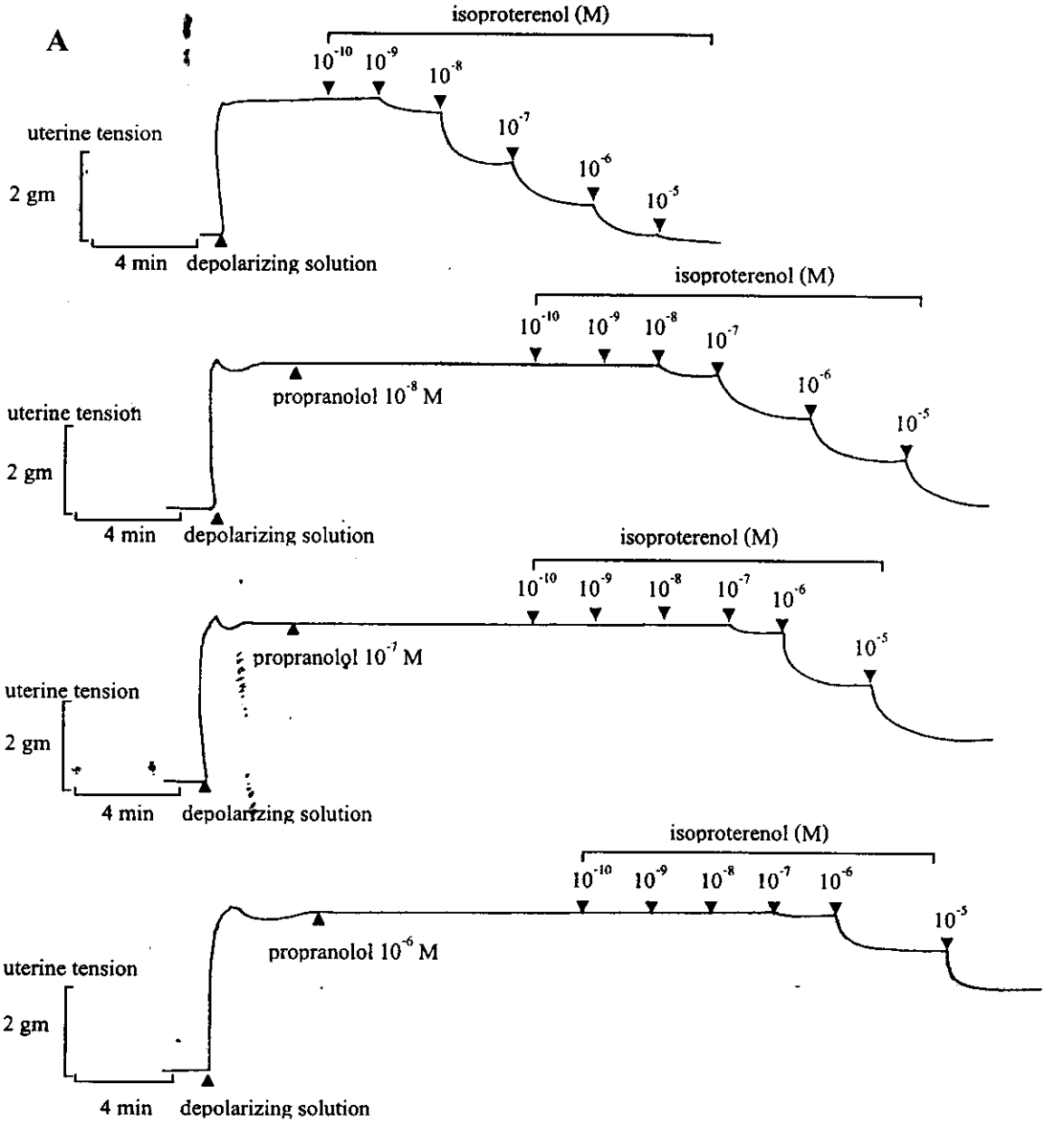
The optimal incubation period of propranolol that inhibited the effect of isoproterenol was determined. Cumulative addition of isoproterenol (10^{-10}-10^{-5} M) produced relaxation on uterine contraction-induced by depolarizing solution. The presence of propranolol (10^{-7} M) for 10, 20 and 30 minutes significantly blocked the relaxing effect of isoproterenol as observed by a shift of log concentration-response curves of isoproterenol to the right. EC_{50} of isoproterenol in the presence of propranolol (10^{-7} M) preincubated at 10, 20 or

30 minutes are 3.46 ± 0.87x10^{-6}, 2.50 ± 0.81 x 10^{-6} and 4.02 ± 0.89x10^{-6}, respectively (Table 7) which were not significant differences from the EC_{50} value obtained with isoproterenol alone ($p > 0.05$, ANOVA). The 20-minute incubation period of propranolol (10^{-7} M) was then selected to use in the next experiment. The representative tracings of blocking activity of propranolol at various times were demonstrated in Figure 50 and the time relationship curves illustrated in Figure 51.

