

CHAPTER 4

RESULTS

1. Extraction of alkaloid from *C. papaya* L. leaves

The yield and texture of crude alkaloid and purified alkaloid from *C. papaya* L. leaves are shown in Table 6 and Figure 14. Dried papaya leaves (7.5 kg) were pulverized and submitted to a conventional acid-base method to obtain 21.3 g (0.284%) of crude alkaloid. Successive chromatography of the crude alkaloid (15 g) on a silica gel column and elution with solvents of increasing polarity afforded the isolation alkaloid. Crystalline carpaine (1.3292 grams, 0.1194%) was obtained and identified by ^1H and ^{13}C NMR spectroscopy. ^1H and ^{13}C NMR spectra of isolated carpaine were shown in Appendix B and C. TLC of the crude alkaloid extract, however, showed 4 positive spots by Dragendorff's reagent. The R_f values of carpaine, unknown 1, unknown 2 and unknown 3 are 0.62, 0.82, 0.71 and 0.17 in solvent system (methanol/chloroform 5:95 v/v), respectively. Isolation and purification of the unknowns were achieved by column chromatography using 3 solvent systems. Both compounds appear as viscous oils and the other one as the cluster of short needles. However, these minor alkaloids were obtained in small amounts and were not studied by NMR. Purified carpaine was then used for the entire experiments in this thesis.

Table 6. Yield and texture of crude alkaloid and purified alkaloid from *C. papaya* L. leaves

Alkaloid	Started material (g)	solvent system	Physical description		Molecular weight	Yield (g)
			Texture	Color		
crude alkaloid	7500 (dried leaves)	Chlor	semi-solid	dark green	–	21.3
purified alkaloid	15 (crude alkaloid)					
carpaine	–	Chlor/Acet(40:60)	cubes	white	478.71	1.3292
unknown1	–	Chlor/Acet(40:60)	viscous oil	yellow	–	–
unknown2	–	MeOH/Chlor(20:80)	viscous oil	yellow	–	–
unknown3	–	MeOH(100)	needle	yellow	–	–

Chlor: chloroform; Acet; acetone; MeOH: methanol

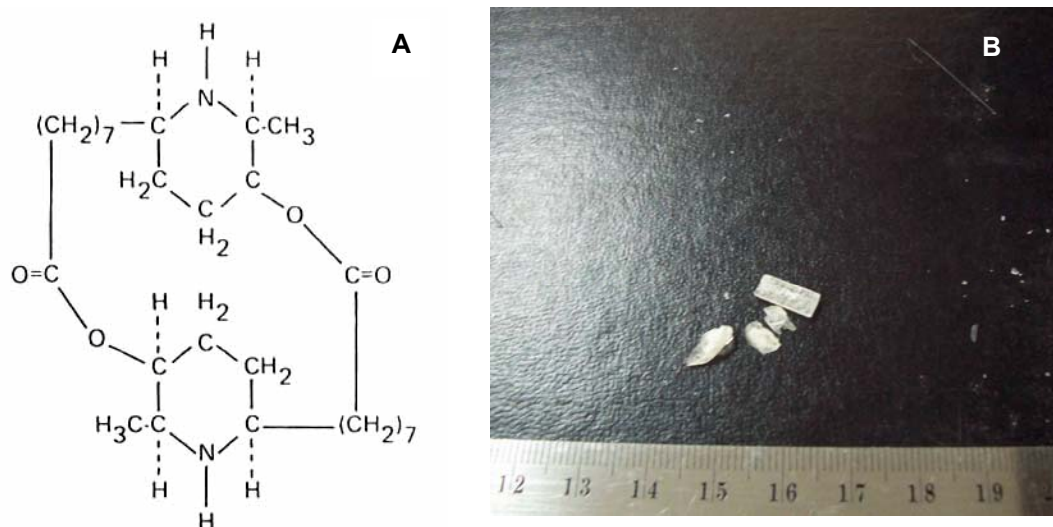


Figure 14. The chemical formula (A) and texture (B) of carpaine, the purified alkaloid from *C. papaya* L. leaves according to Coke and Rice (1965)

2. Effect of carpaine on uterine relaxation

2.1 Effect of carpaine or crude alkaloid on uterine contraction induced by depolarizing solution

2.1.1 Determination of optimum KCl concentration in depolarizing solution

This study was performed to determine optimal KCl concentration in depolarizing solution. The representative tracing of the uterine contraction induced by various concentration of KCl was demonstrated in Figure 15. The force of uterine contraction and KCl concentration relationship were shown in Figure 16. The replacement of Jalon-Ringer solution with depolarizing solution caused a rapid phasic contraction, then relaxed and reached a prolong plateau contraction (tonic phase). Various concentrations of KCl (10, 15, 20, 25, 30, 36.3, 46.3, 56.3, 66.3 and 76.3 mM) containing in depolarizing solution were tested and all concentrations of KCl caused rapid a phasic contraction. KCl (10, 15 and 20 mM) induced a rapid phasic contraction transiently, the effect of which decline rapidly. KCl (25 mM) produced approximately 70-80% of maximum contraction was then chosen for the induction of uterine contraction in the next experiments. Furthermore, 25 mM KCl also produced regular amplitude of contraction for at least 2 hours, which was long enough to complete a series of

experiment. However, after phasic contraction, the depolarizing solution containing 30 and 36.3 mM of KCl rapidly caused a large relaxation and then contracted again with a minor fluctuation in contraction of the uterus in a tonic phase. This contraction appeared to decline slowly with time. In contrast, KCl at the concentration of 46.3, 56.3, 66.3 and 76.3 mM caused a prolonged plateau contraction in tonic phase which remained stable for a long period of time. The tonic contraction induced by depolarizing solution containing 56.3 KCl remain unchange for at least 2 hours or in some case declined very slowly with no more than 10% of the initial contraction within 2 hours. The concentration at 56.3 mM was then chosen for the preparation of depolarizing solution in the following studies. The representative tracings of uterine contraction induced by depolarizing with 56.3 mM KCl were demonstrated in Figure 17, 29, 51, and 55 respectively.

2.1.2 Effect of carpaine or crude alkaloid on uterine contraction induced by depolarizing solution

The representative tracings of relaxing effects of carpaine or crude alkaloid on uterine contraction induced by depolarizing solution (KCl 56.3 mM) were illustrated in Figure 17. The addition of the vehicle of carpaine or crude alkaloid had no effects on uterine contraction induced by depolarizing solution. In contrast, the addition of carpaine (10^{-6} - 3×10^{-3} M) or crude alkaloid (3×10^{-7} - 10^{-3} M) inhibited this contraction in a concentration-dependent manner. A significant inhibition on uterine contraction occurred with carpaine and crude alkaloid at the concentrations of 3×10^{-5} M and 3×10^{-5} g/ml, respectively, while at the concentration of carpaine (1×10^{-3} M) and crude alkaloid (1×10^{-3} g/ml) completely abolished the contraction. The IC_{50} of carpaine and crude alkaloid were $1.31 \pm 0.07 \times 10^{-4}$ M and $1.42 \pm 0.11 \times 10^{-5}$ gm/ml respectively. The cumulative inhibitory concentration-response curve of carpaine and crude alkaloid on uterine contraction induced by depolarizing solution was illustrated in Figure 18.

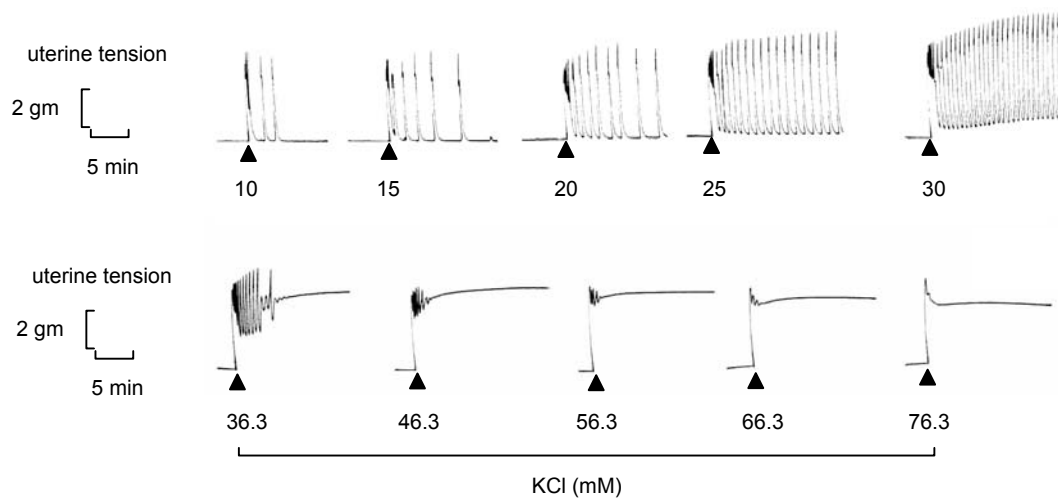


Figure 15. The representative tracing of the uterine contraction induced by depolarizing solutions with various concentrations of KCl in isolated rat uterus

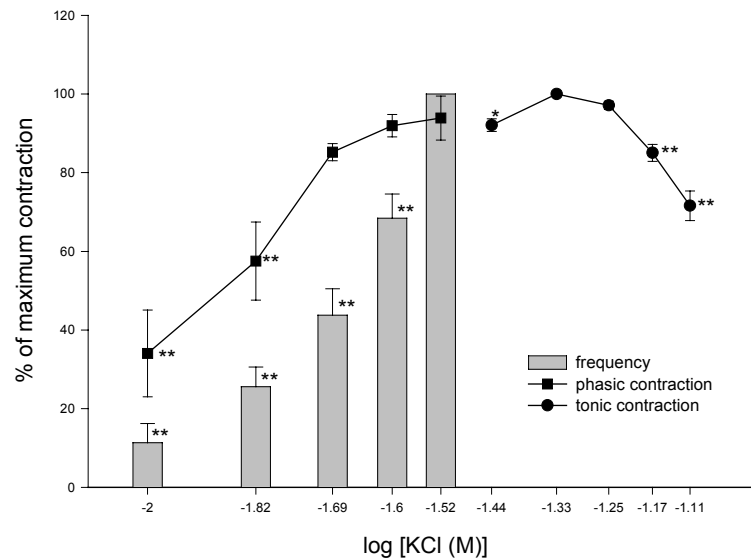


Figure 16. The concentration-response relationship of KCl-induced contraction. The uteri were exposed for 15 minutes to various concentration of KCl in depolarizing solution. Each value was expressed as a percentage of the maximal contraction in response to depolarizing solution with 30 and 46.3 mM of KCl for phasic and tonic contraction respectively. Each datum point represents the mean \pm S.E.M of ten experiments. (** $p < 0.05$, ** $p < 0.01$ as compared to control (Dunnett's test))

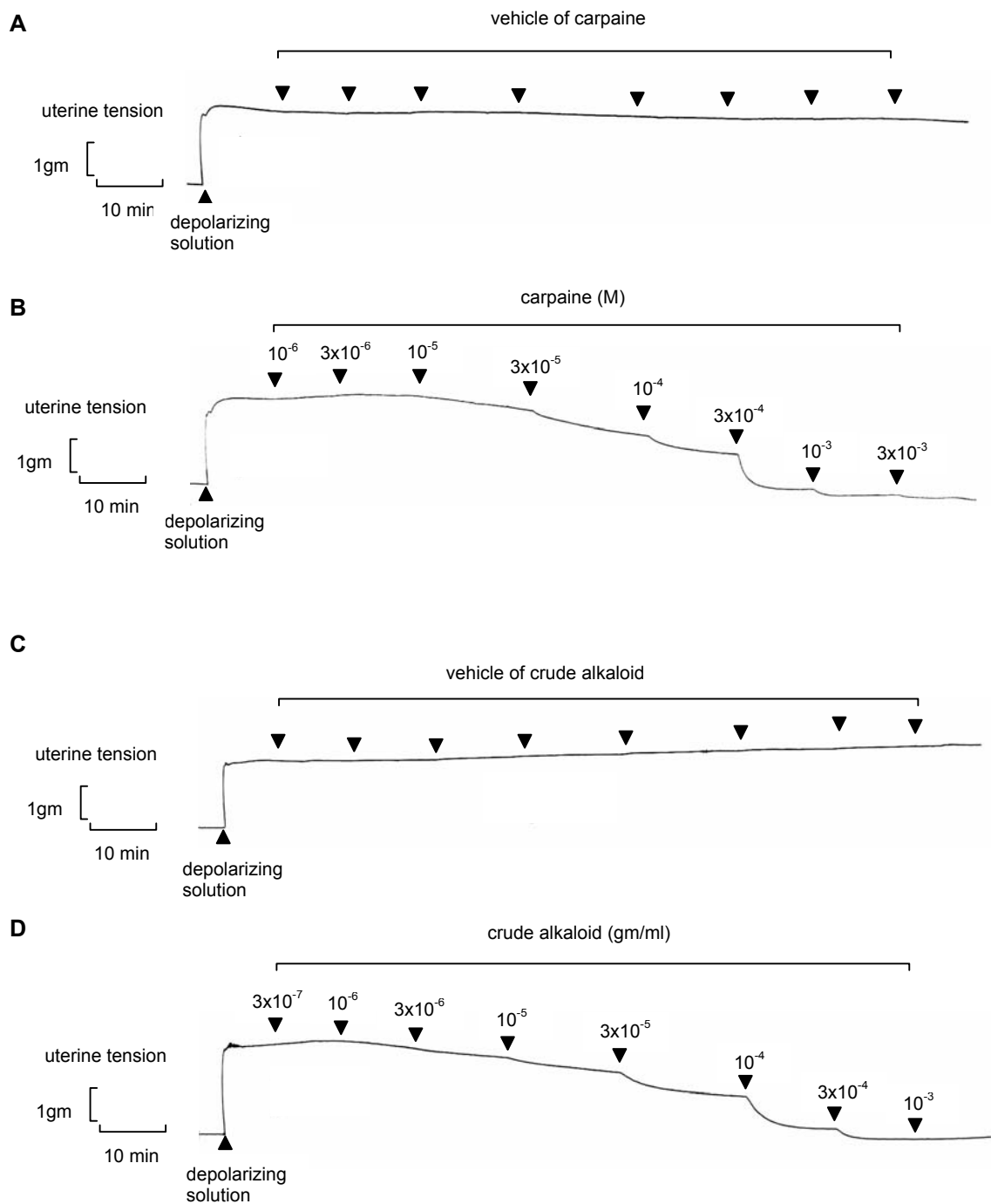


Figure 17. The representative tracing of the relaxing effect of cumulative concentrations of carpaine and crude alkaloid on isolated rat uterus precontracted with depolarizing solution (KCl 56.3 mM); A= time control carpaine, B = carpaine-treated, C = time control crude alkaloid, D = crude alkaloid-treated.

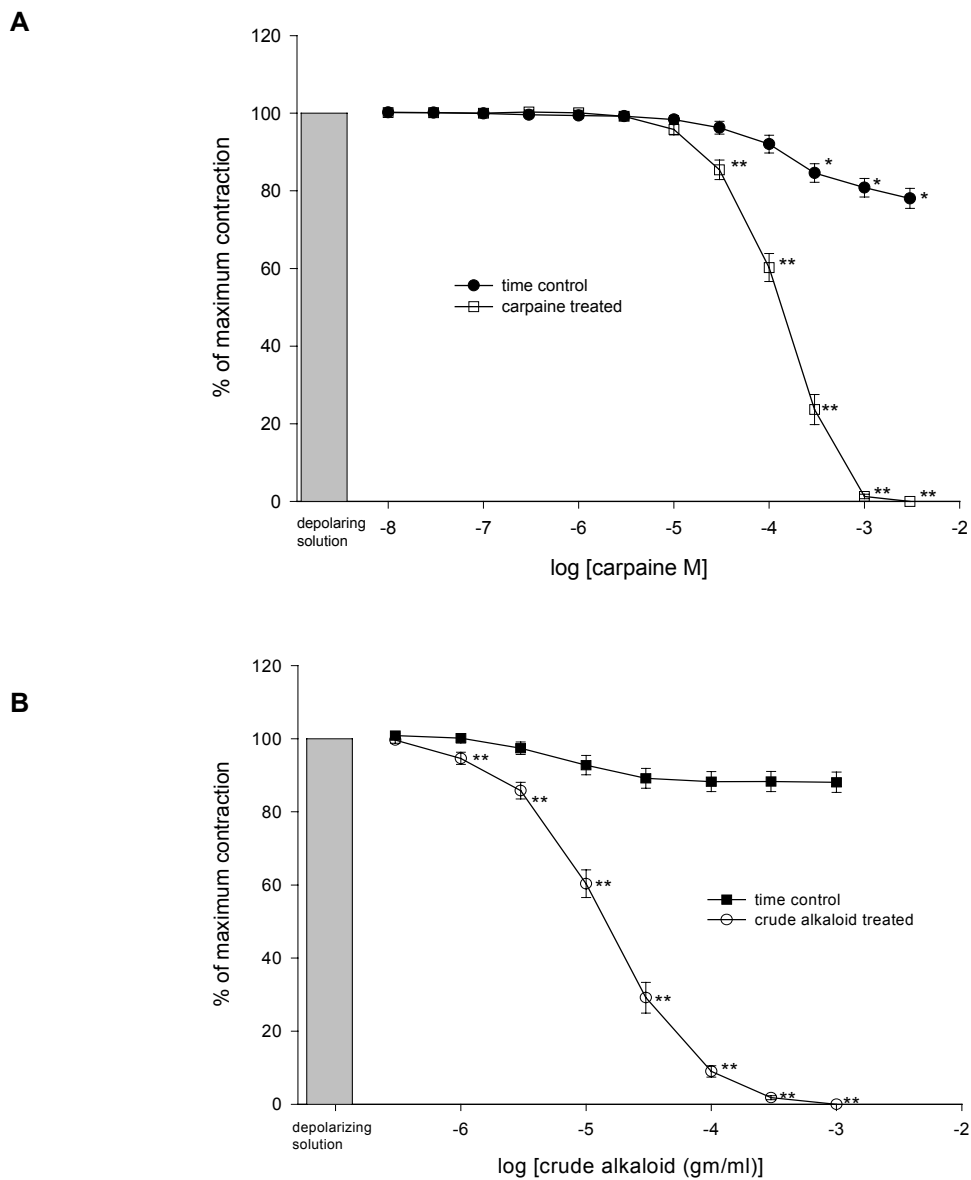


Figure 18. The concentration-response relationship of carpaine (A) and crude alkaloid (B) - induced uterine relaxation on isolated rat uterus precontracted with depolarizing solution (56.3 mM). Each value is expressed as a percentage of the initial maximal contraction in response to depolarizing solution. Each datum point represents the mean \pm S.E.M of ten experiments. (** $p < 0.05$, ** $p < 0.01$ as compared to control (independent sample t -test))

2.2 Effect of carpaine 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 contraction was reached, cumulative concentration of verapamil (10^{-9} - 10^{-3} M) or its vehicle was then added. The addition of vehicle of verapamil had no effect 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 10^{-9} M of verapamil ($p < 0.05$) while, verapamil at the concentration of 10^{-4} M completely abolished this contraction.

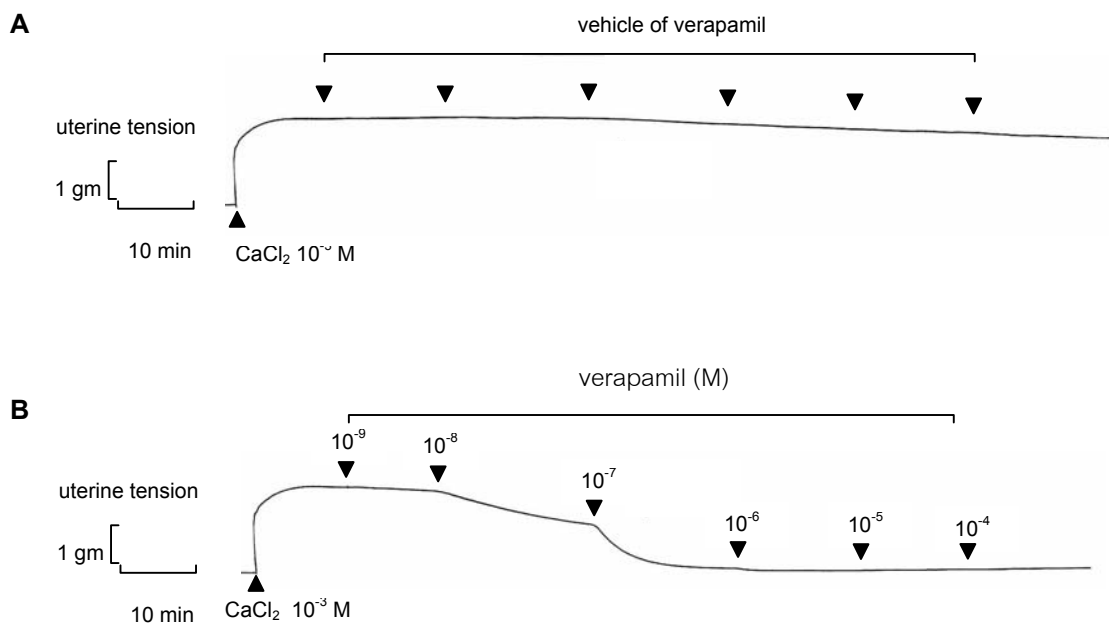


Figure 19. The representative tracing of the relaxing effect of the cumulative concentrations of verapamil on isolated rat uterus precontracted with CaCl_2 (10^{-3} M); A = time control, B = verapamil-treated.

The representative tracing and the log concentration-response relationship of verapamil induced uterine relaxation were demonstrated in Figure 19 and 20 respectively. Verapamil at the concentration of 10^{-9} , 10^{-8} and 10^{-7} M which produced uterine relaxation to approximately 10-90% of maximum contraction was then chosen to use in the next experiment.

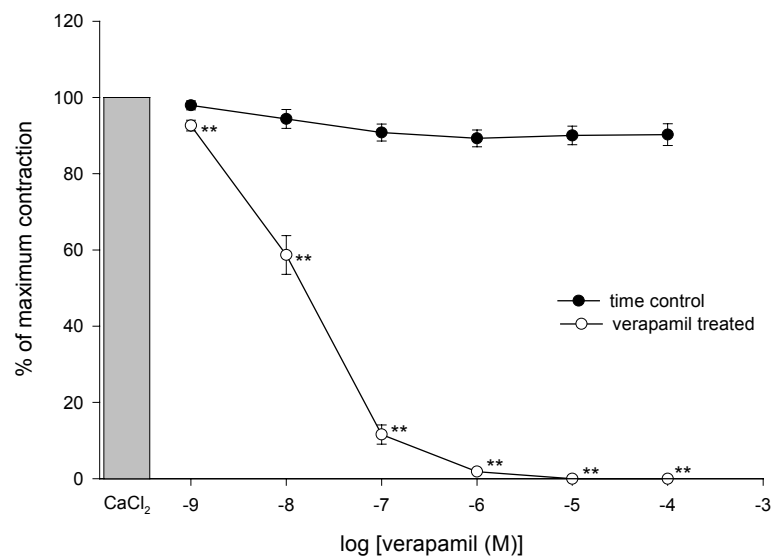


Figure 20. The concentration-response curves of verapamil-induced relaxation on isolated rat uterus precontracted with CaCl_2 (10^{-3} M). Each value is expressed as a percentage of the maximal contraction in response to CaCl_2 . Each datum point represents the mean \pm S.E.M of ten experiments. (** $p < 0.01$ as compared to control (independent sample t -test))

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

The representative tracing of the effect of carpaine and its vehicle, verapamil and its vehicle on uterine contraction induced by CaCl_2 (1×10^{-6} - 3×10^{-2} M) was demonstrated in Figure 21 and 22 respectively. By the use of Ca^{2+} -free high KCl (60 mM) solution containing 0.02 mM of EDTA, the addition of cumulative concentration of CaCl_2 (1×10^{-6} - 3×10^{-2} M) produced uterine contraction in a concentration dependent manner (Figure 23). In the presence of various concentration of carpaine (3×10^{-5} , 1×10^{-4} and 3×10^{-4} M), the uterine contraction induced by CaCl_2 (10^{-6} - 3×10^{-2} M) was significantly reduced ($p < 0.01$ for 3×10^{-5} , 1×10^{-4} and 3×10^{-4} M, ANOVA). The log concentration-response relationship of CaCl_2 was shifted to the right, the maximum concentration was also depressed. EC_{50} of CaCl_2 alone was $2.39 \pm 0.20 \times 10^{-4}$ M, but the value was changed to $9.43 \pm 1.07 \times 10^{-4}$, $2.67 \pm 0.44 \times 10^{-3}$ M and $9.39 \pm 3.08 \times 10^{-3}$ M ($p < 0.01$) in the presence of carpaine at the concentration of 3×10^{-5} , 1×10^{-4} and 3×10^{-4} M, respectively. Similarly, verapamil also inhibited the uterine contraction induced by CaCl_2 in the concentration-dependent manner. The pretreated uterine horn by verapamil (10^{-9} , 10^{-8} and 10^{-7} M) significantly reduced the contraction induced by CaCl_2 ($p < 0.01$ for 10^{-9} , 10^{-8} and 10^{-7} M). The log concentration-response curve of CaCl_2 (10^{-6} - 3×10^{-2} M) was 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 $2.33 \pm 0.24 \times 10^{-4}$ M, but the value was also significantly suppressed to $1.52 \pm 0.29 \times 10^{-3}$ M and $8.38 \pm 3.60 \times 10^{-3}$ M when the uterus was pretreated with verapamil 10^{-9} and 10^{-8} M, respectively. The EC_{50} value of CaCl_2 in the presence of verapamil (10^{-7} M) was not determined, because the maximum contraction is less than 50% of the initial contraction. The log concentration-response curve of CaCl_2 in the presence or absence carpaine or verapamil was illustrated in the Figure 23, EC_{50} was presented in Table 7 and 8.

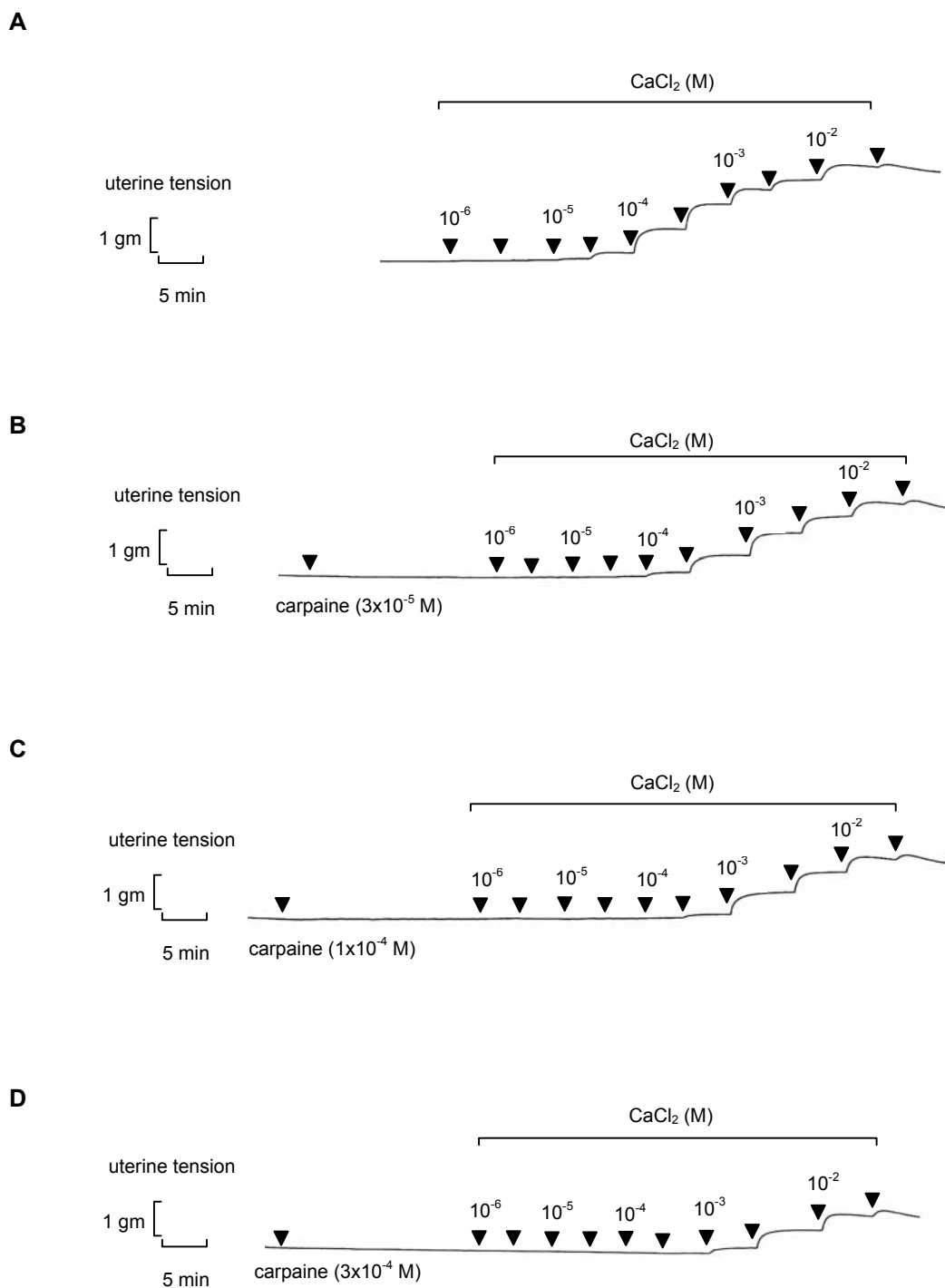


Figure 21. The representative tracings of the contractile response of isolated rat uterus to cumulative concentrations of CaCl₂ in Ca²⁺-free high KCl solution in the presence or absence of carpaine. A = CaCl₂ alone; B, C or D preincubation with carpaine at 3x10⁻⁵, 10⁻⁴ and 3x10⁻⁴ M, respectively for 20 minutes.

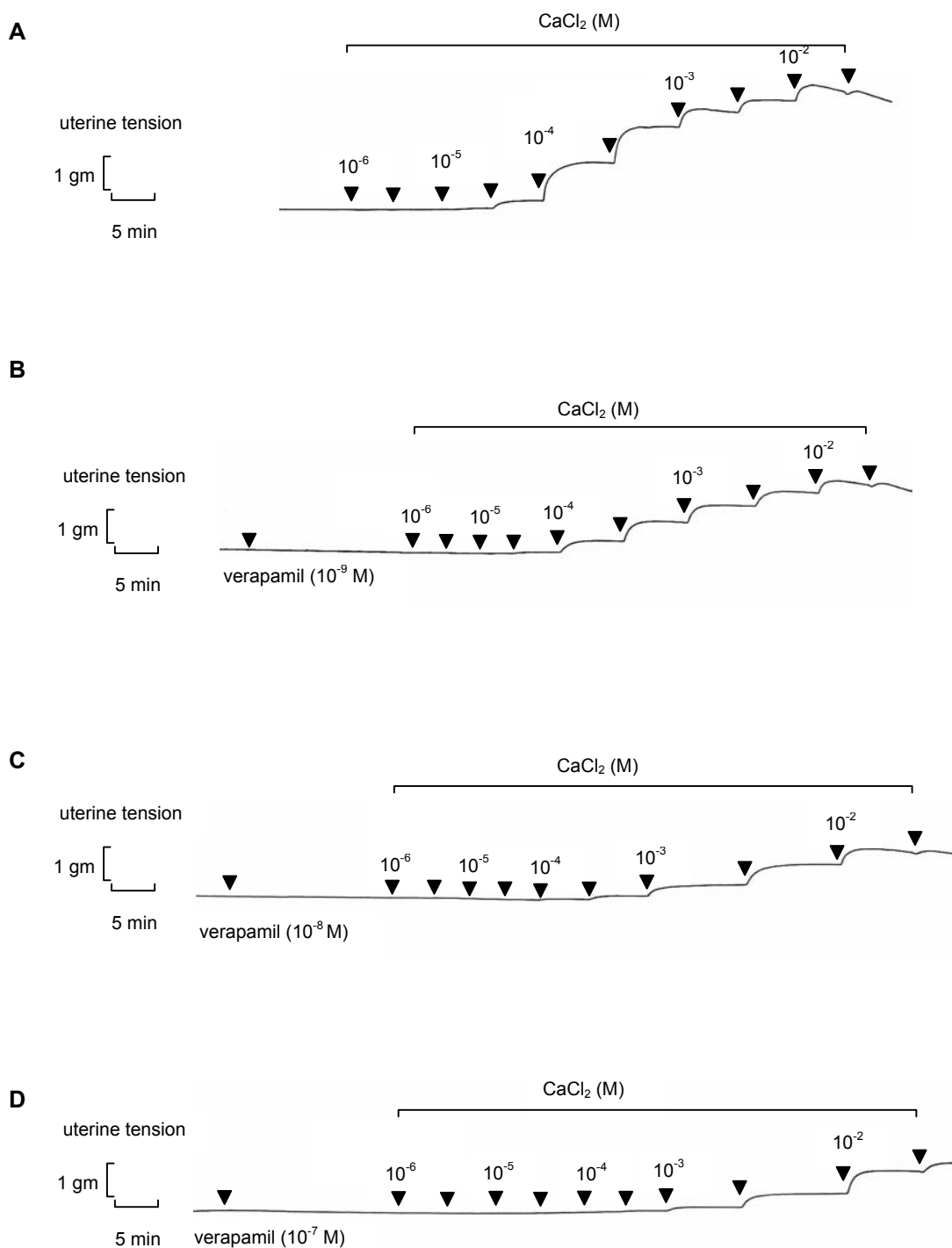
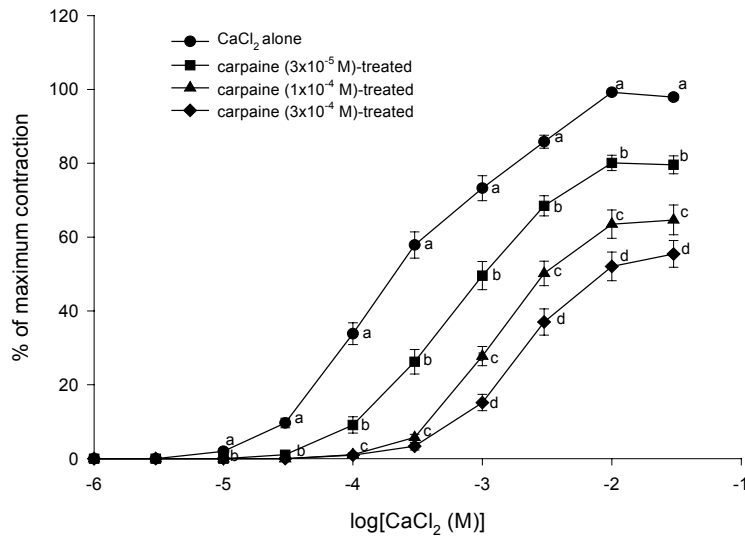


Figure 22. The representative tracings of the contractile response of isolated rat uterus to cumulative concentrations of CaCl₂ in Ca²⁺-free high KCl solution in the presence or absence of verapamil. A=CaCl₂ alone, B, C and D = preincubation with verapamil at 10⁻⁹, 10⁻⁸ and 10⁻⁷ M, respectively for 20 minutes.

A



B

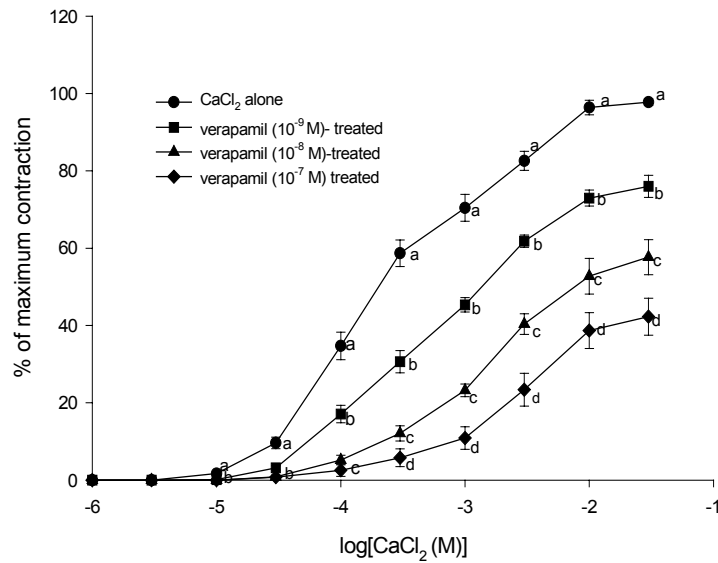


Figure 23. The concentration-response relationship of CaCl_2 (10^{-6} - 3×10^{-2})-induced contraction on isolated rat uterus in the presence or absence of carpaine (A) or verapamil (B). Each value is expressed as percentage of the maximal contraction. Each datum point represents the mean \pm S.E.M of ten experiments. Different characters (a, b, c and d) mean significant difference among the comparing means which in the same treatment group (CaCl_2 concentration) at $p < 0.05$ (Duncan's multiple range test).

Table 7. Comparison of EC₅₀ value of CaCl₂-induced contraction in the presence or absence of carpaine in isolated rat uterus.

EC ₅₀ (Mean ± S.E.M)			
CaCl ₂ alone	carpaine-treated (M)		
	1x10 ⁻⁴	3x10 ⁻⁴	1x10 ⁻³
2.39 ± 0.20 x10 ⁻⁴	**9.43 ± 1.07 x10 ⁻⁴	**2.67 ± 0.44 x10 ⁻³	**9.39 ± 3.08 x10 ⁻³

(** $p < 0.01$ as compared to carpaine alone (Dunnett's test))

Table 8. Comparison of EC₅₀ values of CaCl₂-induced contraction in the presence or absence of verapamil in isolated rat uterus.

EC ₅₀ (Mean ± S.E.M)			
CaCl ₂ alone	verapamil-treated (M)		
	10 ⁻⁹	10 ⁻⁸	10 ⁻⁷
2.33 ± 0.24 x10 ⁻⁴	**1.52 ± 0.29 x10 ⁻³	**8.38 ± 3.60 x10 ⁻³	ND

(** $p < 0.01$ as compared to verapamil alone (Dunnett's test))

ND = not determined, maximum contraction was less than 50% of maximum response

2.3 Effect of carpaine on the uterine contraction induced by uterine stimulants

2.3.1 Determination of optimal concentration of oxytocin.

The effect of oxytocin on uterine contraction was determined. Cumulatively addition of oxytocin (0.001-10 mU/ml) caused uterine contraction in a concentration dependent manner. The representative tracing of oxytocin induced uterine contraction was demonstrated in Figure 24. Oxytocin at the concentration of 1 mU/ml produced approximately 70-80% of the maximum contraction. In addition, this concentration of oxytocin also produced regular amplitude of contraction for at least 2 hours which was long enough to complete a series of experiment. This concentration of oxytocin was then chosen for the induction of uterine contraction in the next experiments. In some experiments, the frequency of contraction induced by oxytocin (1 mU/ml) declined very slowly with time, however, at the end of experiment, with no more than 10 % of the initial frequency. The representative tracing of the frequency of contraction induced by oxytocin and the time control were demonstrated in Figure 26, 41, 43 and 44.

2.3.2 Effect of carpaine and verapamil on uterine contraction induced by oxytocin

The representative tracing of the relaxing effects of carpaine and its vehicle, or verapamil and its vehicle on uterine contraction induced by oxytocin (1 mU/ml) were demonstrated in Figure 26. 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 carpaine or verapamil had no effects on uterine contraction induced by oxytocin. In contrast, the addition of cumulative concentration of carpaine (10^{-6} - 10^{-3} M) or verapamil (10^{-9} - 10^{-4} M) significantly inhibited both frequency and amplitude of contraction ($p < 0.05$). The frequency of contraction was more sensitive to carpaine than the amplitude of contraction, as observed by the first concentration of carpaine that significantly affected to the force and frequency of contraction. Carpaine at the concentration of 10^{-5} M significantly reduced the frequency of contraction ($p < 0.05$), while the first concentration of carpaine that significantly inhibited the force of contraction was 3×10^{-5} M

($p < 0.05$). Similarly, in the uterus treated with verapamil, the frequency of contraction was more sensitive to verapamil than the amplitude of contraction. The cumulative inhibitory concentration-response relationship of carpaine and verapamil on uterine contraction induced by oxytocin was illustrated in Figure 27 and 28, IC_{50} value was presented in Table 9.

2.4 The effect of carpaine on the role of K^+ channels in isolated rat uterus.

2.4.1 Effect of cromakalim on KCl-elicited contraction

The response of the uterine to 56.3 mM K^+ -depolarizing solution was biphasic with a rapid and distinct phasic contraction of about 3.0 ± 0.48 g followed by greater sustained tonic contraction of 2.9 ± 0.21 g. On the other hand, a monophasic contraction developed in response to 25 mM K^+ -depolarizing solution (3.2 ± 0.73 g). Control contraction was stable over the time of cumulative addition of cromakalim. In some experiments, the frequency of contraction induced by 25 mM K^+ declined very slowly with time, however, with no more than 10 % of the initial frequency at the end of experiment. Cromakalim relaxed the contractions induced by 25 mM K^+ in preference to those induced by 56.3 mM K^+ (Figure. 29). The mean EC_{50} value for cromakalim (10^{-8} - 3×10^{-4} M) induced relaxation in uterus precontracted with 56.3 mM K^+ is shown in Table 10. Cumulative addition of cromakalim (10^{-8} - 3×10^{-5} M) produced a concentration-related inhibitory on amplitude and frequency of 25 mM K^+ -induced contraction, with complete inhibition occurring at 10^{-5} M. The mean EC_{50} values for cromakalim inhibition of amplitude and frequency were $9.72 \pm 1.12 \times 10^{-7}$ M and $6.26 \pm 3.73 \times 10^{-5}$ M (Table 10.) respectively. The cumulative inhibitory concentration-response relationship of cromakalim on uterine contraction induced by 25 and 56.3 mM K^+ was illustrated in Figure 30, EC_{50} values were presented in Table 10.