2.4.3 Effect of propranolol on uterine relaxation induced by PA or isoproterenol

The representative tracings of PA and isoproterenol induced uterine relaxation precontracted with depolarizing solution were illustrated in Figure 52 and 53, respectively. The uterine contraction induced by depolarizing solution (KCl 56.3 mM) produced a stable plateau of tonic contraction throughout the experiment as observed in the time control. The addition of vehicle of PA or isoproterenol had no effects on uterine contraction induced by depolarizing solution. The addition of cumulative concentration of PA inhibited this contraction with IC_{50} value of 6.28 ± 0.62x10^{-4} gm/ml. In the presence of 10^{-7} M of propranol, PA also inhibited this contraction with IC_{50} value of 6.35 ± 0.32 x 10^{-4} gm/ml which was not significantly different from that obtained in the absence of propanolol ($p > 0.05$, ANOVA). The addition of cumulative concentration of isoproterenol inhibited the uterine contraction in a concentration-dependent manner with IC_{50} of 1.39 ± 0.23x10^{-8} M. In the presence of 10^{-7} M of propranolol, the relaxing effect of isoproterenol was significantly inhibited ($p < 0.01$, ANOVA) as observed by the shift of log

concentration-response curve of isoproterenol to the right with IC_{50} of 1.72 ± $0.38x10^{-6}$ M. The log concentration-response relationship of PA and isoproterenol in the presence or absence of propranolol (10^{-7} M) were demonstrated in Figure 54. IC_{50} values were presented in Table 8.



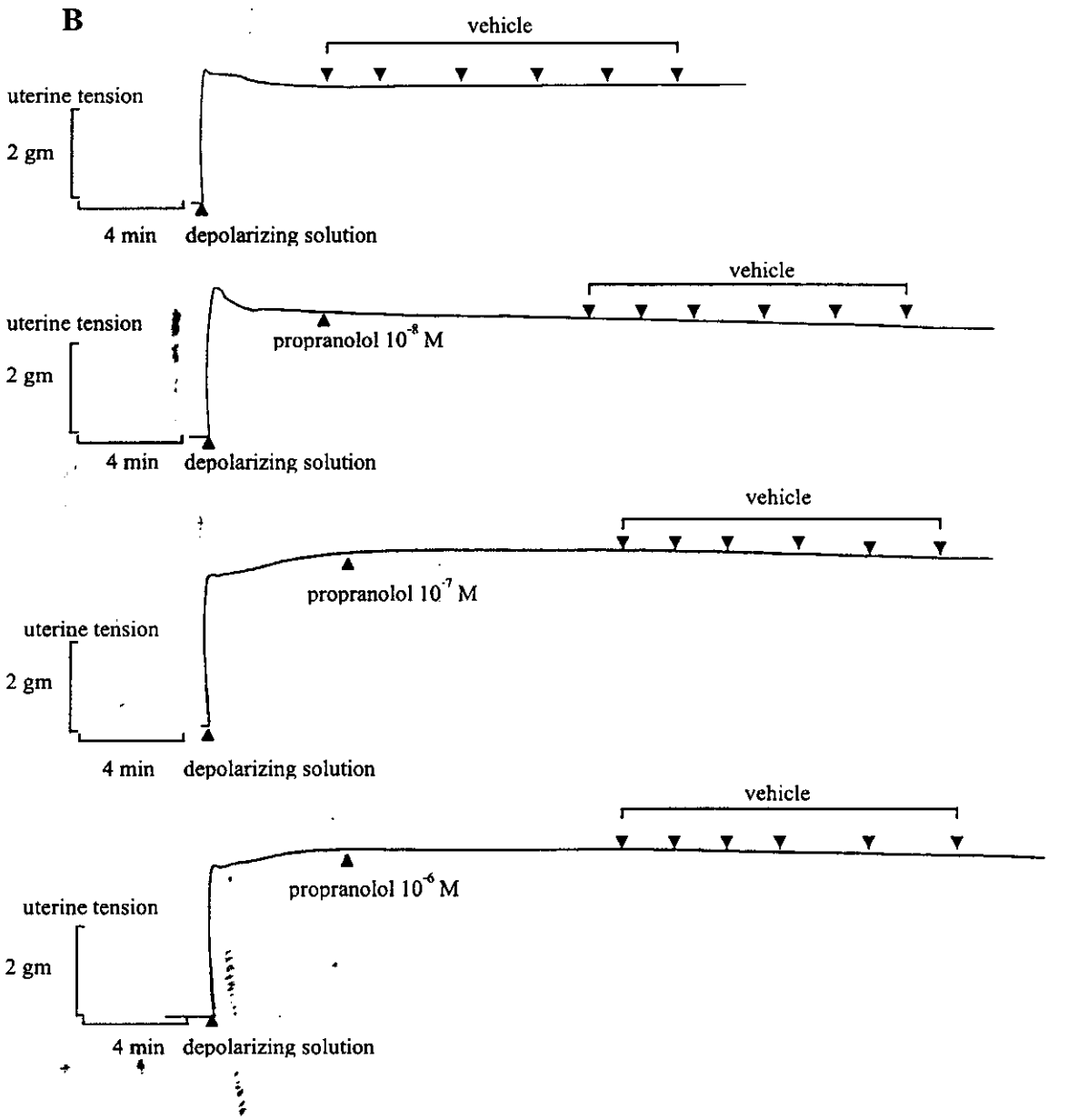
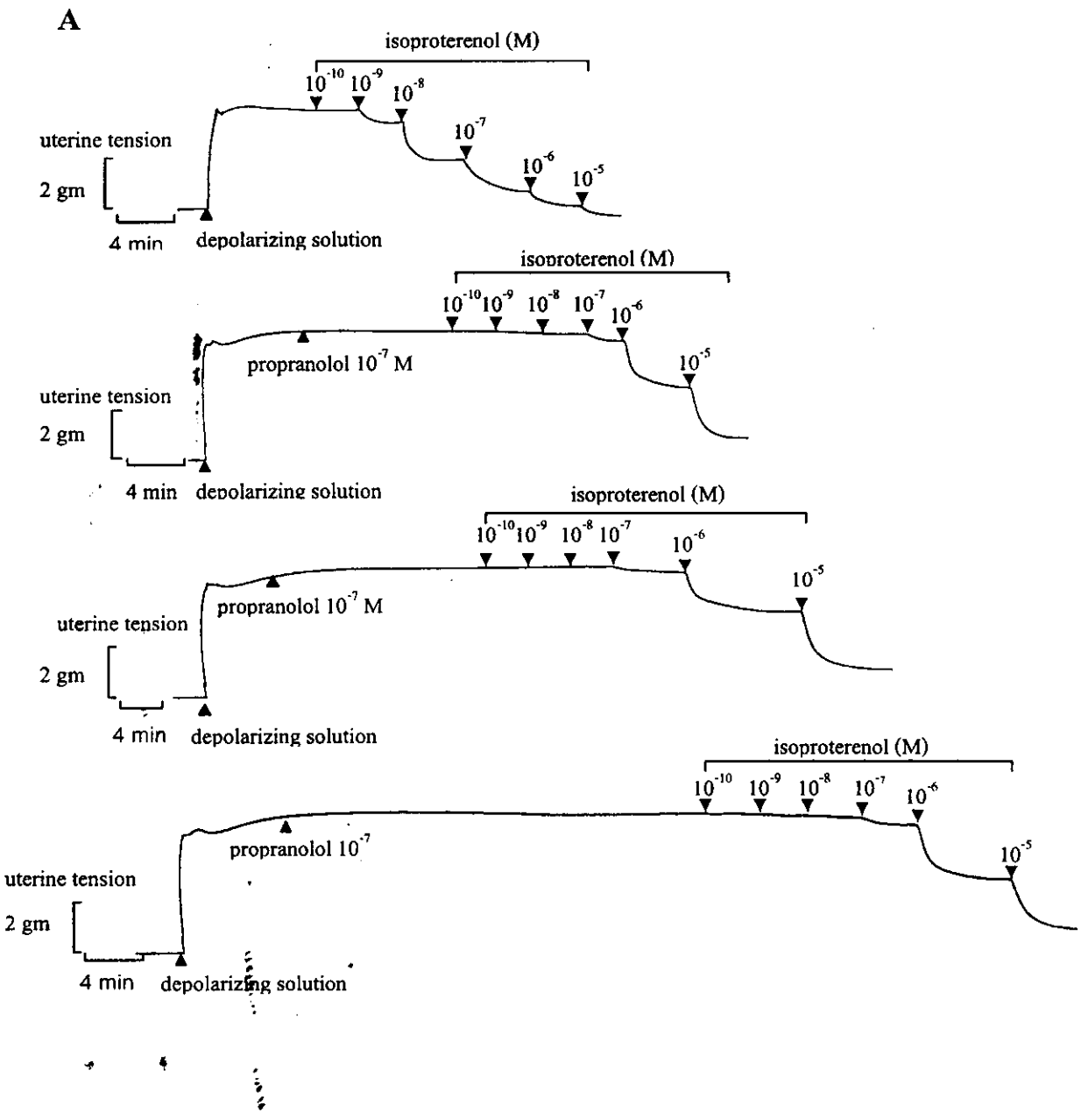


Figure 49. The representative tracings of the inhibitory effect of propranolol (10 min) on the relaxing effect of isoproterenol in isolated rat uterus precontracted with the depolarizing solution (KCl 56.3 mM). A = isoproterenol alone and 10^{-8}, 10^{-7}, 10^{-6} M propranolol-treated respectively, B = time control of each experiment.