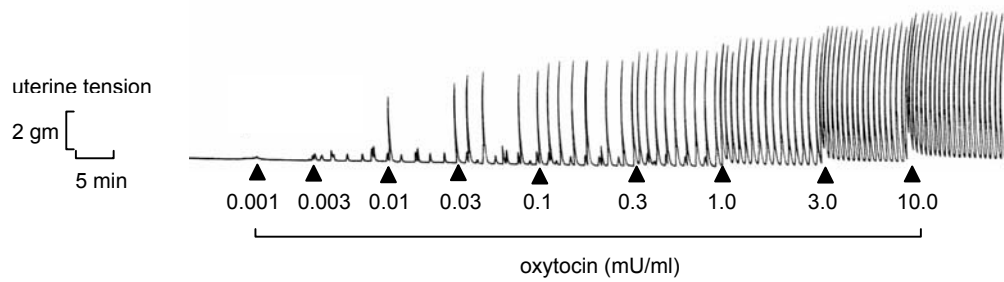


Figure 24. The representative tracing of the contractile response of isolated rat uterus to cumulative concentrations of oxytocin.

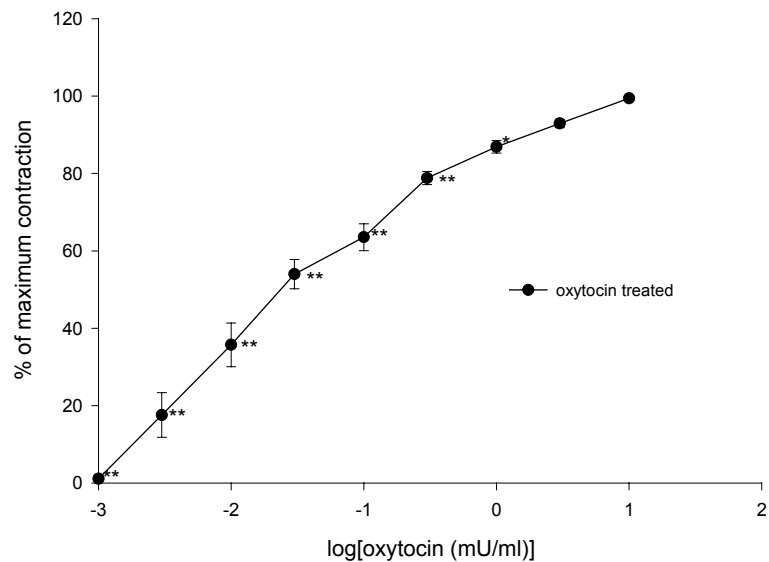


Figure 25. The concentration-response relationship of cumulative concentrations of oxytocin (0.001-10.0 mU/ml) on isolated rat uterus. Each value is expressed as a percentage of the initial maximal contraction in response to depolarizing solution. Each datum point represents the mean \pm S.E.M of ten experiments. (* $p < 0.05$, ** $p < 0.01$ as compared to maximum contraction (Dunnett's test))

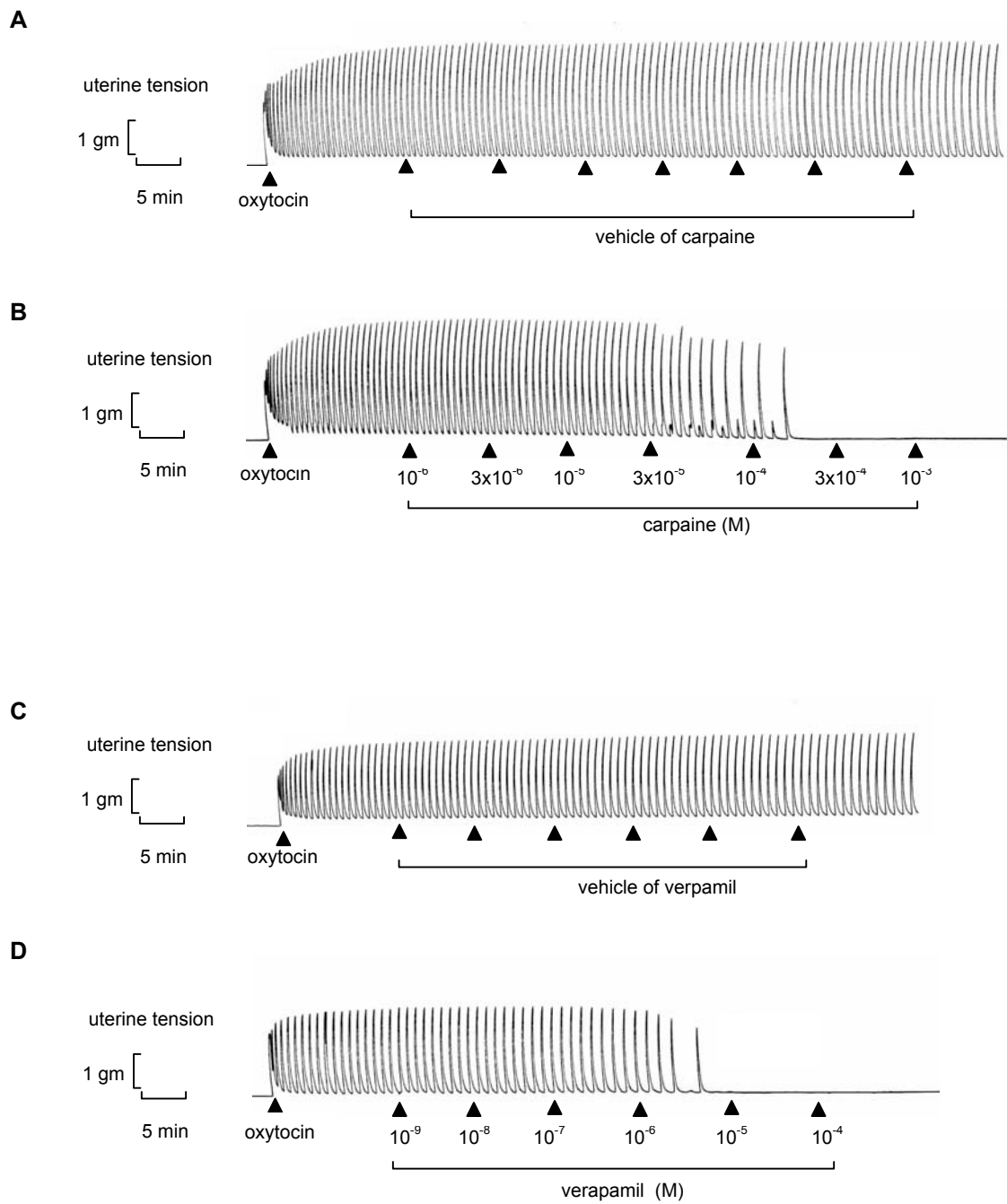


Figure 26. The representative tracings of the relaxing effect of the cumulative concentrations of carpaine (A) and its time control (B), verapamil (C) and its time control (D) on uterine contraction induced by oxytocin (1mU/ml) in isolated rat uterus.

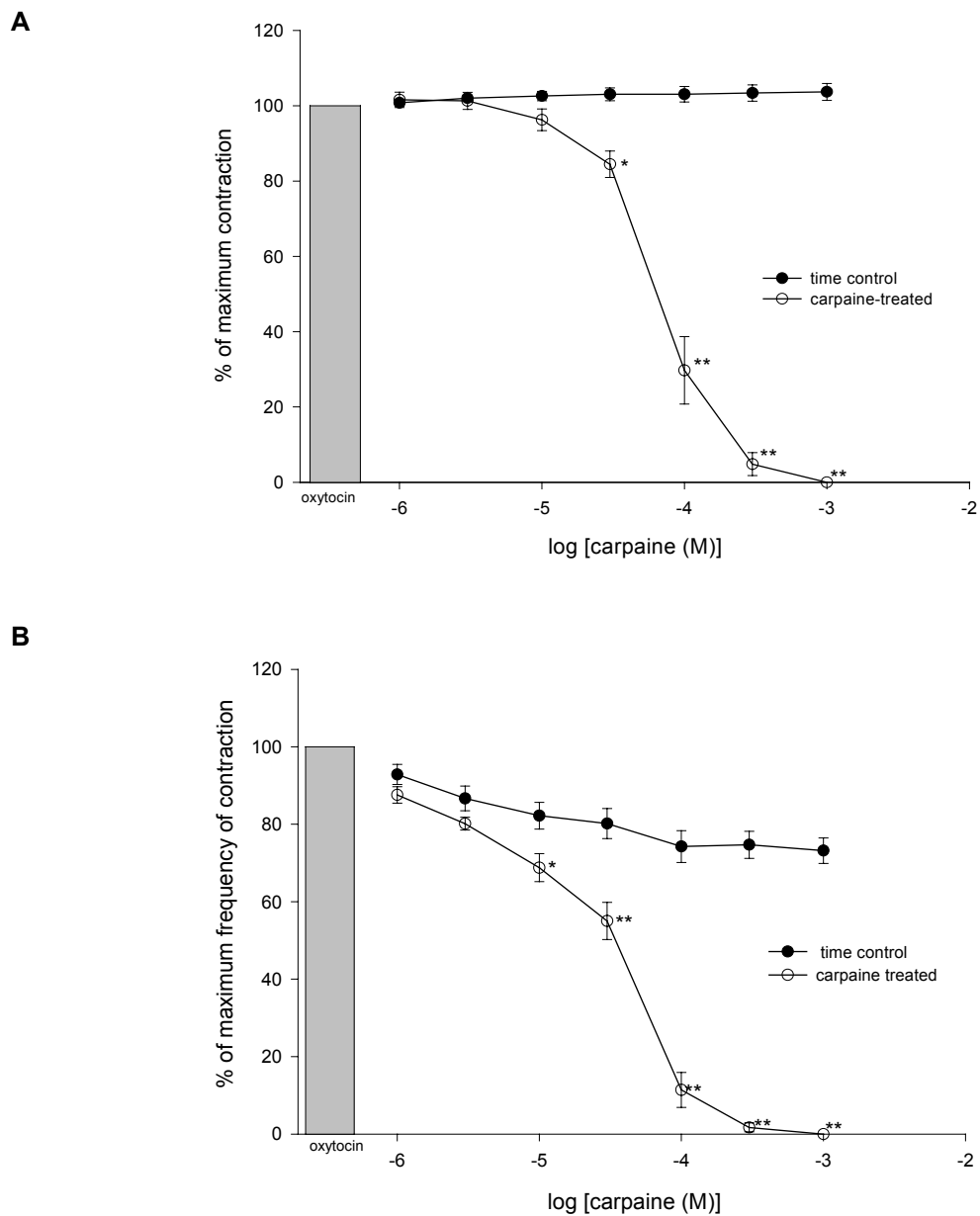


Figure 27. The concentration-response relationship of carpine and its relaxing effect on isolated rat uterus precontracted with oxytocin (1mU/ml). (A) The effect of carpine on force of contraction and (B) the effect of carpine on frequency of contraction. Each value is expressed as a percentage of the initial maximal contraction in response to oxytocin. Each datum point represents the mean \pm S.E.M of ten experiments. (* $p < 0.05$, ** $p < 0.01$ as compared to control (independent sample t -test)).

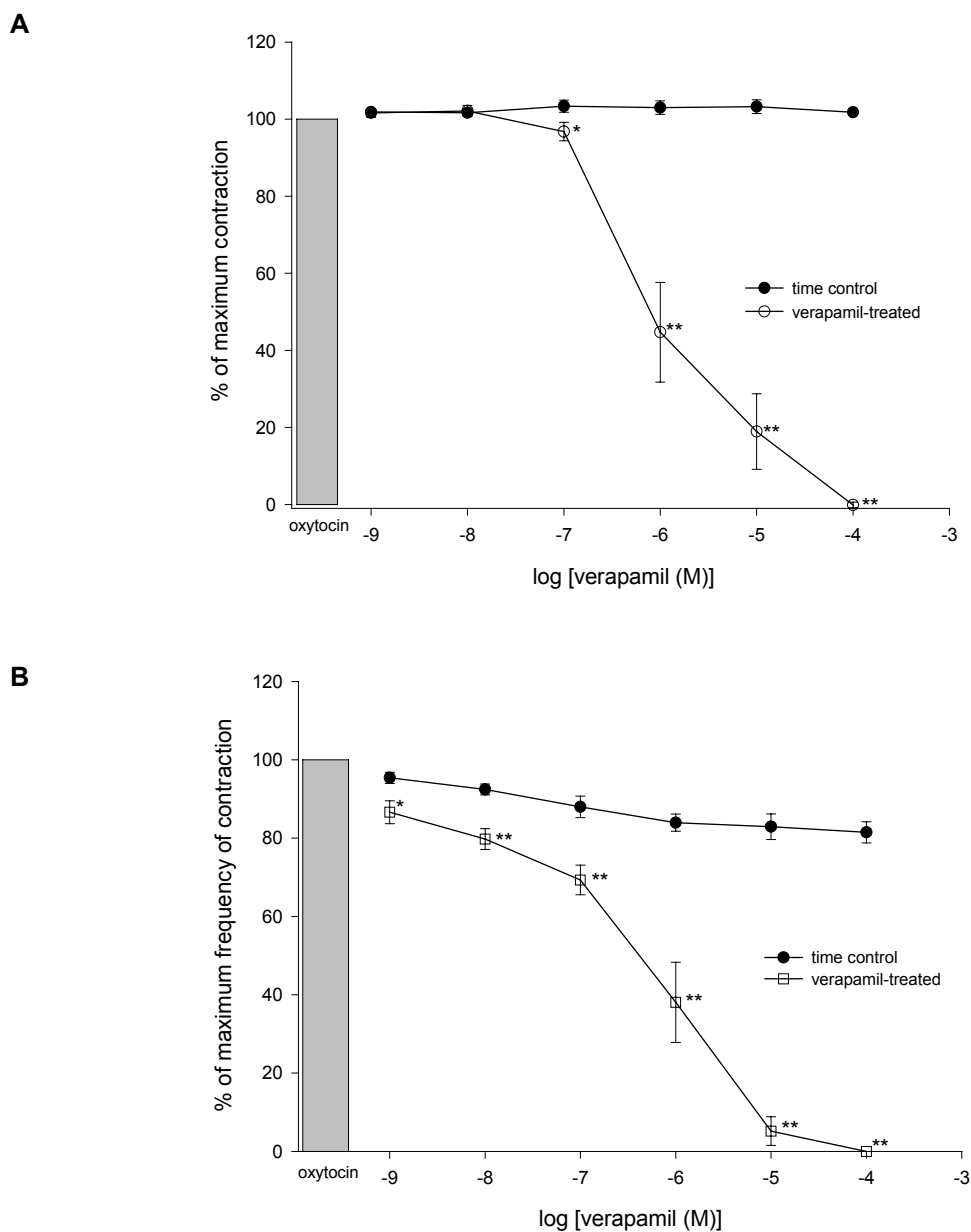


Figure 28. The concentration-response relationship of verapamil and its relaxing effect on isolated rat uterus precontracted with oxytocin (1mU/ml). (A) The effect of verapamil on force of contraction and (B) the effect of verapamil on frequency of contraction. Each value is expressed as a percentage of the initial maximal contraction in response to oxytocin. Each datum point represents the mean \pm S.E.M of ten experiments. (* $p < 0.05$, ** $p < 0.01$ as compared to control (independent sample t -test))

Table 9. Comparison of the IC₅₀ values of carpaine and verapamil on frequency and amplitude of contraction on isolated rat uterus precontracted with oxytocin (1mU/ml)

Compounds	IC ₅₀ (Mean ± S.E.M)	
	Force of contraction	Frequency of contraction
carpaine	6.58 ± 0.58 x10 ⁻⁵	3.97 ± 0.67 x10 ⁻⁵
verapamil	9.01 ± 2.83 x10 ⁻⁷	7.11 ± 2.07 x10 ⁻⁷

Table 10. Comparison of EC₅₀ values of cromakalim-induced relaxation on the isolated rat uterus precontracted with 56.3 mM or 25 mM KCl depolarizing solution.

KCl concentration (M)	EC ₅₀ (Mean ± S.E.M)	
	Force of contraction	Frequency of contraction
25	9.72 ± 1.12 x10 ⁻⁷	6.26 ± 3.73 x10 ⁻⁵
56.3	9.98 ± 0.95 x10 ⁻⁵	-

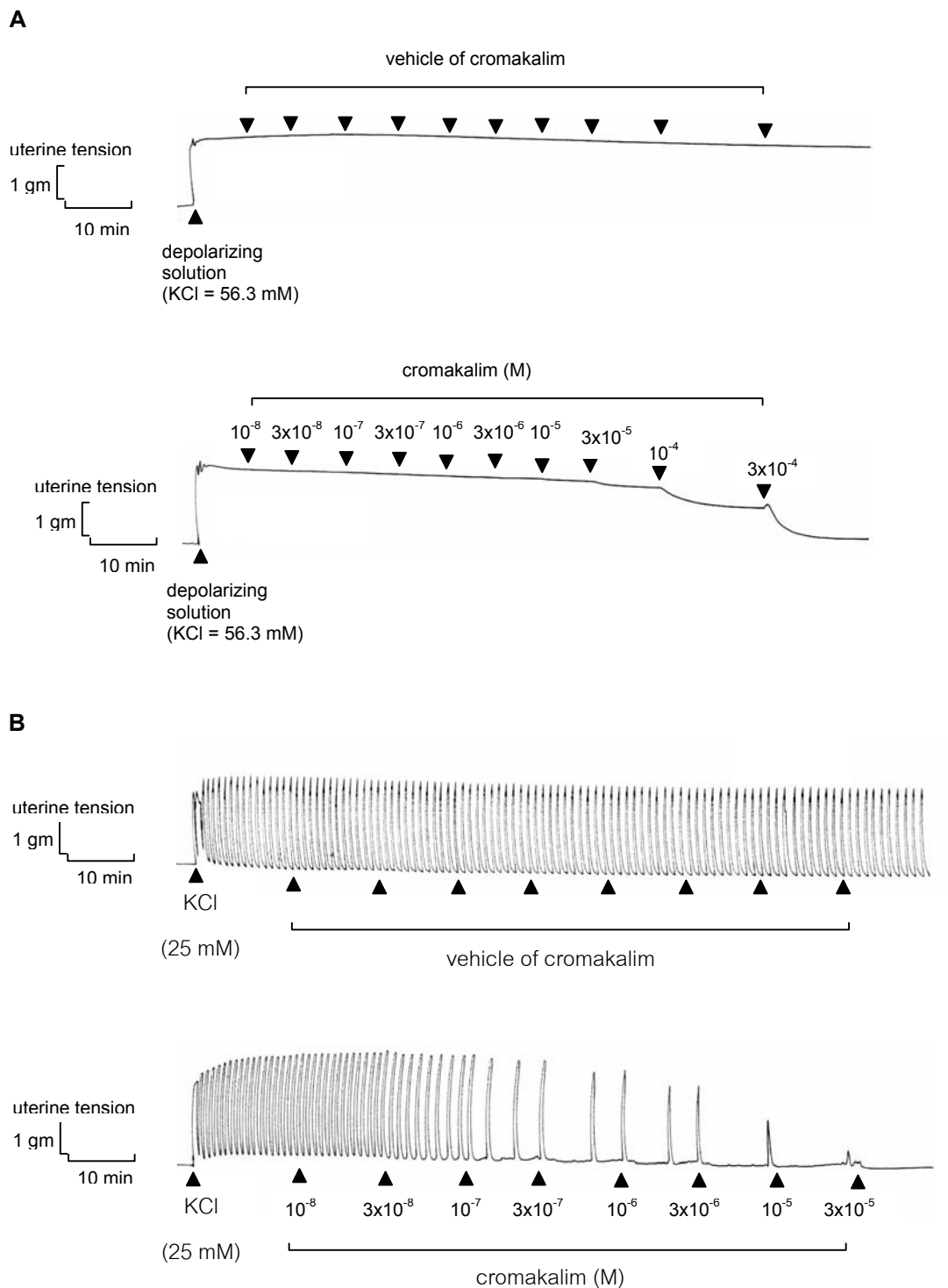


Figure 29. The representative tracing of the relaxing effect of cromakalim and its vehicle on isolated rat uterus precontracted with the 56.3 mM KCl (A) or 25 mM KCl (B) depolarizing solution.