A
isoproterenol (M)
10^{-10} 10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5}
uterine tension
2 gm
4 min
depolarizing solution
isoproterenol (M)
10^{-10} 10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5}
propranolol 10^{-7} M
uterine tension
2 gm
4 min
depolarizing solution
isoproterenol (M)
10^{-10} 10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5}
propranolol 10^{-7} M
uterine tension
2 gm
4 min
depolarizing solution
isoproterenol (M)
10^{-10} 10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5}
propranolol 10^{-7}
uterine tension
2 gm
4 min
depolarizing solution

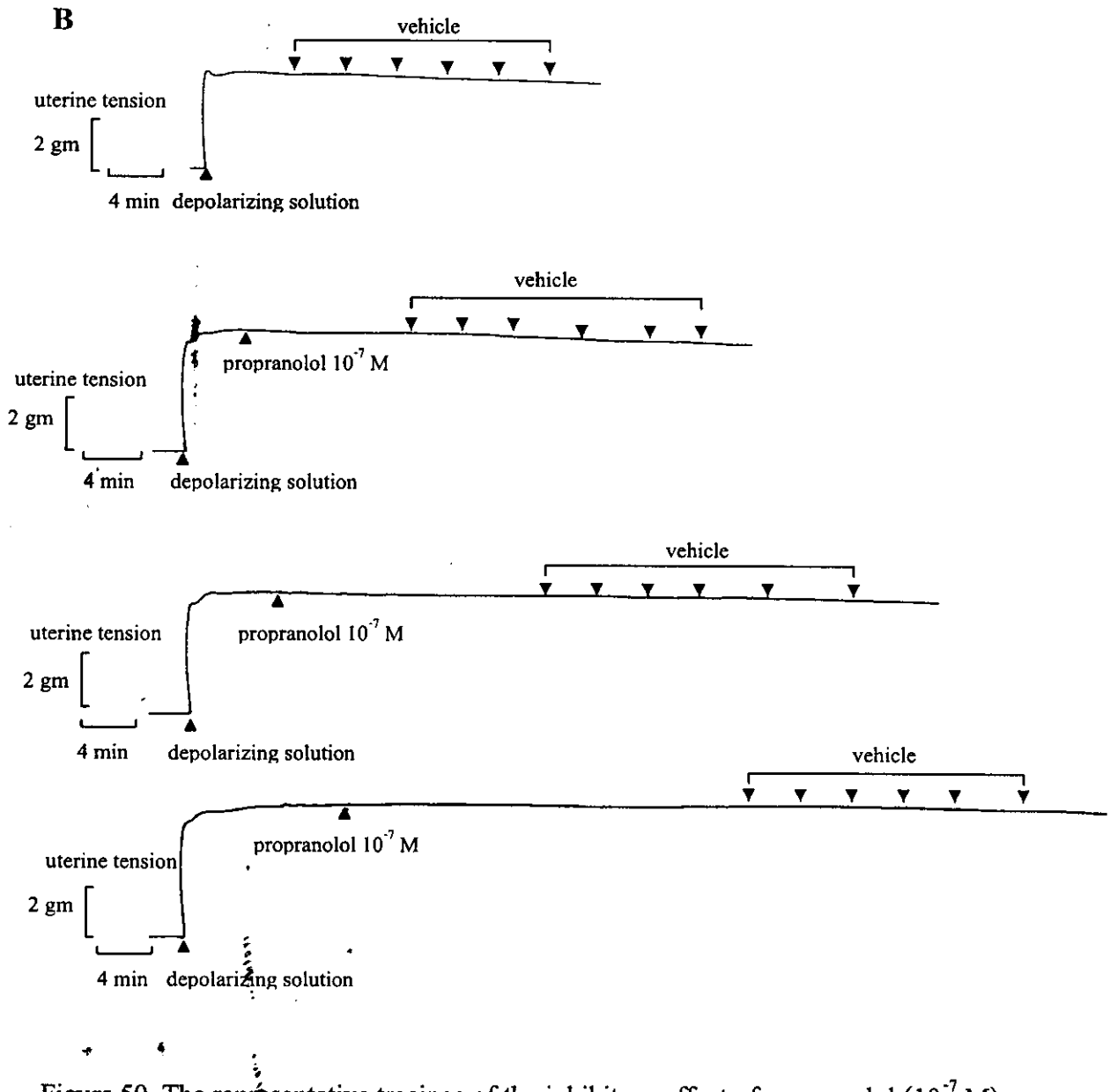


Figure 50. The representative tracings of the inhibitory effect of propanolol (10^{-7} M) on the relaxing effect of isoproterenol at various incubation times (10, 20 and 30 minutes) in isolated rat uterus precontracted with the depolarizing solution (KCl 56.3 mM). A = isoproterenol alone and propranolol-treated, B = time control of each experiment.

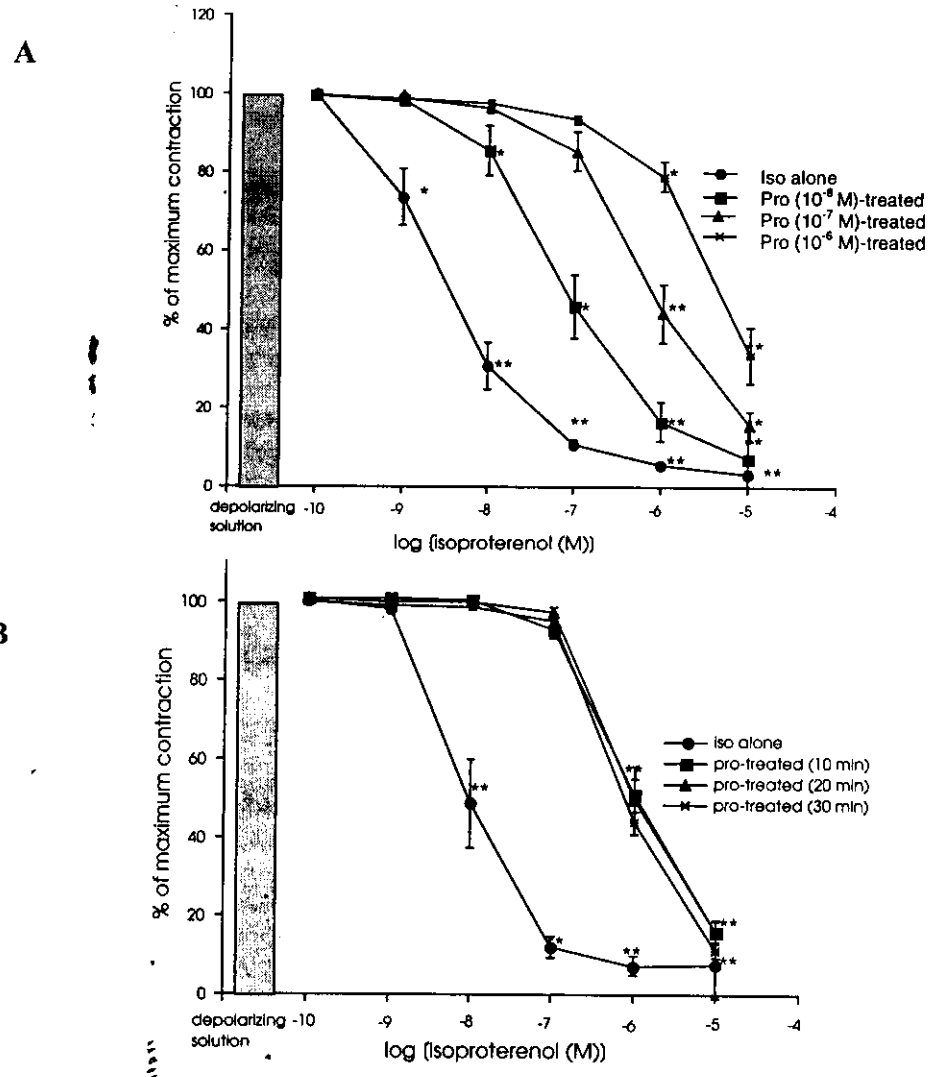


Figure 51. The concentration-response relationship of the inhibitory effect of propranolol on relaxing effect of isoproterenol in isolated rat uterus, the uteri were precontrated with depolarizing solution (KCl 56.3 mM). (A) Effects of various concentrations of propranolol (10^{-8}, 10^{-7} and 10^{-6} M, 10 minutes) and (B) effect of propranolol (10^{-7} M) at various incubation times (10, 20 and 30 minutes). Each value is expressed as a percentage of the maximal contraction in response to depolarizing solution and each data point represents mean ± S.E. of ten experiments. (* $p < 0.05$, ** $p < 0.01$ as compared to the maximum contraction induced by depolarizing solution.)

Table 6 Comparison of the EC_{50} of isoproterenol-induced relaxation in the presence or absence of propranolol (10^{-8}, 10^{-7} and 10^{-6} M) on isolated rat uterus precontracted with depolarizing solution (KCl 56.3 mM).