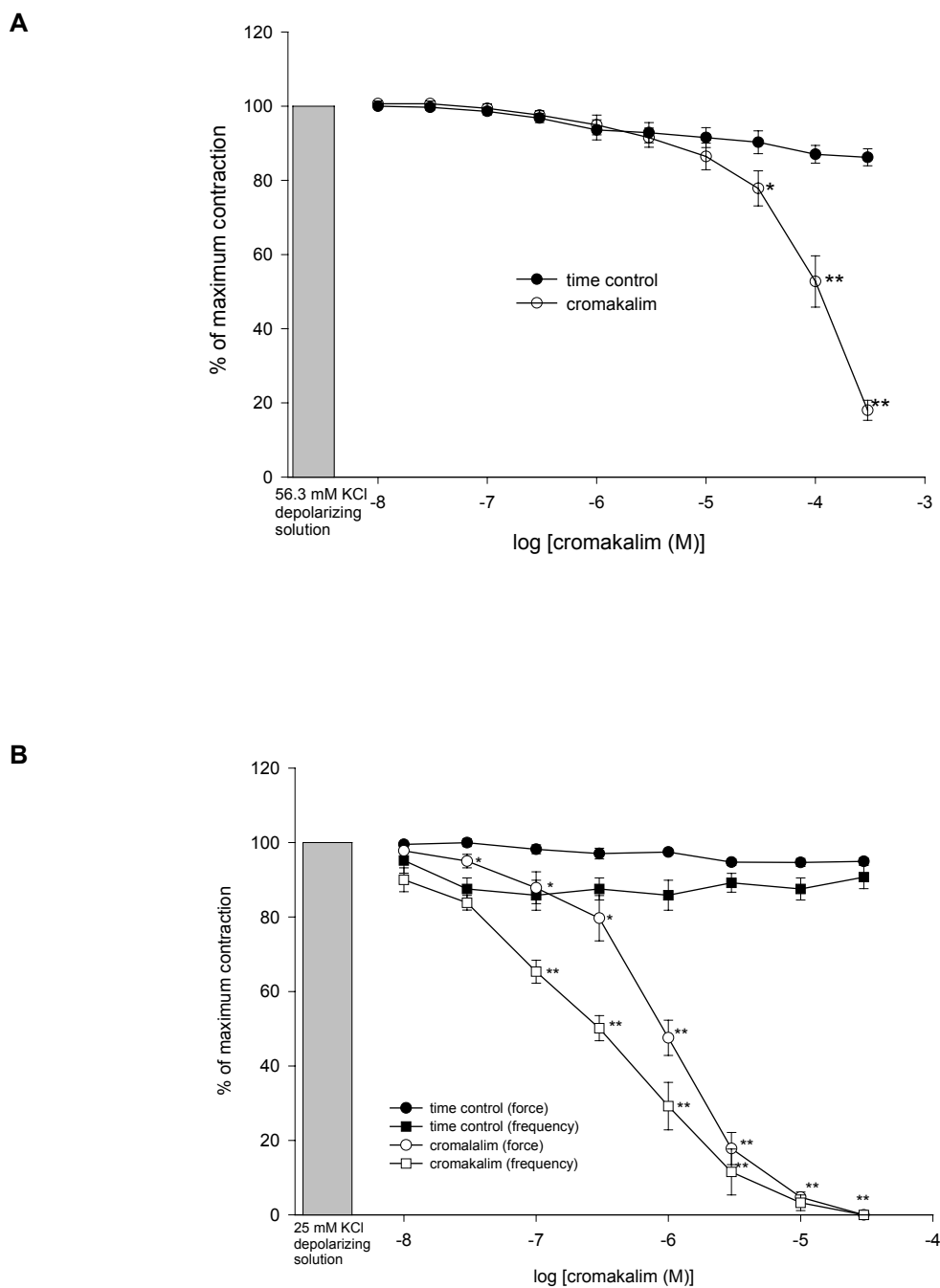


Figure 30. The concentration-response curve of cromakalim-induced relaxation on isolated rat uterus precontracted with depolarizing solution 56.3 mM KCl (A) and 25 mM KCl (B). Each datum point represents the mean \pm S.E.M of ten experiments. (* p < 0.05, ** p < 0.01 as compared to the control (independent sample t -test)).

2.4.2 Effect of carpaine and cromakalim on uterine contraction induced by 25 mM K⁺-depolarizing solution and the influence of K⁺-channel blocker.

2.4.2.1 Tetraethylammonium (TEA)

2.4.2.1.1 Determination of optimum concentration of TEA

Cumulative addition of cromakalim (10^{-8} - 3×10^{-5} M) produced a concentration-related inhibitory effect on the amplitude and frequency of uterine contraction precontracted with 25 mM K⁺ depolarizing solution. The EC₅₀ values for cromakalim inhibition of amplitude and frequency were $7.38 \pm 1.08 \times 10^{-7}$ M and $2.98 \pm 0.56 \times 10^{-7}$ M, respectively. In the presence of 10^{-5} , 10^{-4} and 10^{-3} M of TEA, the inhibition was inhibited, as observed by a shift of the log concentration-response curve of cromakalim to the right. The vehicle of TEA was without effect. The EC₅₀ values for cromakalim of the amplitude and frequency of contraction in the presence of TEA (10^{-5} , 10^{-4} and 10^{-3} M) were shown in Table 11. The representative tracing of the blocking activity of TEA on uterine relaxing effect of cromakalim at various concentrations was demonstrated in Figure 31 and the log concentration-response relationship was demonstrated in Figure 32. The concentration of TEA at 10^{-3} M was then chosen to use in the next experiment.

2.4.2.1.2 Effect of TEA on uterine relaxation induced by carpaine and cromakalim

The representative tracing of carpaine and cromakalim induced uterine relaxation precontracted with 25 mM K⁺ depolarizing solution were illustrated in Figure 33 and 34, respectively. The addition of vehicle of carpaine or cromakalim had no effects on uterine contraction induced by 25 mM K⁺ depolarizing solution. The addition of cumulative concentration of carpaine inhibited this contraction with IC₅₀ value of $5.93 \pm 0.94 \times 10^{-5}$ M and $1.45 \pm 0.71 \times 10^{-4}$ M for amplitude and frequency, respectively. In the presence of 10^{-3} M TEA, carpaine also inhibited this contraction which was significantly different from that obtained in the absence of TEA ($p < 0.05$). The mean EC₅₀ values for carpaine inhibition of amplitude and frequency were $1.74 \pm 0.24 \times 10^{-4}$ M and $1.02 \pm 0.23 \times 10^{-4}$ M (Table 12.), respectively. The addition of cumulative concentration of cromakalim inhibited the uterine contraction in a

concentration-dependent manner with IC_{50} $1.39 \pm 0.15 \times 10^{-6}$ M and $7.80 \pm 2.97 \times 10^{-6}$ M for amplitude and frequency, respectively. In the presence of 10^{-3} M of TEA, the relaxing effect of cromakalim was significantly inhibited ($p < 0.05$). The mean EC_{50} values for cromakalim inhibition of amplitude and frequency were not determined, because the maximum response is less than 50% of the initial contraction. The log concentration-response relationship of carpaine and cromakalim in the presence of TEA (10^{-3} M) was demonstrated in Figure 35 and 36, respectively.

Table 11. Comparison of EC_{50} values of cromakalim-induced relaxation in the presence or absence of TEA (10^{-5} , 10^{-4} and 10^{-3} M) on isolated rat uterus precontracted with 25 mM KCl depolarizing solution.

Contraction	EC_{50} (Mean \pm S.E.M)			
	cromakalim alone	TEA-treated (M)		
		10^{-5}	10^{-4}	10^{-3}
Force	$7.38 \pm 1.08 \times 10^{-7}$	$1.01 \pm 0.15 \times 10^{-6}$	$4.55 \pm 1.98 \times 10^{-6}$	ND
frequency	$2.98 \pm 0.56 \times 10^{-7}$	$0.60 \pm 4.06 \times 10^{-7}$	$1.32 \pm 2.16 \times 10^{-6}$	ND

(** $p < 0.01$ as compared to cromakalim alone (Dunnett's test))

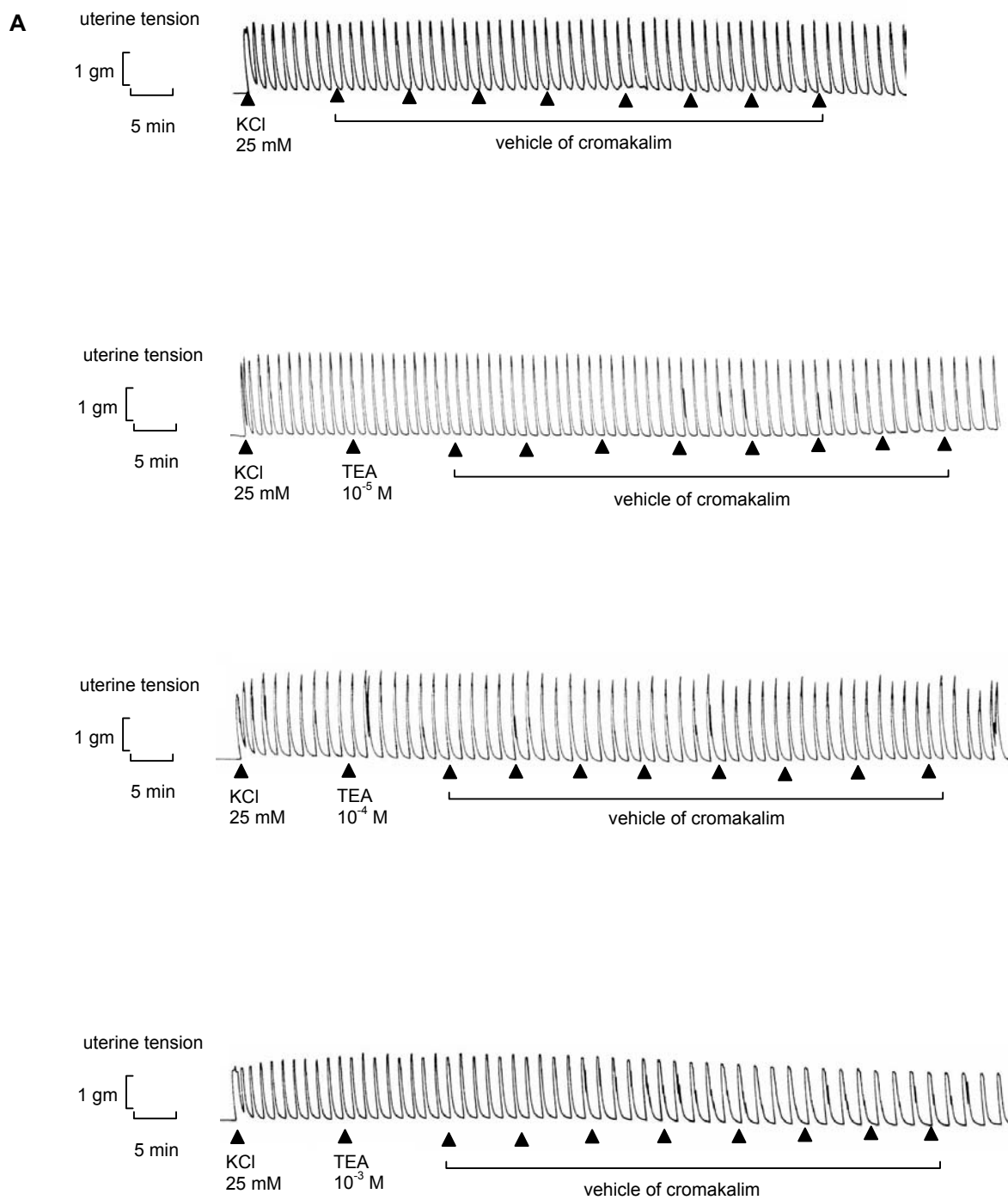
ND = not determined, maximum contraction was less than 50% of maximum response

Table 12. Comparison of IC_{50} values of carpaine or cromakalim-induced relaxation in the presence or absence of TEA (10^{-3} M) on isolated rat uterus precontracted with 25 mM KCl depolarizing solution.

Contraction	IC_{50} of carpaine (Mean \pm S.E.M)		IC_{50} of cromakalim (Mean \pm S.E.M)	
	carpaine alone	TEA-pretreated	cromakalim alone	TEA-treated
Force	$5.93 \pm 0.94 \times 10^{-5}$	$1.74 \pm 0.24 \times 10^{-4}$	$1.39 \pm 0.15 \times 10^{-6}$	ND
Frequency	$1.45 \pm 0.71 \times 10^{-4}$	$1.02 \pm 0.23 \times 10^{-4}$	$7.80 \pm 2.97 \times 10^{-6}$	ND

(* $p < 0.05$, ** $p < 0.01$ as compared to carpaine or cromakalim alone (Dunnett's test))

ND = not determined, maximum contraction was less than 50% of maximum response



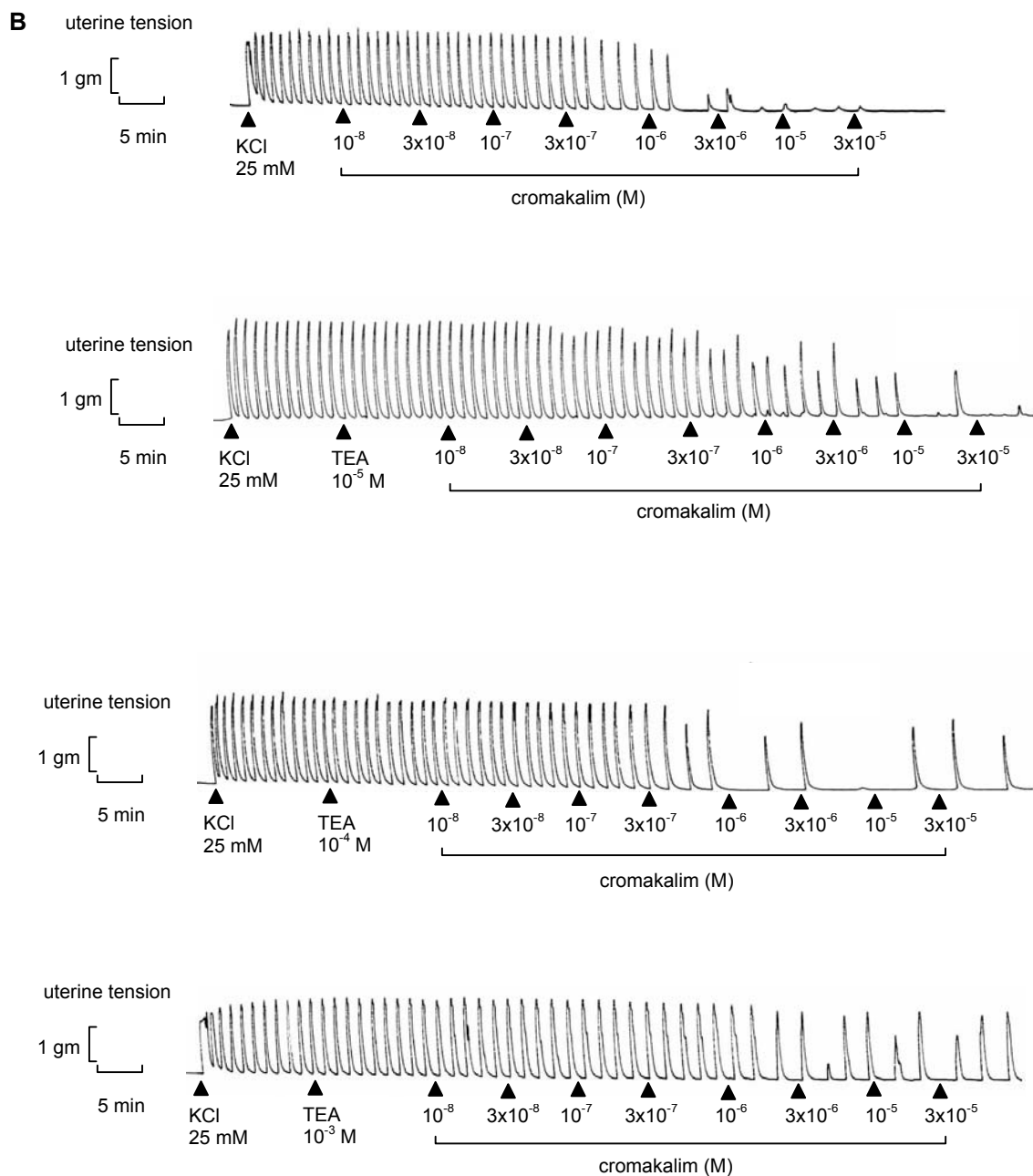


Figure 31. The representative tracing of the inhibitory effect of TEA on relaxing effect of cromakalim on isolated rat uterus precontracted with the depolarizing solution (KCl 25 mM). A = time control of each experiment, B = cromakalim alone and 10^{-5} , 10^{-4} , 10^{-3} M TEA-treated respectively.

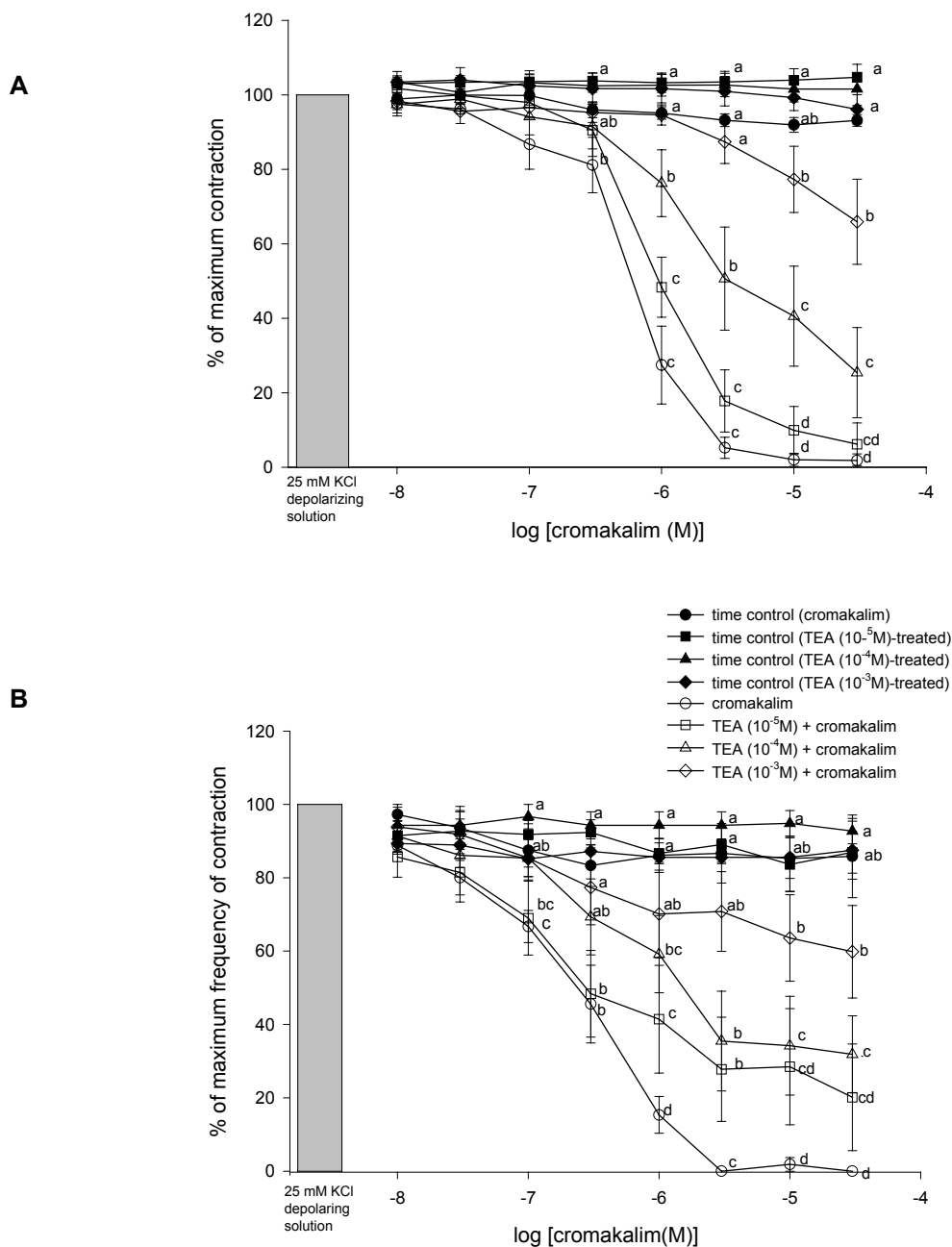


Figure 32. The concentration-response relationship of inhibitory effect of TEA on relaxing effect of cromakalim on isolated rat uterus, the uteri were precontracted with 25 mM KCl. (A) Effect of various concentrations of TEA (10⁻⁵, 10⁻⁴ and 10⁻³ M, 10 minutes) on the effect of cromakalim on the force contraction and (B) the effect of cromakalim on frequency of contraction. Each datum point represents the mean \pm S.E.M of ten experiments. Different characters (a, b, c and d) mean significant difference among the comparing means which in the same treatment group (cromakalim concentration) at $p < 0.05$ (Duncan's multiple range test).