EC_{50} values (Mean ± S.E.)			
isoproterenol alone	propranolol-pretreated (10 min)		
	10^{-8} M	10^{-7} M	10^{-6} M
$6.62 \pm 1.25 \times 10^{-9}$	$5.21 \pm 0.76 \times 10^{-9*}$	$7.60 \pm 0.89 \times 10^{-9**}$	$7.09 \pm 1.10 \times 10^{-9**}$

(* $p < 0.05$, ** $p < 0.01$ as compared to isoproterenol alone)

Table 7 Comparison of the EC_{50} of isoproterenol-induced relaxation in the presence or absence of propranolol (10^{-7} M) at 10, 20 or 30 minutes on isolated rat uterus precontracted with depolarizing solution (KCl 56.3 mM).

EC_{50} values (Mean ± S.E.)			
isoproterenol alone	propranolol-pretreated (10^{-7} M)		
	10 min	20 min	30 min
$8.74 \pm 1.40 \times 10^{-9}$	$3.46 \pm 0.87 \times 10^{-6**}$	$2.50 \pm 0.81 \times 10^{-6**}$	$4.02 \pm 0.90 \times 10^{-6**}$

(* $p < 0.05$, ** $p < 0.01$ as compared to isoproterenol alone)

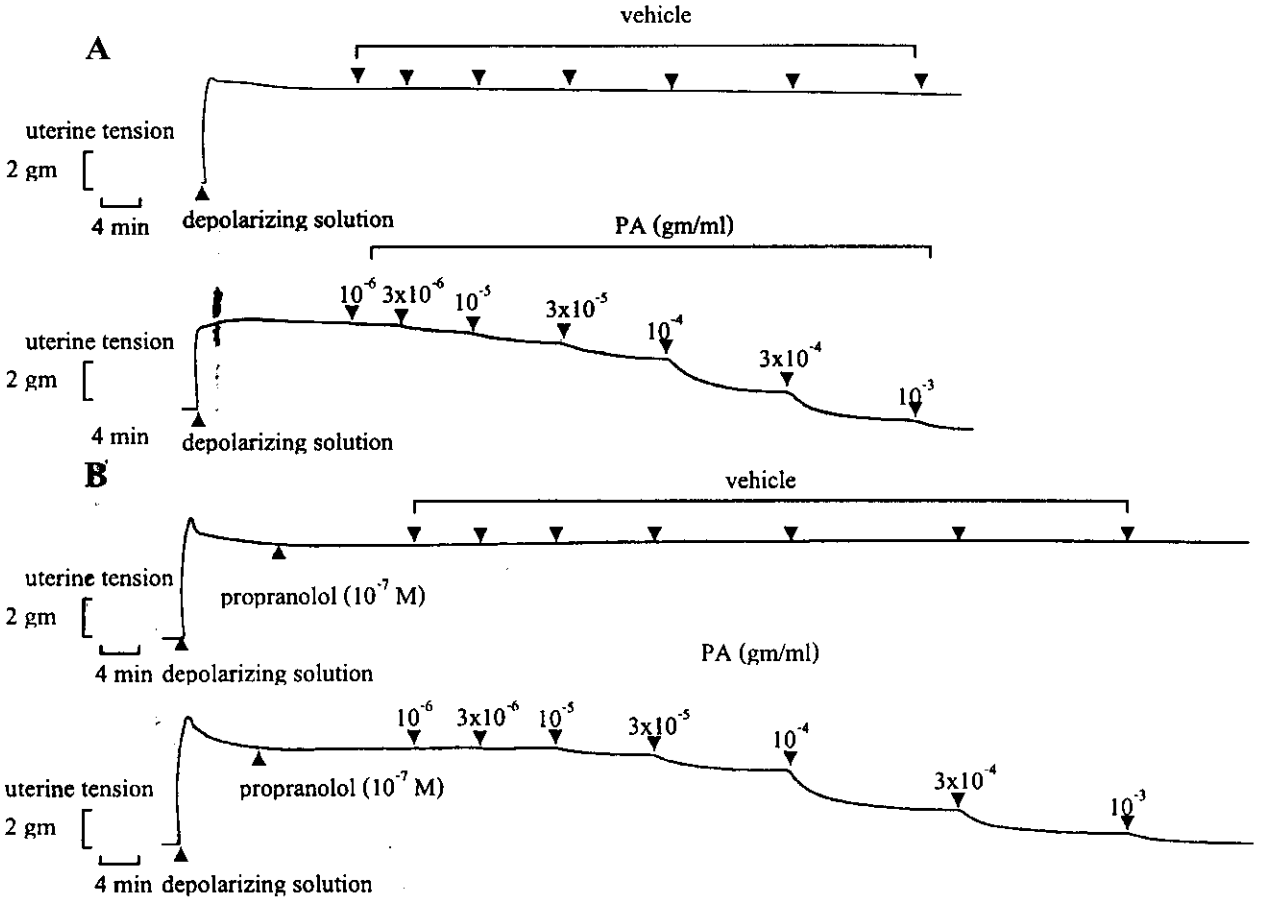


Figure 52. The representative tracings of the relaxing effect of PA and its time control (A), or pretreated with propranolol (10^{-7} M) for 20 minutes and its time control (B) on rat isolated uterus precontracted with depolarizing solution.