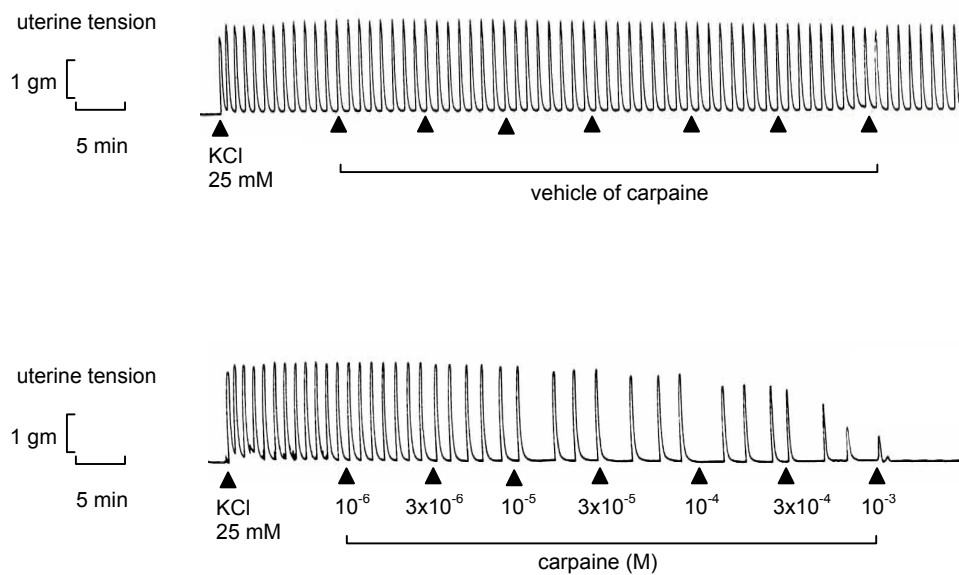
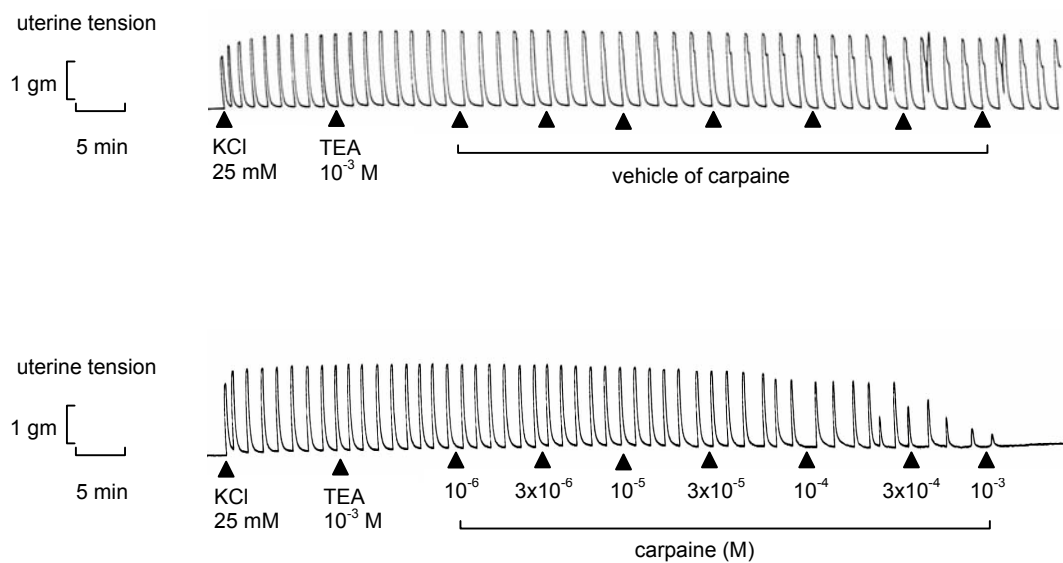
A**B**

Figure 33. The representative tracings of the relaxing effect of carpaine and its time control (A), or pretreated with TEA (10^{-3} M) for 15 minutes and its time control (B) on isolated rat uterus precontracted with 25 mM KCl depolarizing solution.

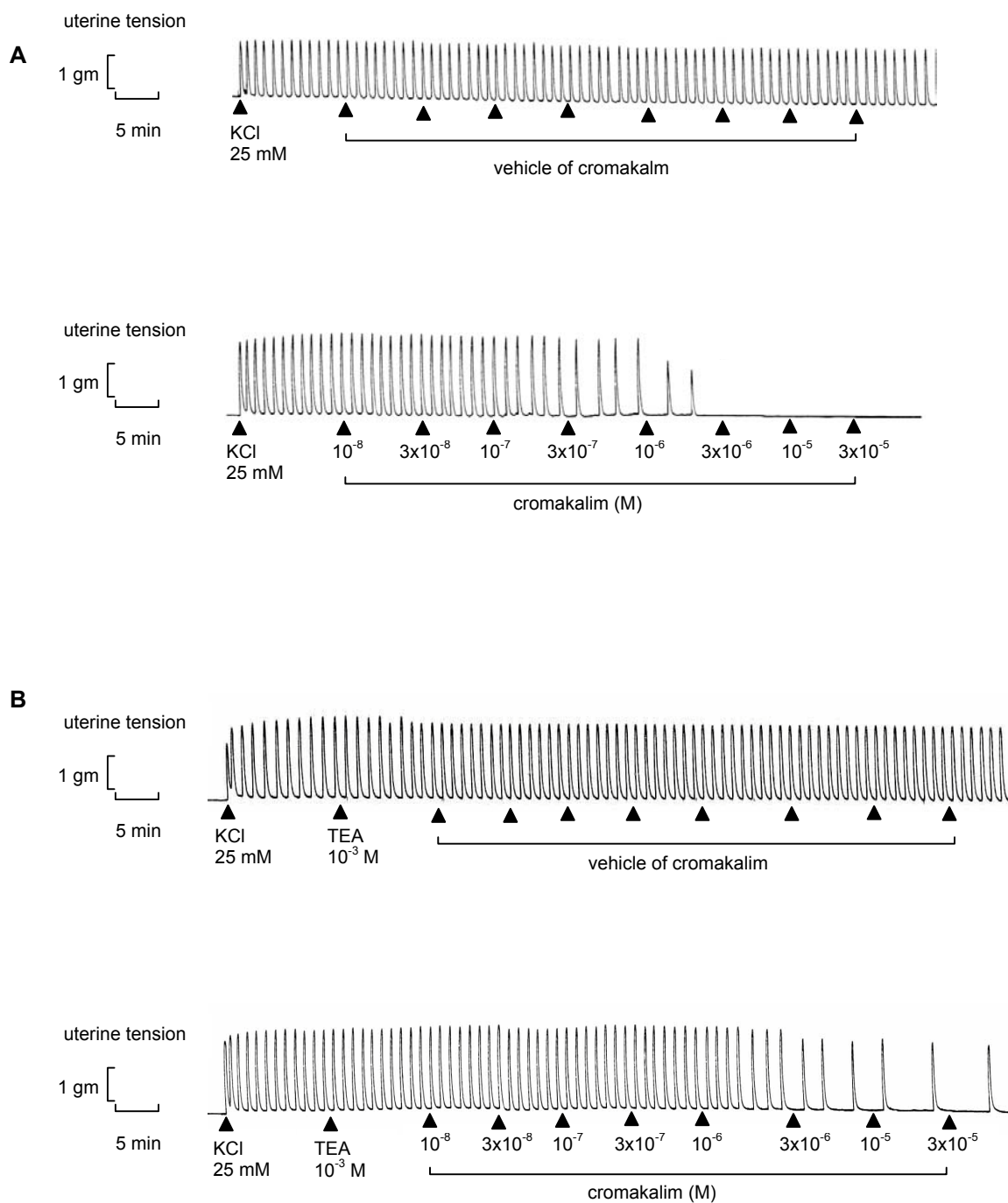
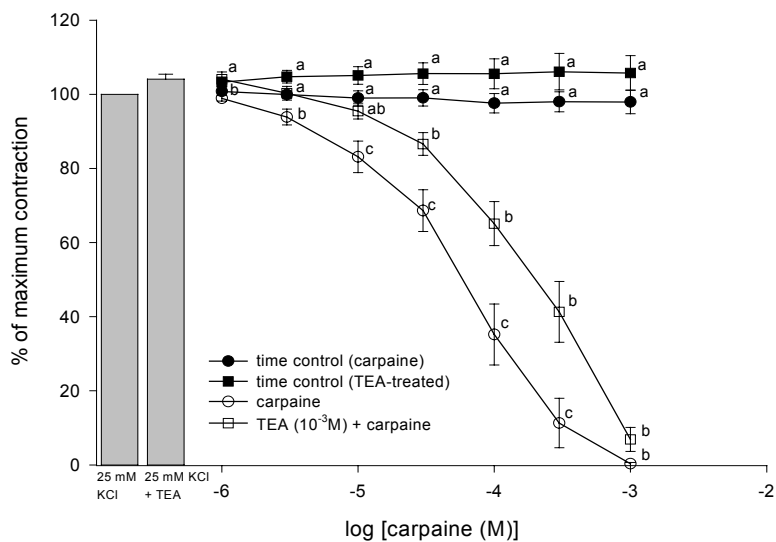


Figure 34. The representative tracings of the relaxing effect of cromakalim and its time control (A), or pretreated with TEA (10^{-3} M) for 15 minutes and its time control (B) on isolated rat uterus precontracted with 25 mM KCl depolarizing solution.

A



B

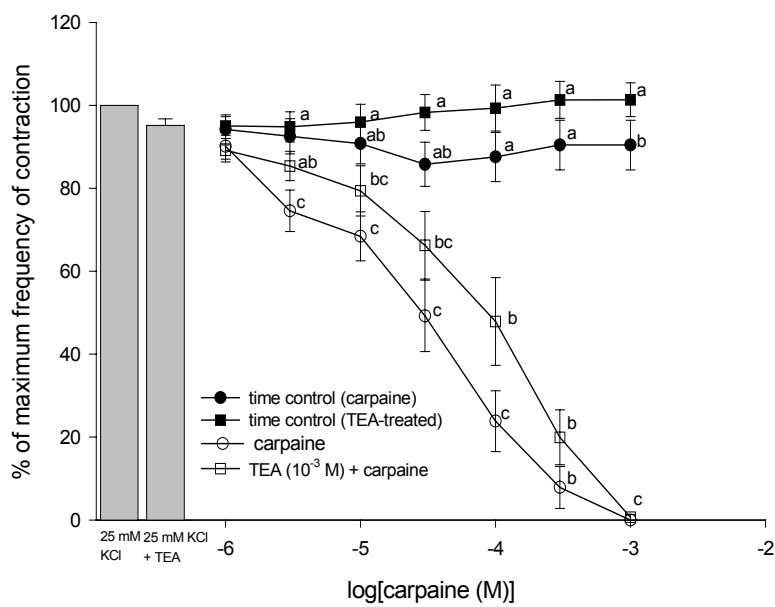
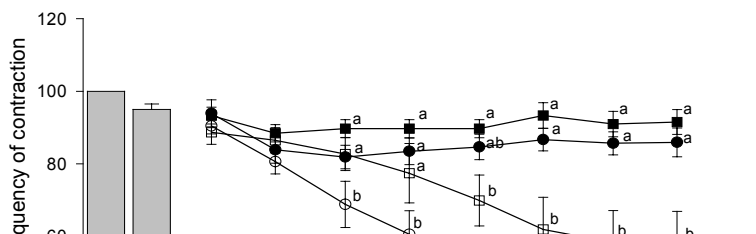
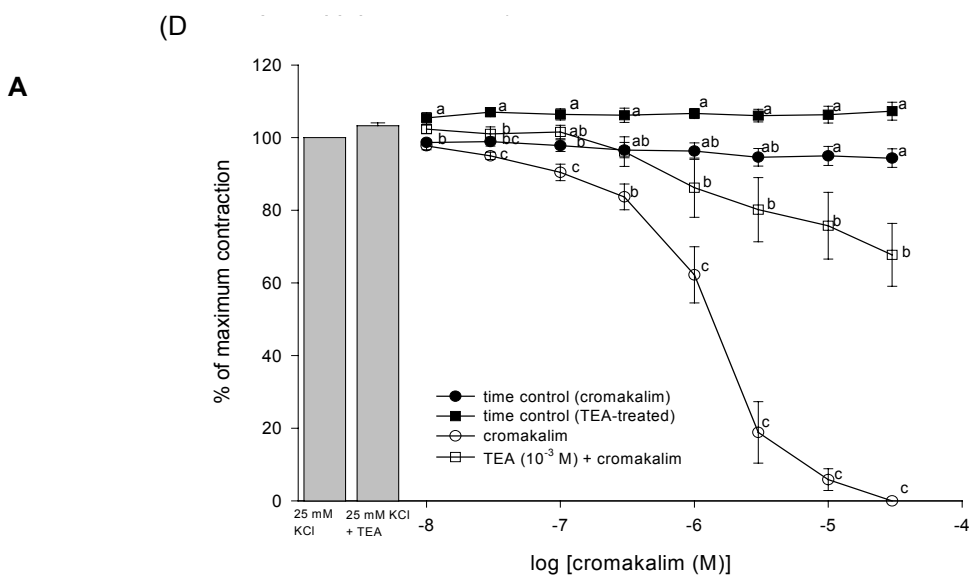


Figure 35. The concentration-response relationship of carpaine induced relaxation in the presence or absence of TEA (10^{-3} M) on isolated rat uterus precontracted with 25 mM KCl. (A) The effect of carpaine on force of contraction and (B) the effect of carpaine on frequency of contraction. Each value is expressed as a percentage of the initial maximal contraction in response to 25 mM KCl. Each datum point represents the mean \pm S.E.M of ten experiments. Different characters (a, b and c) mean significant difference among the comparing means which in the same treatment group (carpaine concentration) at $p < 0.05$



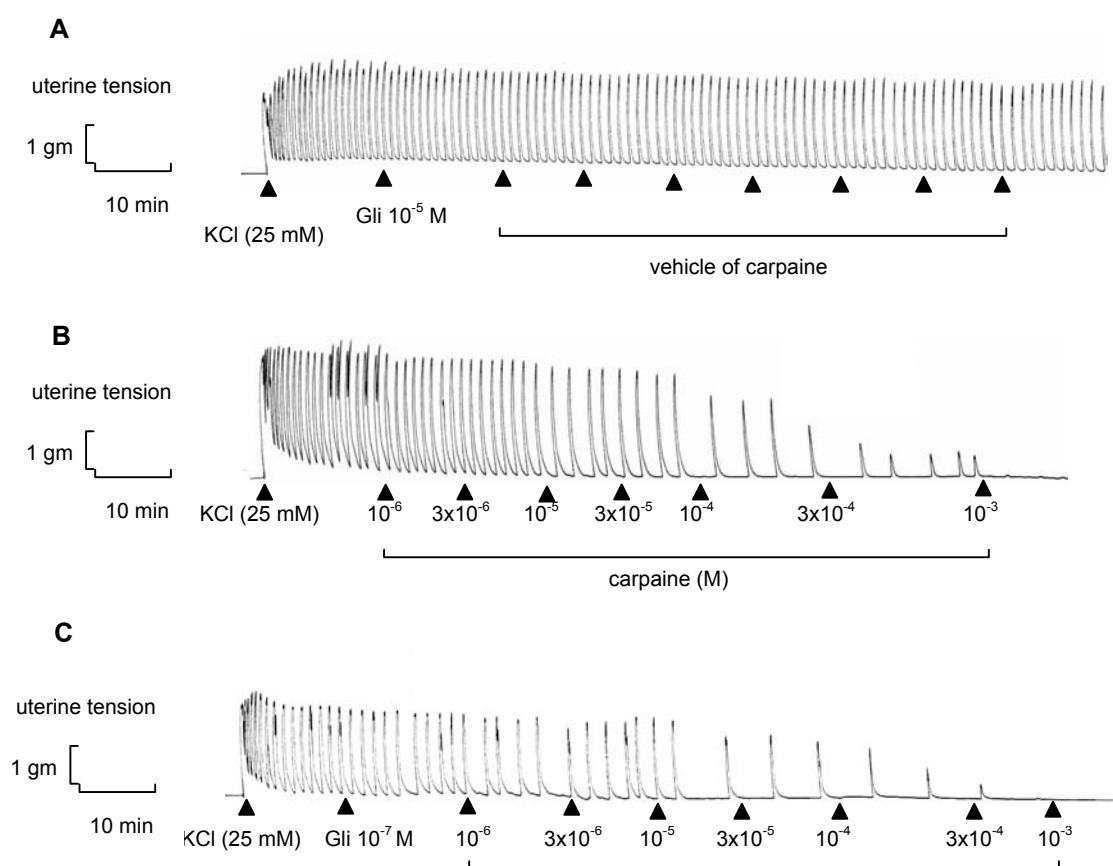
B

Figure 36. The concentration-response relationship of cromakalim induced relaxation in the presence or absence of TEA (10^{-3} M) on isolated rat uterus precontracted with 25 mM KCl. (A) The effect of cromakalim on force of contraction and (B) the effect of cromakalim on frequency of contraction. Each value is expressed as a percentage of the initial maximal contraction in response to 25 mM KCl. Each datum point represents the mean \pm S.E.M of ten experiments. Different characters (a, b and c) mean significant difference among the comparing means which in the same treatment group (cromakalim concentration) at $p < 0.05$ (Duncan's multiple range test).

2.4.2.2 Glibenclamide

The representative tracings of carpine and cromakalim-induced uterine relaxation in the presence of various concentrations of glibenclamide were illustrated in Figure 37 and 38, respectively. In 25 mM KCl-induced uterine contraction experiment, glibenclamide (10^{-7} , 10^{-6} and 10^{-5} M) caused rightward displacements of the concentration-response curves for cromakalim. The vehicle for glibenclamide caused no effect on uterine contraction. However, all concentrations of glibenclamide produced changes in the frequency of contraction induced by 25 mM K^+ . Glibenclamide caused an initial rapid decline about of 10-25% frequency of contraction to follow by a stably contractile response over the time of cumulative of cromakalim and carpine (Figure 37 and 38). The effects of various

concentration of glibenclamide on the concentration-response relationship for cromakalim were shown in Figure 39. In the presence of various concentration of glibenclamide (10^{-7} , 10^{-6} and 10^{-5} M), the relaxing effect of cromakalim on uterine contraction induced by 25 mM K^{+} -depolarizing solution was significantly inhibited ($p < 0.01$ for 10^{-7} M). The mean EC_{50} values for cromakalim inhibition of amplitude and frequency of contraction were shown in Table 13. In contrast, glibenclamide had no effect on the relaxing effect of carpaine on uterine contraction induced by 25 mM K^{+} -depolarizing solution. The IC_{50} values for carpaine inhibition of amplitude and frequency of contraction were not significant differences from the IC_{50} values obtained with in the presence of various concentrations of glibenclamide ($p > 0.05$) (Table 14). The log concentration-response relationship of carpaine in the presence or absence of various concentrations of glibenclamide was demonstrated in Figure 40.



D

Figure 37. The representative tracings of the inhibitory effect of glibenclamide (15 min) on relaxing effect of cromakalim on isolated rat uterus precontracted with 25 mM KCl depolarizing solution. (A) = time control, (B) = cromakalim treated, (C) =

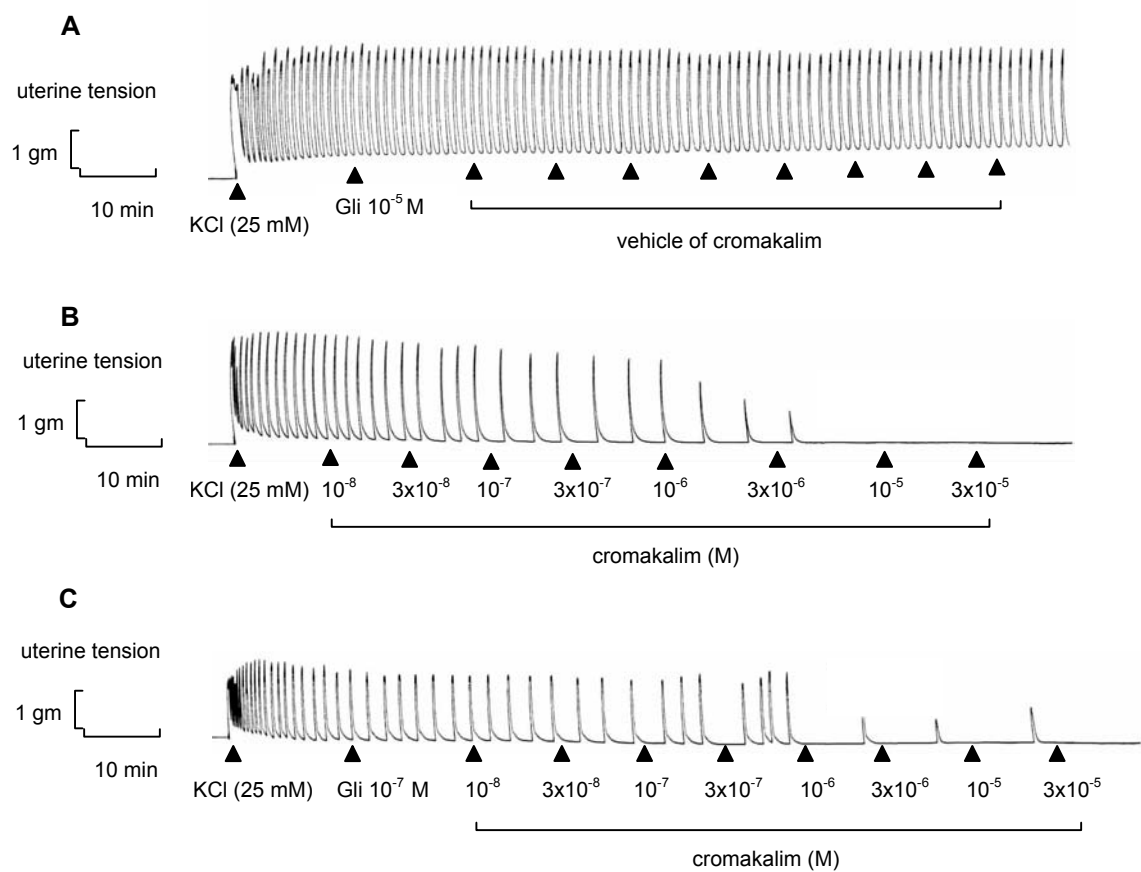


Figure 38. The representative tracings of the inhibitory effect of glibenclamide (15 min) on relaxing effect of cromakalim on isolated rat uterus precontracted with 25 mM KCl depolarizing solution. (A) = time control, (B) = cromakalim treated, (C) = glibenclamide (10^{-7} M) and cromakalim treated, (D) = glibenclamide (10^{-6} M) and cromakalim treated.