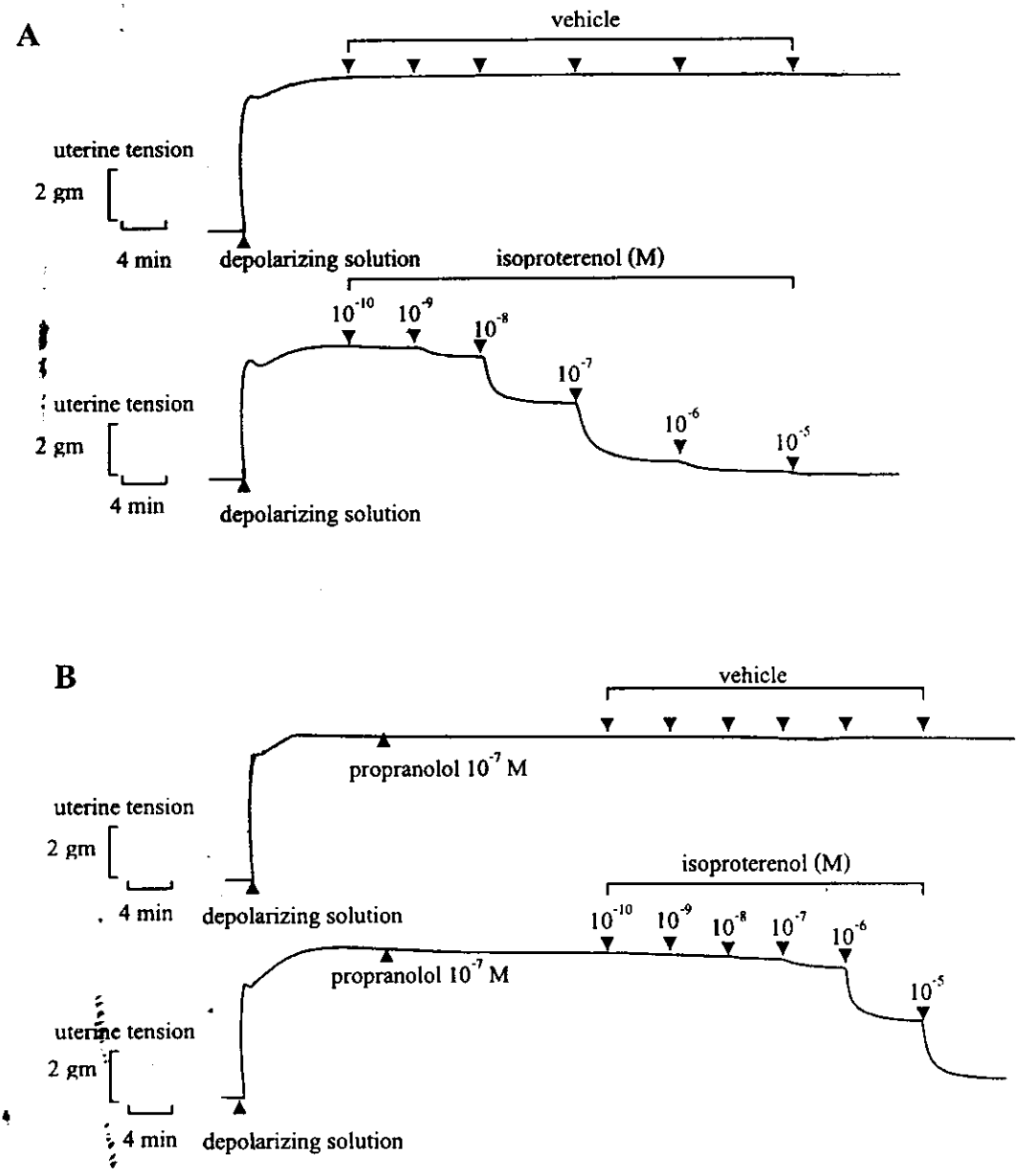


Figure 53. The representative tracings of the relaxing effect of isoproterenol and its time control (A), or pretreated with propranolol (10^{-7} M) for 20 min and its time control (B) on isolated rat uterus precontracted with depolarizing solution.

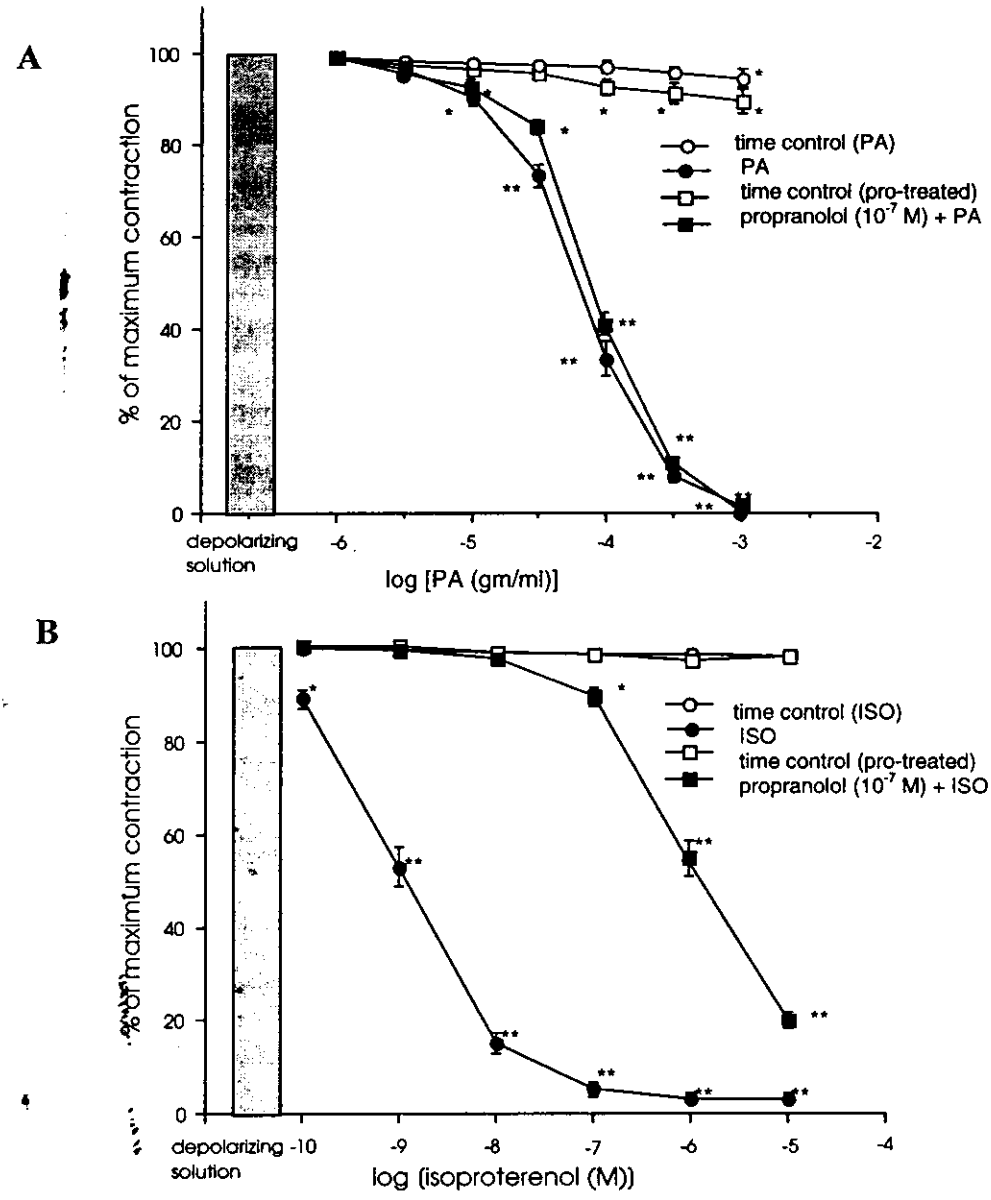


Figure 54. The concentration-response relationship of PA (A) or isoproterenol (B) and uterine relaxation in the presence or absence propranolol (10^{-7} M) in isolated rat uterus precontracted with depolarizing solution. Each value is expressed as a percentage of the initial maximal contraction in response to depolarizing solution. Each data point represents the mean ± S.E. of ten experiments. (* $p < 0.05$, ** $p < 0.01$ as compared to the control)

Table 8 Comparison of the IC_{50} of PA or EC_{50} isoproterenol induced relaxation in the presence or absence of propranolol (10^{-7} M) on isolated rat uterus precontracted with depolarizing solution (KCl 56.3 mM).

IC_{50} of PA (gm/ml) (Mean ± S.E.)		EC_{50} of isoproterenol (M) (Mean ± S.E.)	
PA alone	propranolol-pretreated	isoproterenol alone	propranolol-pretreated
$8.28 \pm 0.66 \times 10^{-4}$	$8.35 \pm 0.42 \times 10^{-4}$	$1.39 \pm 0.23 \times 10^{-9}$	$2.72 \pm 0.38 \times 10^{-6**}$

(* $p < 0.05$, ** $p < 0.01$ as compared to PA or isoproterenol alone)