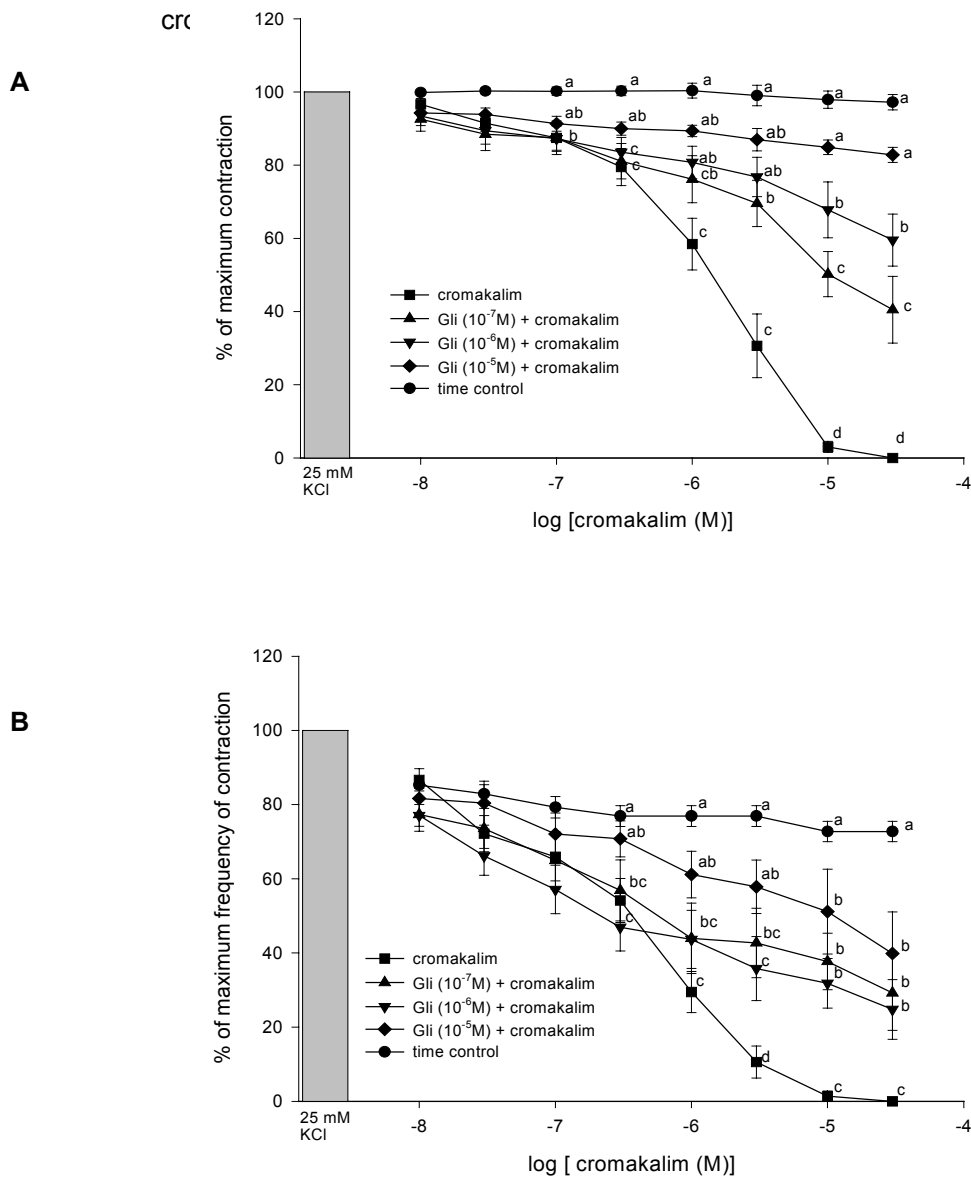
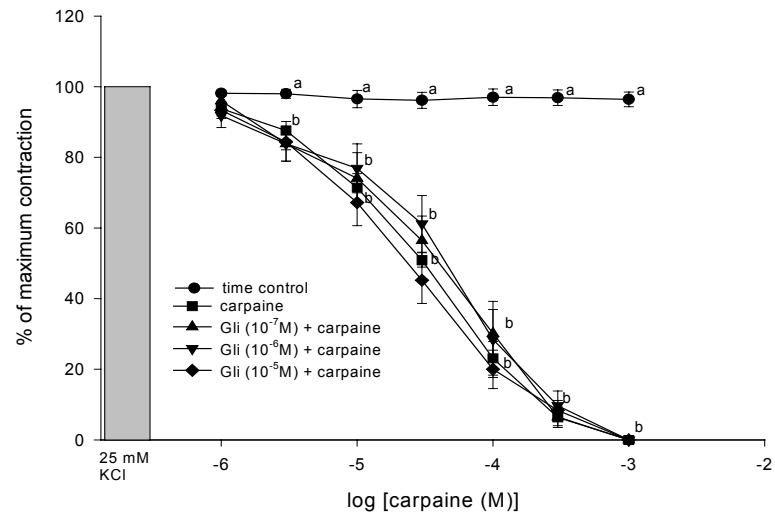


Figure 39. The concentration-response relationship of inhibitory effect of glibenclamide on relaxing effect of cromakalim on isolated rat uterus. The uteri were precontracted with 25 mM KCl. (A) Effect of various concentrations of glibenclamide (10^{-7} , 10^{-6} and 10^{-5} M, 15 minutes) on the effect of cromakalim on force contraction and (B) on frequency of contraction. Each datum point represents the mean \pm S.E.M of

ten experiments. Different characters (a, b, c and d) mean significant difference among the comparing means which in the same treatment group (cromakalim concentration) at $p < 0.05$ (Duncan's multiple range test).

A



B

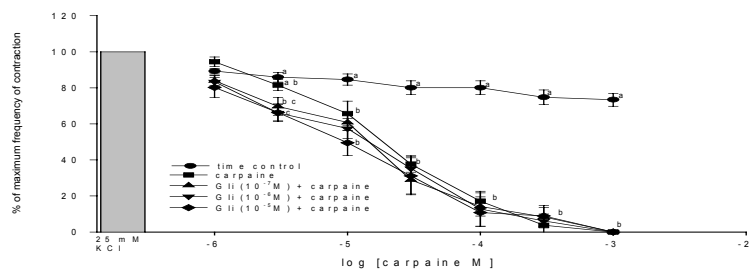


Figure 40. The concentration-response relationship of inhibitory effect of glibenclamide on relaxing effect of carpaine on isolated rat uterus. The uteri were precontracted with 25 mM KCl. (A) Effect of various concentrations of glibenclamide (10^{-7} , 10^{-6} and 10^{-5} M, 15 minutes) on the effect of carpaine on force contraction and (B) on frequency of contraction. Each datum point represents the mean \pm S.E.M of ten experiments. Different characters (a and b) mean significant difference among the comparing means which in the same treatment group (carpaine concentration) at $p < 0.05$ (Duncan's multiple range test).

Table 13. Comparison of EC_{50} values of cromakalim -induced relaxation in the presence or absence of glibenclamide (10^{-7} , 10^{-6} and 10^{-5} M) on isolated rat uterus precontracted with depolarizing solution containing 25 mM KCl.

EC ₅₀ (Mean \pm S.E.M)				
contraction	cromakalim alone	Glibenclamide-treated (M)		
		10^{-7}	10^{-6}	10^{-5}
Force	$1.50 \pm 0.21 \times 10^{-6}$	** $1.72 \pm 0.61 \times 10^{-5}$	ND	ND
Frequency	$5.00 \pm 1.21 \times 10^{-7}$	$0.69 \pm 7.92 \times 10^{-7}$	$0.5 \pm 1.21 \times 10^{-7}$	$0.32 \pm 6.22 \times 10^{-6}$

(* $p < 0.05$, ** $p < 0.01$ as compared to cromakalim alone (Dunnett's test))

ND = not determined, maximum contraction was less than 50% of maximum response

Table 14. Comparison of EC_{50} values of carpaine-induced relaxation in the presence or absence of glibenclamide (10^{-7} , 10^{-6} and 10^{-5} M) on isolated rat uterus precontracted with depolarizing solution containing 25 mM KCl.

EC ₅₀ (Mean \pm S.E.M)				
Contraction	carpaine alone	Glibenclamide-treated (M)		
		10^{-7}	10^{-6}	10^{-5}
Force	$3.22 \pm 0.39 \times 10^{-5}$	$4.22 \pm 0.87 \times 10^{-5}$	$5.36 \pm 1.16 \times 10^{-5}$	$2.47 \pm 0.52 \times 10^{-5}$
Frequency	$1.93 \pm 0.38 \times 10^{-5}$	$1.77 \pm 0.59 \times 10^{-5}$	$4.23 \pm 2.14 \times 10^{-5}$	$1.21 \pm 0.81 \times 10^{-5}$

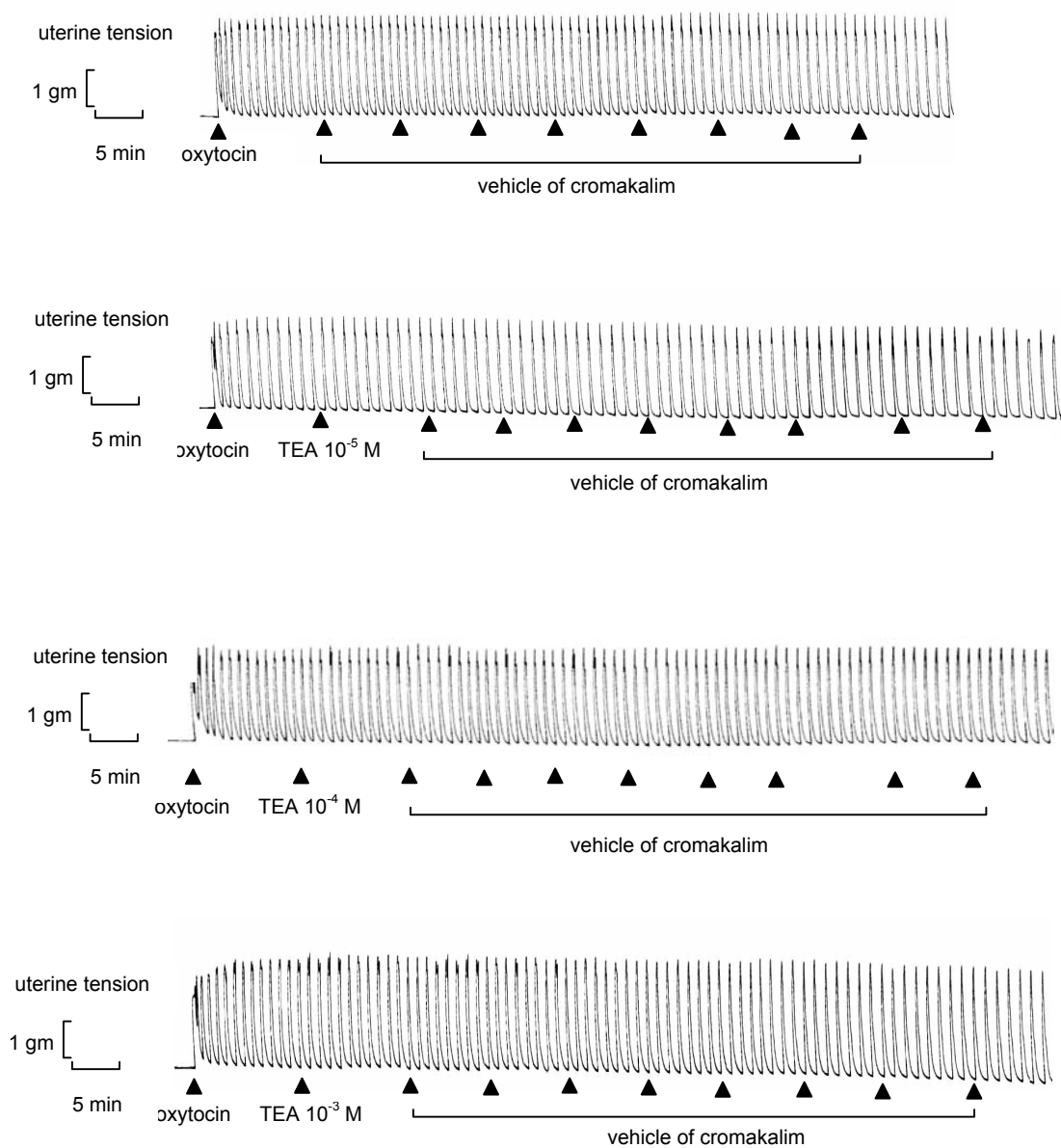
2.4.3 Effect of carpaine and cromakalim on uterine contraction induced by oxytocin (1mU/ml) and the influence of K⁺-channel blocker.

2.4.3.1. Tetraethylammonium (TEA)

2.4.3.1.1 Determination of optimum concentration of TEA

The representative tracing of the relaxing effects of cromakalim and its vehicle on uterine contraction induced by oxytocin (1 mU/ml) were demonstrated in Figure 41. 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 cromakalim had no effects on uterine contraction induced by oxytocin (1 mU/ml). Cumulative addition of cromakalim (10^{-8} - 3×10^{-5} M) significantly produced a concentration-related inhibitory effect on the amplitude and frequency of uterine contraction precontracted with oxytocin (1mU/ml) ($p < 0.01$). The amplitude of contraction was more sensitive to cromakalim than the frequency of contraction. The EC₅₀ values of cromakalim for the suppression of amplitude and frequency were $1.31 \pm 0.24 \times 10^{-6}$ M and $0.15 \pm 0.54 \times 10^{-6}$ M, respectively. In the presence of 10^{-5} , 10^{-4} and 10^{-3} M of TEA, the inhibition was antagonized, as observed by a shift of the log concentration response curve of cromakalim to the right. The IC₅₀ values for cromakalim inhibition of amplitude of contraction in the presence of TEA (10^{-5} , 10^{-4} and 10^{-3} M) was significantly different only at the concentration 10^{-3} M ($p < 0.05$) (Table 15). The IC₅₀ value for cromakalim for the inhibition of frequency of contraction were not significant different form the IC₅₀ values obtained in the presence of various concentrations of TEA ($p > 0.05$) (Table 15). The representative tracing of the blocking activity of TEA on uterine relaxing effect of cromakalim (10^{-8} - 3×10^{-5} M) was demonstrated in Figure 41 and the log concentration-response relationship was demonstrated in Figure 42. The concentration of TEA at 10^{-3} M was then chosen to use in the next experiment.

A



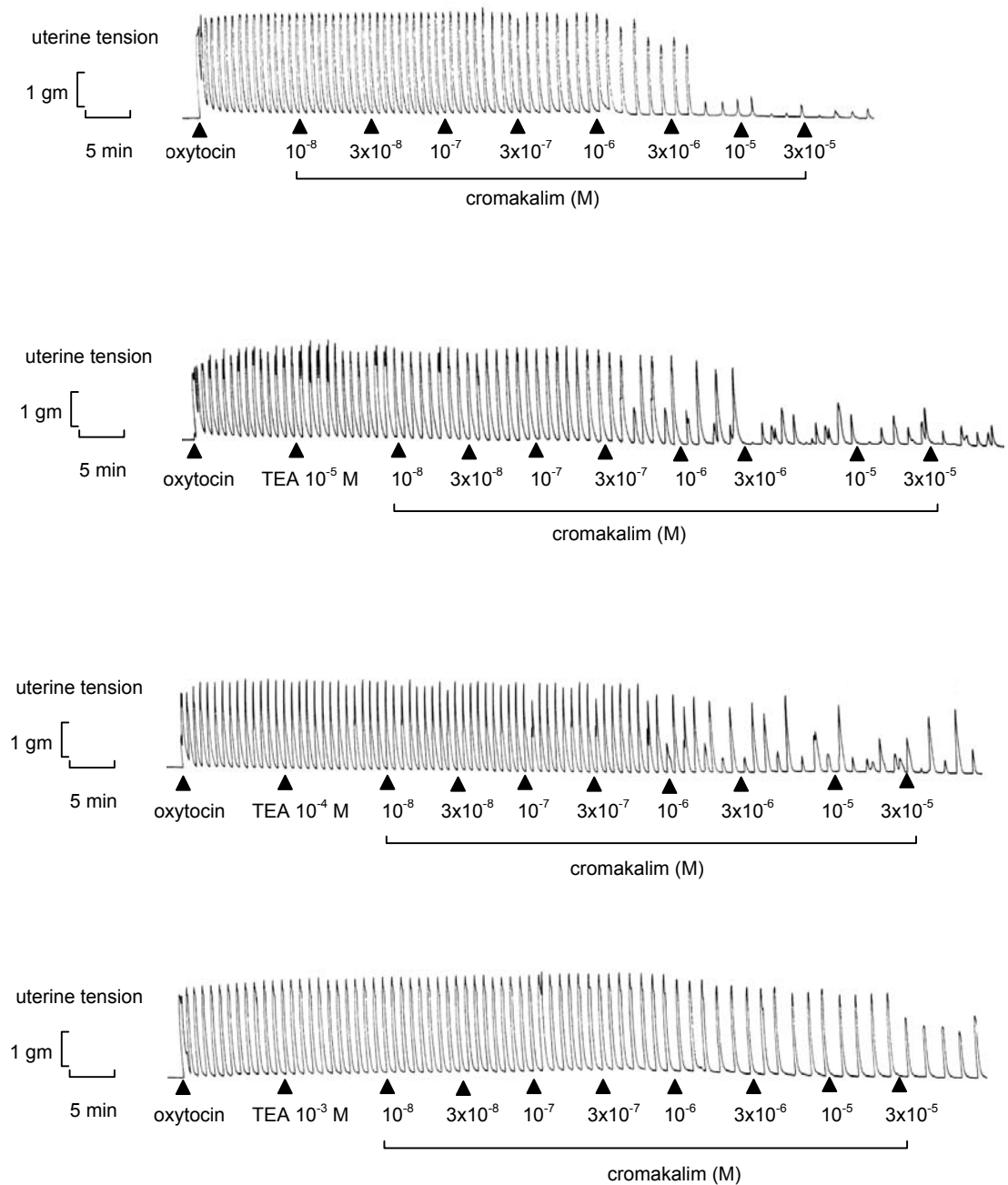
B

Figure 41. The representative tracing of the inhibitory effect of TEA on relaxing effect of cromakalim on isolated rat uterus precontracted with oxytocin (1mU/ml). (A) time

control of each experiment and (B) cromakalim alone and 10^{-5} , 10^{-4} , 10^{-3} M TEA-treated respectively.

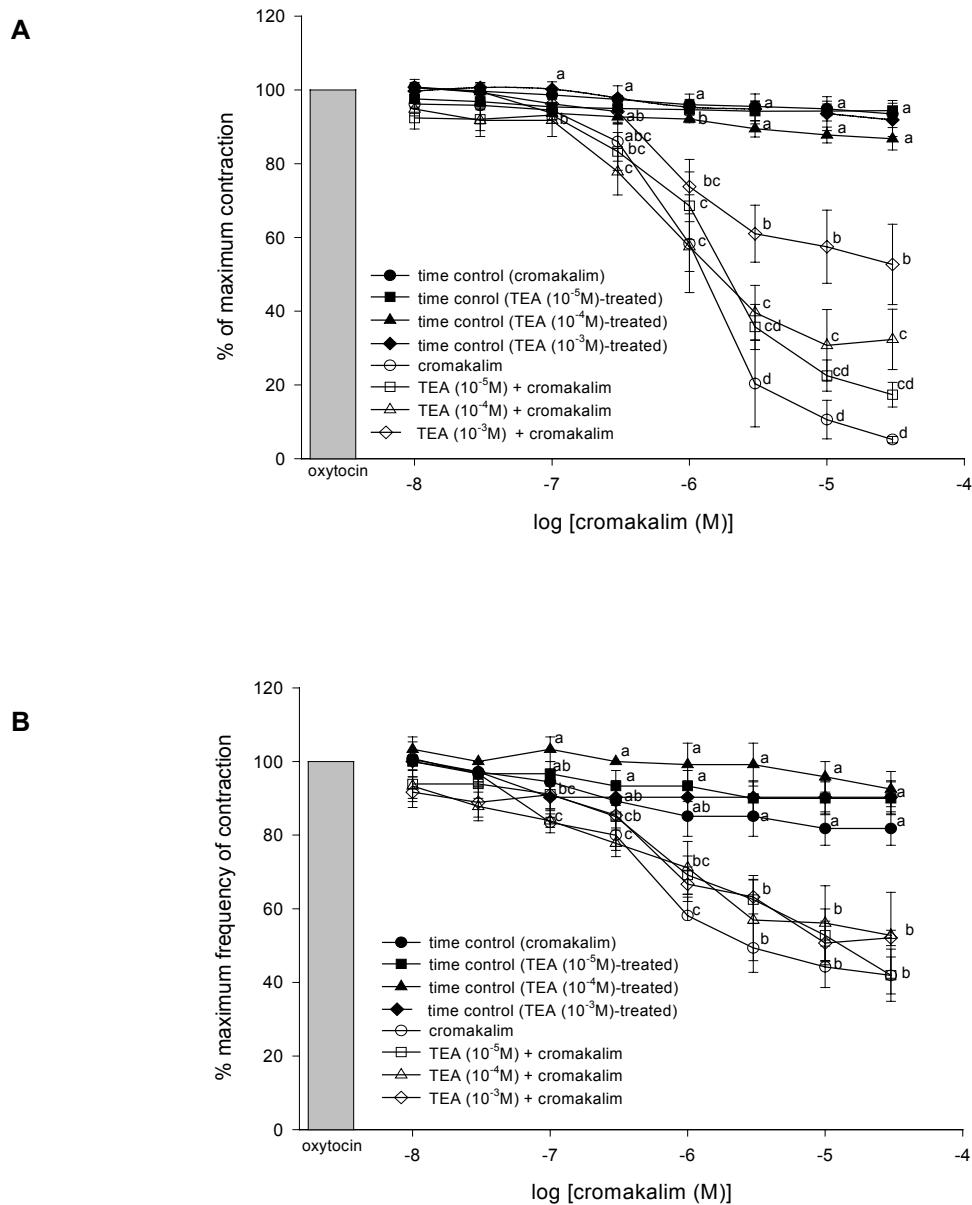


Figure 42. The concentration-response relationship of inhibitory effect of TEA on relaxing effect of cromakalim on isolated rat uterus. The uteri were precontracted with oxytocin (0.1 mU/ml). (A) Effect of various concentrations of TEA (10^{-5} , 10^{-4} and 10^{-3} M, 10 minutes) on the effect of cromakalim on force contraction and (B) on frequency of contraction. Each datum point represents the mean \pm S.E.M of ten experiments. Different characters (a, b, c and d) mean significant difference

among the comparing means which in the same treatment group (cromakalim concentration) at $p < 0.05$ (Duncan's multiple range test).

Table 15. Comparison of IC_{50} values of cromakalim-induced relaxation in the presence or absence of TEA (10^{-5} , 10^{-4} and 10^{-3} M) on isolated rat uterus precontracted with oxytocin (1mU/ml).

IC_{50} (Mean \pm S.E.M)				
Contraction	cromakalim alone	TEA-treated (M)		
		10^{-5}	10^{-4}	10^{-3}
Force	$1.31 \pm 0.24 \times 10^{-6}$	$2.39 \pm 0.49 \times 10^{-6}$	$1.67 \pm 1.2 \times 10^{-6}$	** $0.89 \pm 1.1 \times 10^{-5}$
Frequency	$0.15 \pm 0.54 \times 10^{-6}$	$1.04 \pm 0.56 \times 10^{-5}$	$0.13 \pm 1.28 \times 10^{-5}$	$1.39 \pm 1.40 \times 10^{-5}$

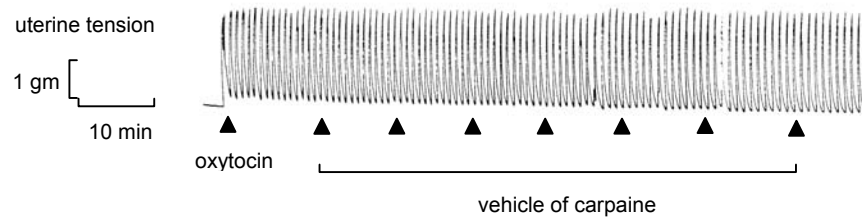
(** $p < 0.01$ as compared to cromakalim alone (Dunnett' test))

2.4.3.1.2 Effect of TEA on uterine relaxation induced by carpaine and cromakalim on isolated rat uterus precontracted with oxytocin

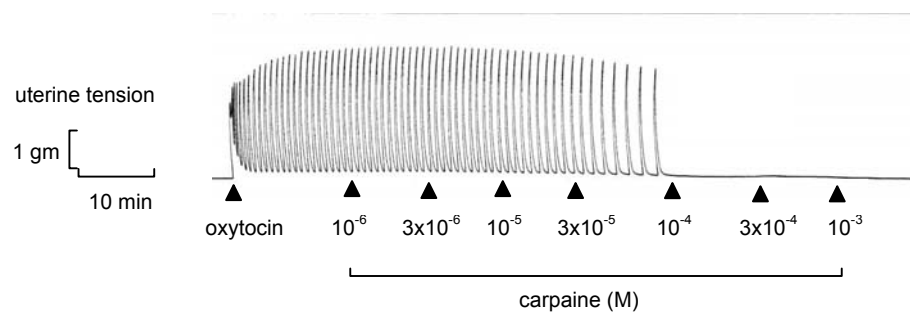
The representative tracing of carpaine and cromakalim induced uterine relaxation precontracted with oxytocin (1mU/ml) were illustrated in Figure 43 and 44, respectively. The addition of vehicle of carpaine or cromakalim had no effects on uterine contraction induced by oxytocin (1mU/ml). The addition of cumulative concentration of carpaine inhibited this contraction with IC_{50} value of $7.29 \pm 0.57 \times 10^{-5}$ M and $5.00 \pm 1.04 \times 10^{-5}$ M for amplitude and frequency, respectively. In the presence of 10^{-3} M of TEA, carpaine inhibited amplitude of contraction which was significantly different from that obtained in the absence of TEA ($P < 0.05$). The mean IC_{50} values for carpaine inhibition of amplitude and frequency were $1.95 \pm 0.34 \times 10^{-4}$ M and $4.99 \pm 2.82 \times 10^{-5}$ M (Table 16.), respectively. The addition of cumulative concentration of cromakalim inhibited the uterine contraction in a concentration-dependent manner with IC_{50} of $1.17 \pm 0.17 \times 10^{-6}$ M and $0.55 \pm 0.96 \times 10^{-6}$ M for amplitude and frequency, respectively. In the presence of 10^{-3} M TEA, the relaxing effect of cromakalim on the amplitude of contraction was significantly inhibited ($p < 0.05$) but with no effect on the frequency of contraction. The mean IC_{50} values for cromakalim inhibition on the amplitude and frequency were $0.55 \pm 0.96 \times 10^{-6}$ M and $0.56 \pm 0.88 \times 10^{-5}$ M, respectively

(Table 16.). The log concentration-response relationship of carpaine and cromakalim in the presence of TEA (10^{-3} M) was demonstrated in Figure 45 and 46, respectively.

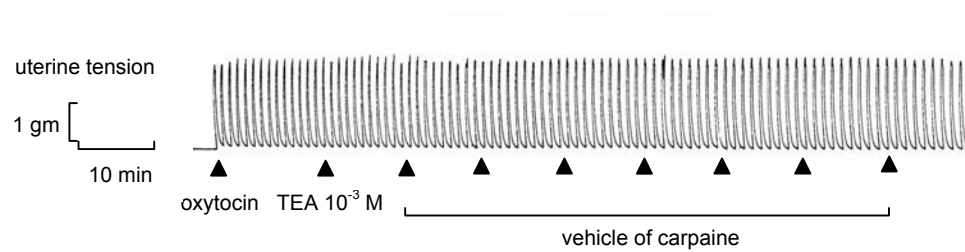
A



B



C



D

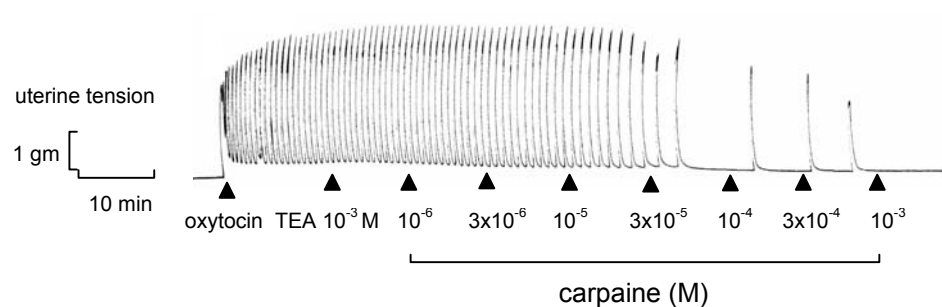


Figure 43. The representative tracings of the relaxing effect of carpaine in the presence or absence of TEA (10^{-3} M) on isolated rat uterus precontracted with oxytocin (1mU/ml). (A) time control (B) carpaine alone (C) time control of carpaine treated

in the presence of TEA (10^{-3} M) and (D) cromakalim treated in the presence of TEA (10^{-3} M) for 15 minutes.

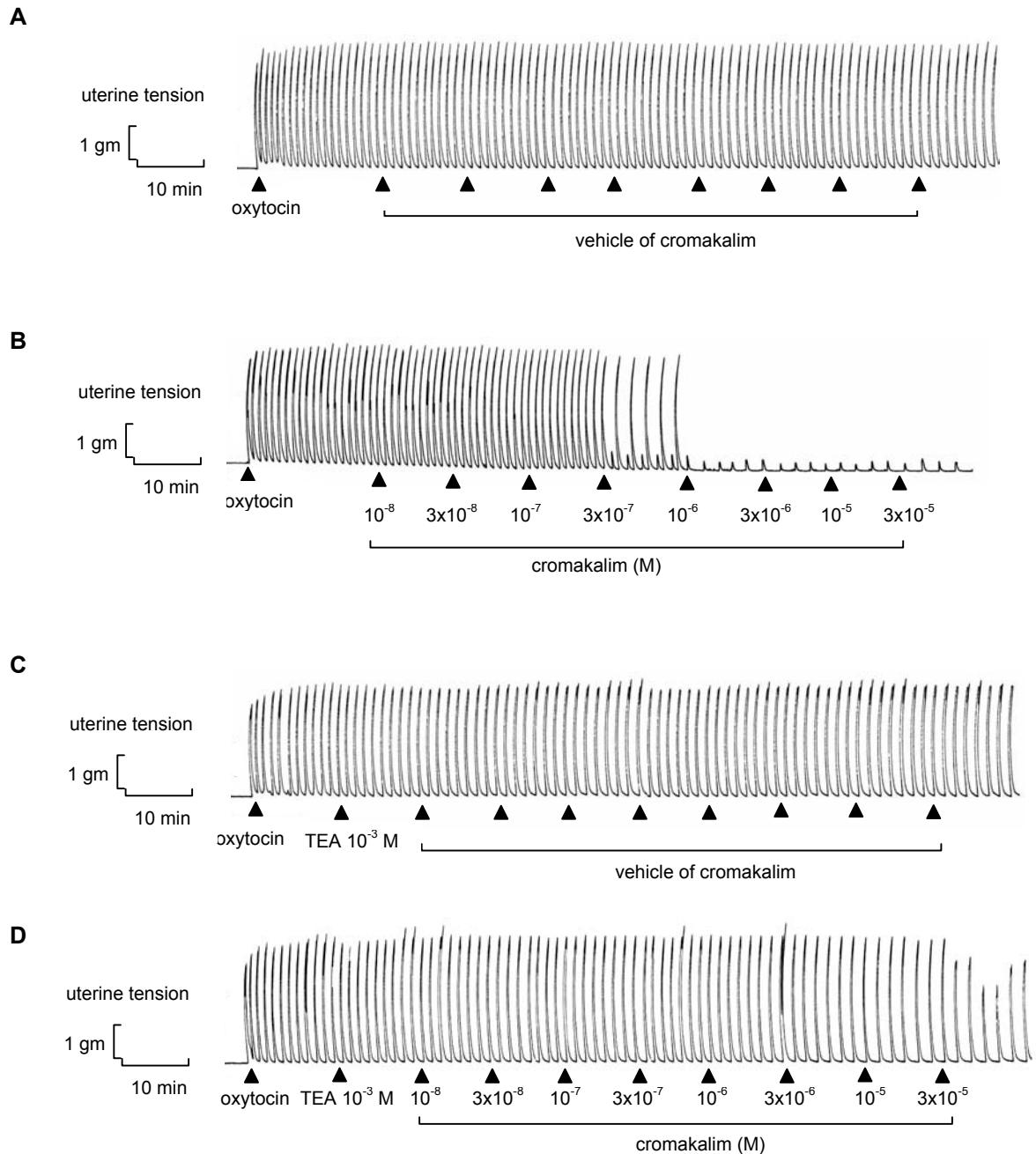


Figure 44. The representative tracings of the relaxing effect of cromakalim on isolated rat uterus precontracted with oxytocin (1mU/ml). (A) time control (B) cromakalim alone (C) time control of cromakalim treated in the presence of TEA (10^{-3} M) and (D) cromakalim treated in the presence of TEA (10^{-3} M) for 15 minutes.

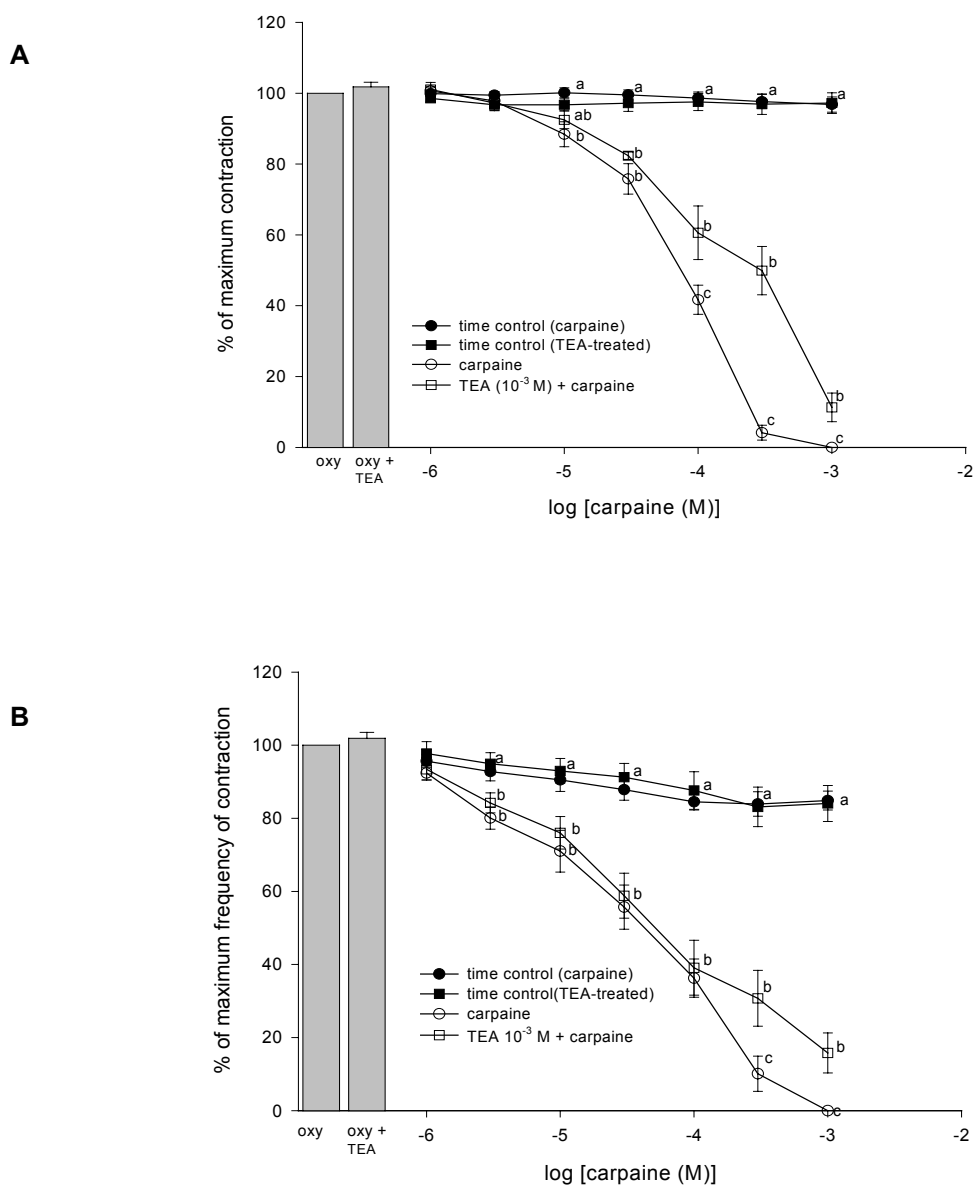


Figure 45. The concentration-response relationship of carpaine and its relaxing effect in the presence or absence of TEA (10^{-3} M) on isolated rat uterus precontracted with oxytocin (1mU/ml). (A) The effect of carpaine on force of contraction and (B) the effect of carpaine on frequency of contraction in the presence or absence TEA (10^{-3} M). Each value is expressed as a percentage of the initial maximal contraction in response to oxytocin. Each datum point represents the mean \pm S.E.M of ten experiments. Different characters (a, b and c) mean significant

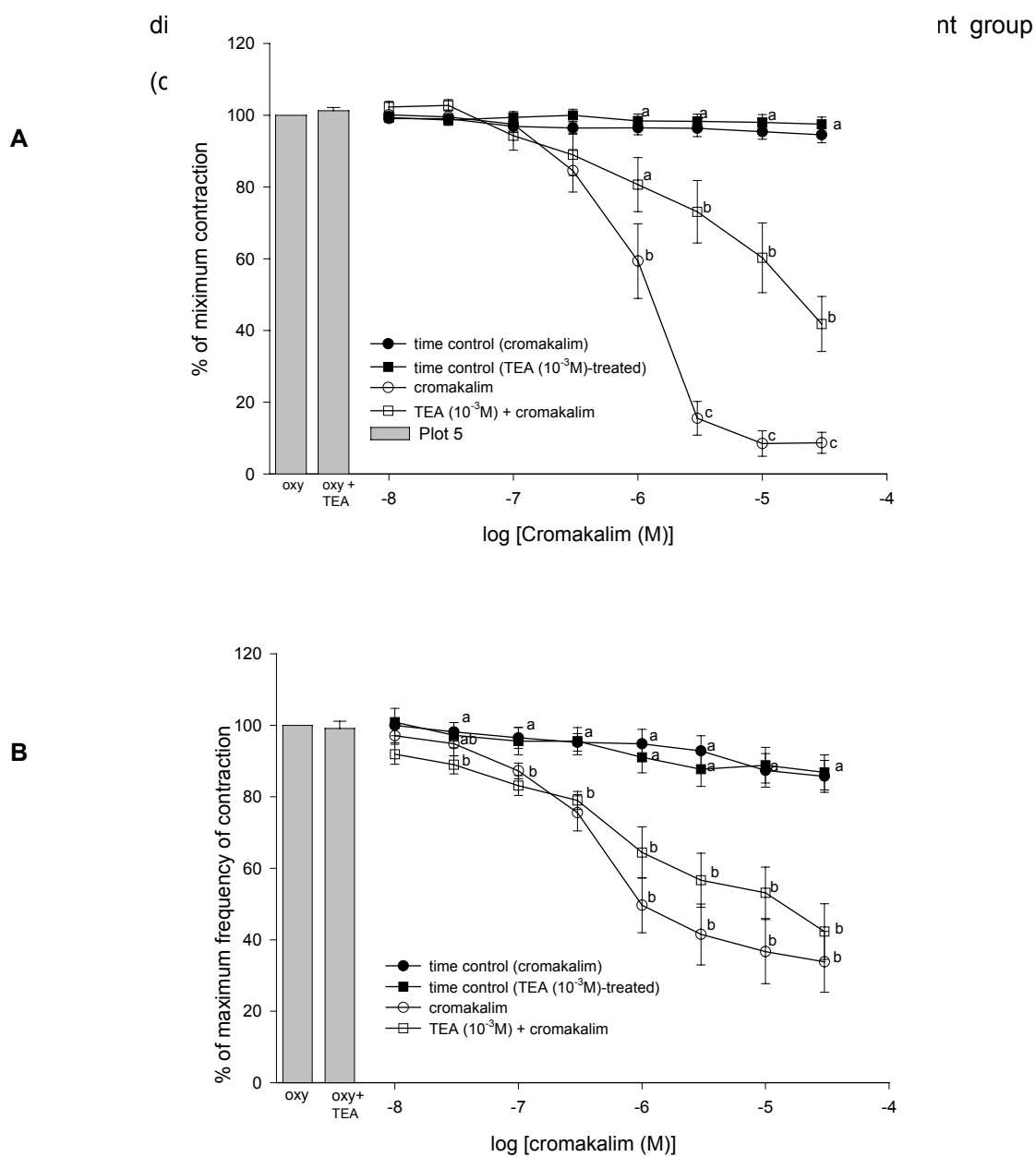


Figure 46. The concentration-response relationship of cromakalim and its relaxing effect in the presence or absence of TEA (10^{-3} M) on isolated rat uterus precontracted with oxytocin (1mU/ml). (A) The effect of cromakalim on force of contraction and (B) the effect of cromakalim on frequency of contraction in the presence or absence TEA (10^{-3} M). Each value is expressed as a percentage of the initial maximal contraction in response to oxytocin. Each datum point represents the mean \pm S.E.M of ten experiments. Different characters (a, b and c) mean

significant difference among the comparing means which in the same treatment group (cromakalim concentration) at $p < 0.05$ (Duncan's multiple range test).

Table 16. Comparison of IC_{50} values of carpaine or cromakalim-induced relaxation in the presence or absence of TEA (10^{-3} M) on isolated rat uterus precontracted with oxytocin (1mU/ml).

IC ₅₀ of (Mean ± S.E.M)				
Contraction	carpaine (M)		cromakalim (M)	
	carpaine alone	TEA-pretreated	cromakalim alone	TEA-treated
Force	$7.29 \pm 0.57 \times 10^{-5}$	$**1.95 \pm 0.34 \times 10^{-4}$	$1.17 \pm 0.17 \times 10^{-6}$	$*1.49 \pm 0.63 \times 10^{-5}$
Frequency	$5.00 \pm 1.04 \times 10^{-5}$	$4.99 \pm 2.82 \times 10^{-5}$	$0.55 \pm 0.96 \times 10^{-6}$	$0.56 \pm 0.88 \times 10^{-5}$

(* $p < 0.05$, ** $p < 0.01$ as compared to carpaine or cromakalim alone (Dunnett's test))

2.4.3.2. Glibenclamide

2.4.3.2.1 Effect of glibenclamide on uterine relaxation induced by cromakalim or carpaine

The representative tracings of the effect of carpaine and cromakalim in the presence or absence of various concentrations of glibenclamide on isolated rat uterus precontracted with oxytocin (1 mU/ml) were illustrated in Figure 47 and 48, respectively. The log concentration-response relationship of inhibitory effect of various concentration of glibenclamide on the relaxing effect of carpaine and cromakalim was demonstrated in Figure 49 and 50, respectively. IC_{50} values of carpaine or cromakalim-induced uterine relaxation in the presence or absence of glibenclamide were shown in Table 17 and 18, respectively. In oxytocin (1mU/ml)-contracted preparation, glibenclamide (10^{-7} , 10^{-6} and 10^{-5} M) caused rightward displacements of the concentration-response curves for cromakalim. The vehicle for glibenclamide was no effect on uterine contraction. Figure 50 was shown the effect of glibenclamide on the concentration response relationship for cromakalim. In the presence of various concentration of glibenclamide (10^{-7} , 10^{-6} and 10^{-5} M), the relaxing effect of cromakalim on uterine contraction induced by oxytocin (1mU/ml) was significantly inhibited ($p > 0.05$ for 10^{-7} , $p < 0.01$ for 10^{-6} and $p < 0.01$ for 10^{-5} M, Duncan's test). Glibenclamide, at the

same concentrations, also caused non-parallel rightward shifts of concentrations-response curves for carpaine. The IC_{50} values for carpaine inhibition of amplitude and frequency of contraction were significant differences from the IC_{50} values obtained in the presence of various concentrations of glibenclamide ($p < 0.05$) (Table 17). The log concentration-response relationship of carpaine was demonstrated in Figure 49.

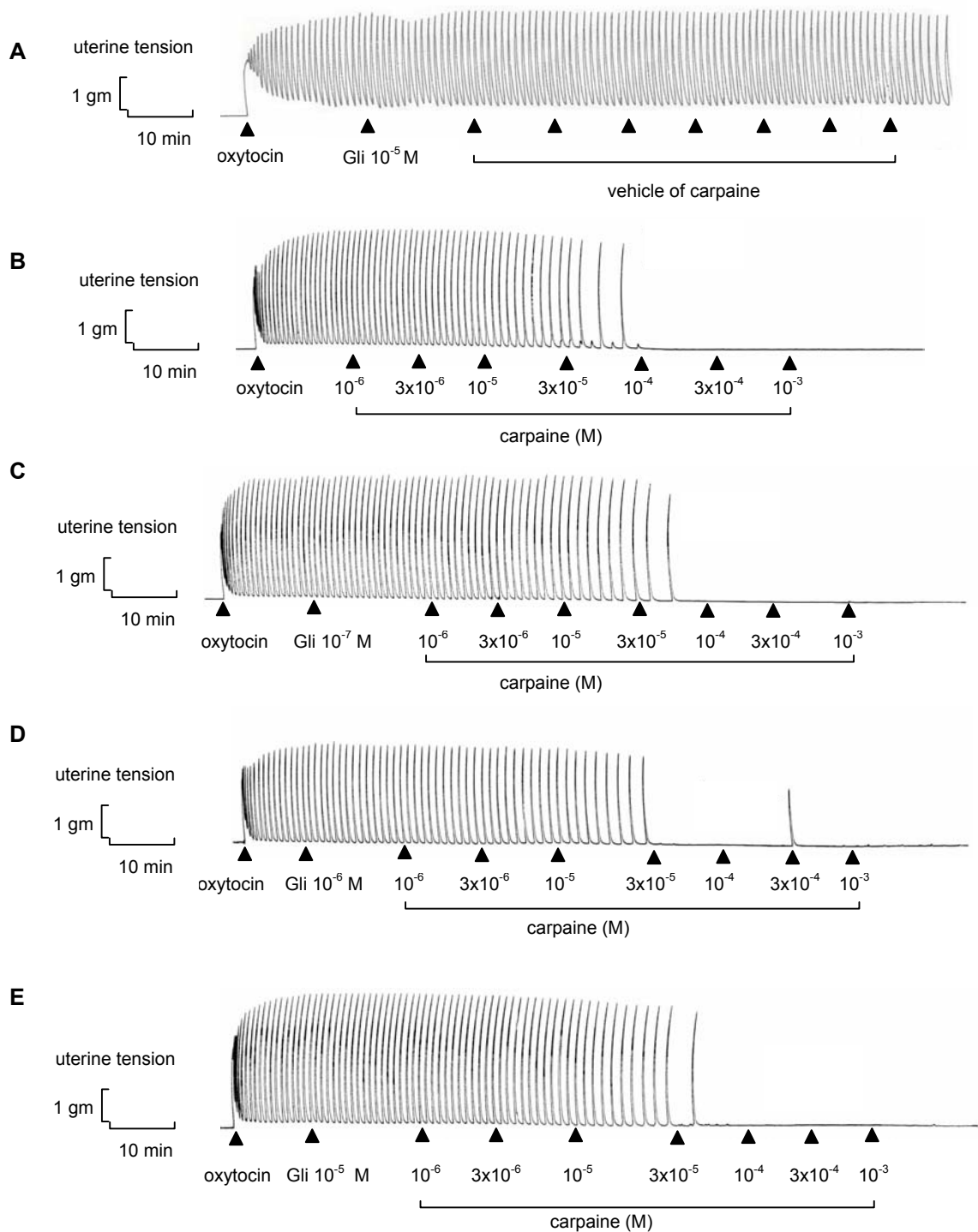


Figure 47. The representative tracings of the inhibitory effect of glibenclamide (15 min) on relaxing effect of carpaine on isolated rat uterus precontracted with oxytocin (1 mU/ml). (A) = time control, (B) = carpaine treated, (C) = glibenclamide (10^{-7} M)

and carnaine treated. (D) = olibenclamide (10^{-6} M) and carnaine treated and (E)

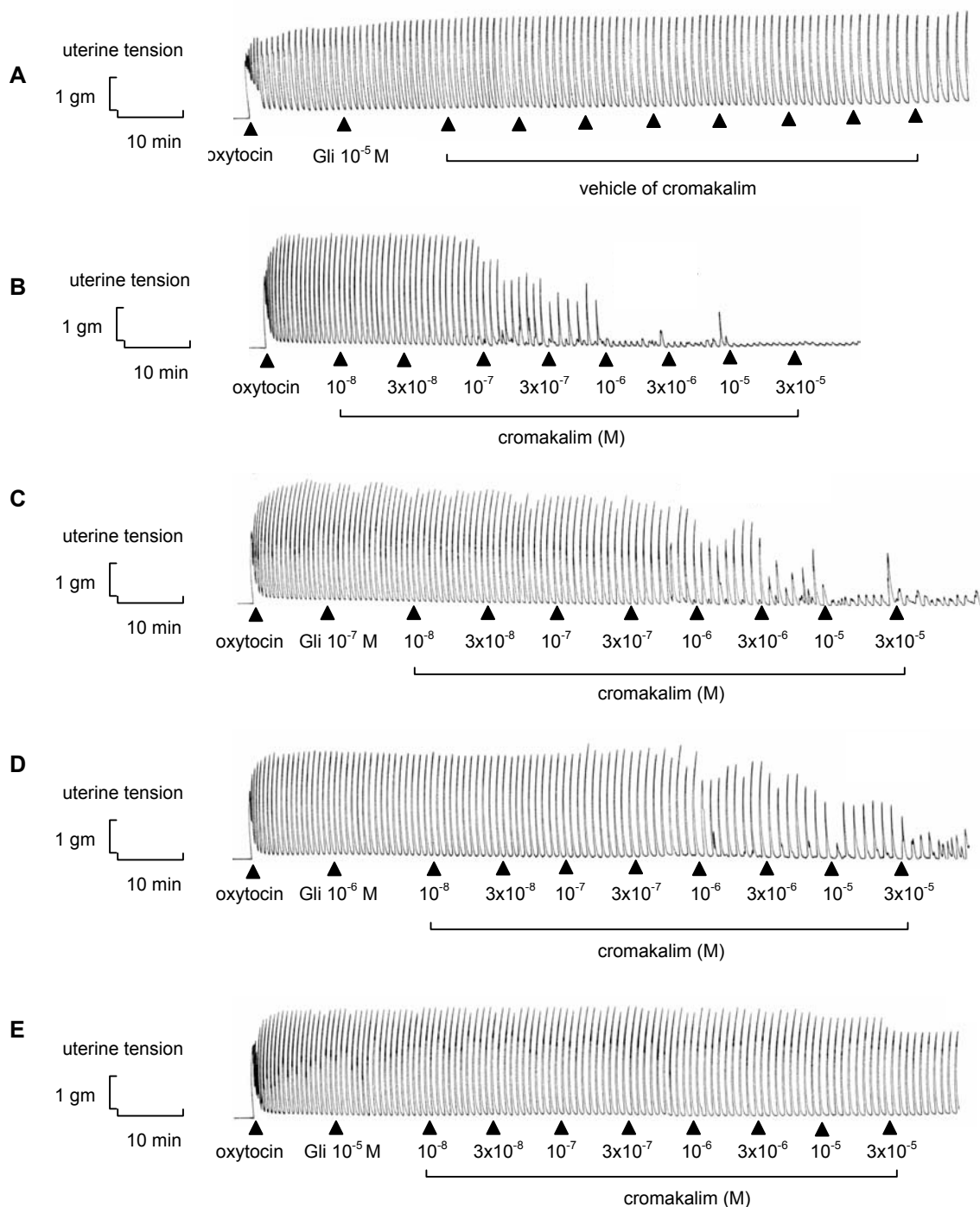
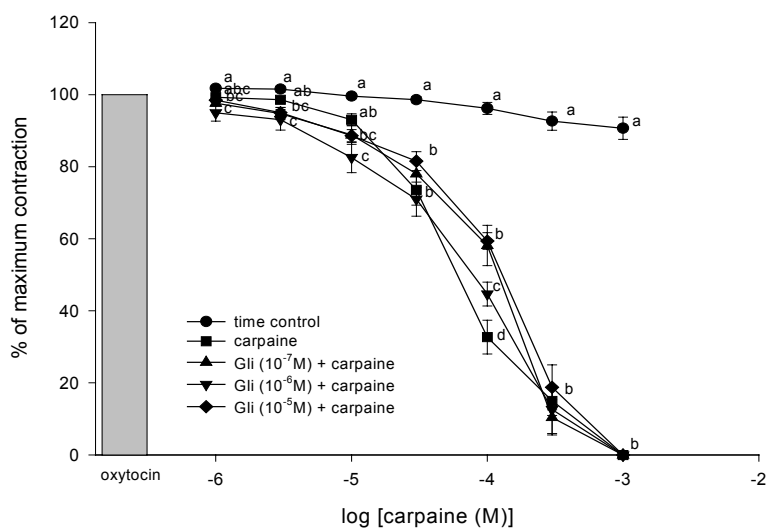


Figure 48. The representative tracings of the inhibitory effect of glibenclamide (15 min) on relaxing effect of cromakalim on isolated rat uterus precontracted with oxytocin (1 mU/ml). (A) = time control, (B) = cromakalim treated, (C) = glibenclamide (10^{-7}

M) and cromakalim treated, (D) = glibenclamide (10^{-6} M) and cromakalim treated and (E) = glibenclamide (10^{-5} M) and cromakalim treated.

A



B

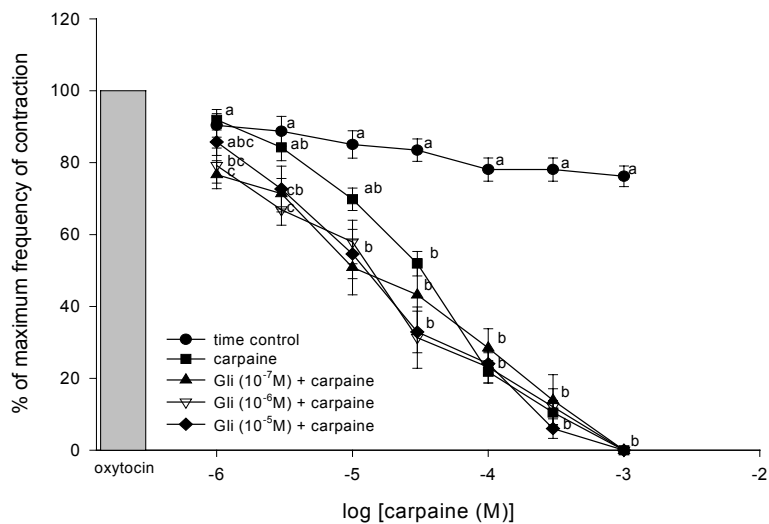


Figure 49. The concentration-response relationship of inhibitory effect of glibenclamide on relaxing effect of carpaine on isolated rat uterus precontracted with oxytocin (1 mU/ml). (A) Effect of various concentrations of glibenclamide (10^{-7} , 10^{-6} and 10^{-5} M, 15 minutes) on the effect of carpaine on force of contraction and (B) the effect of carpaine on frequency of contraction. Each datum point represents the mean

\pm S.E.M of ten experiments. Different characters (a, b, c and d) mean significant difference among the comparing means which in the same treatment group

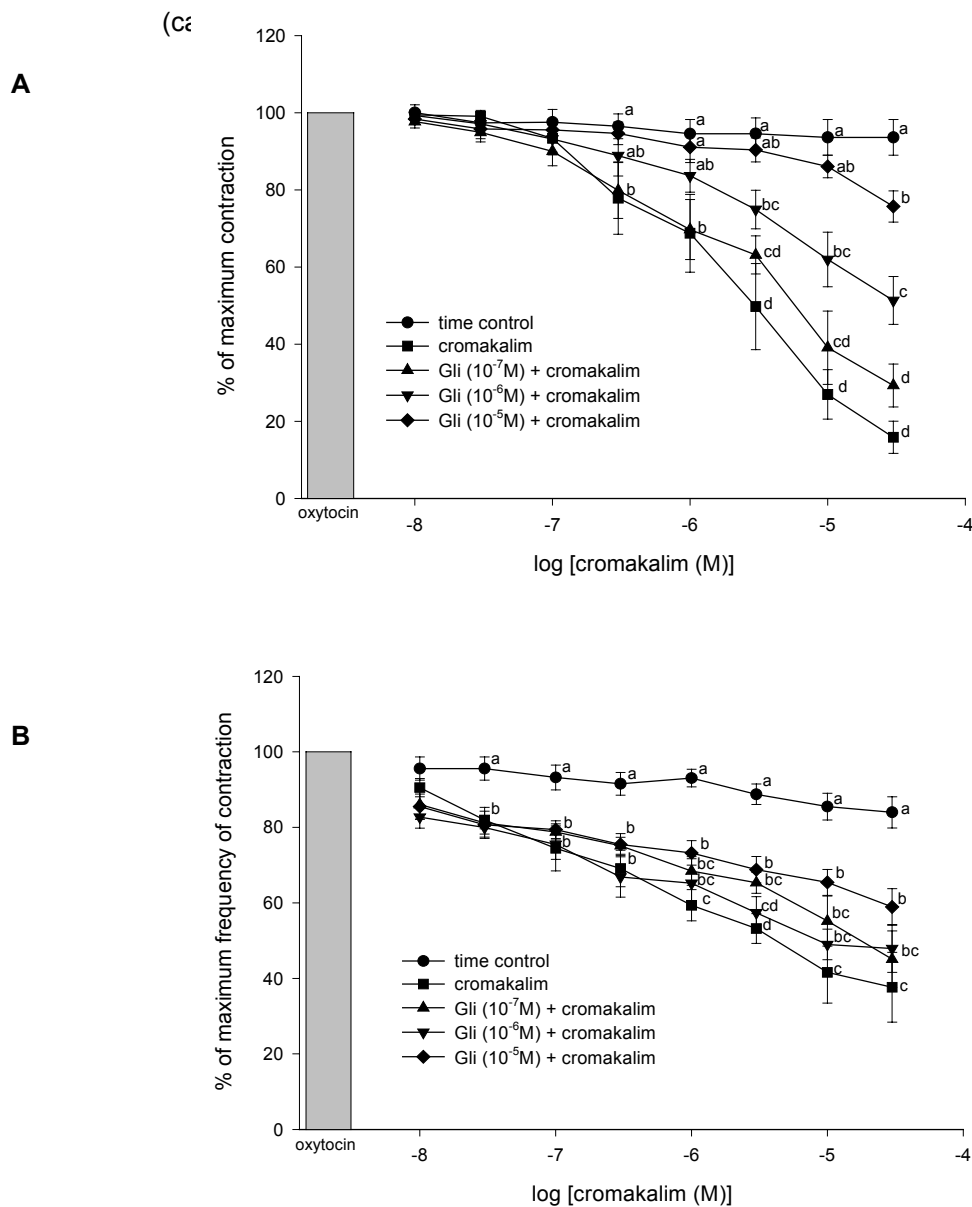


Figure 50. The concentration-response relationship of inhibitory effect of glibenclamide on relaxing effect of cromakalim on isolated rat uterus precontracted with oxytocin (1 mU/ml). (A) Effect of various concentrations of glibenclamide (10^{-7} , 10^{-6} and 10^{-5} M, 15 minutes) on the effect of cromakalim on force of contraction and (B) the effect of cromakalim on frequency of contraction. Each datum point represents the mean \pm S.E.M of ten experiments. Different characters (a, b, c

and d) mean significant difference among the comparing means which in the same treatment group (cromakalim concentration) at $p < 0.05$ (Duncan's multiple range test).

Table 17. Comparison of IC_{50} values of carpine-induced relaxation in the presence or absence of glibenclamide (10^{-7} , 10^{-6} and 10^{-5} M) on isolated rat uterus precontracted with oxytocin (1mU/ml).

Contraction	IC_{50} (Mean \pm S.E.M)			
	carpine alone	Glibenclamide-treated (M)		
		10^{-7}	10^{-6}	10^{-5}
Force	$6.24 \pm 0.52 \times 10^{-5}$	* $1.19 \pm 0.10 \times 10^{-4}$	$8.17 \pm 1.03 \times 10^{-5}$	* $1.30 \pm 0.09 \times 10^{-4}$
Frequency	$3.37 \pm 0.46 \times 10^{-5}$	$2.86 \pm 1.69 \times 10^{-5}$	$1.65 \pm 1.15 \times 10^{-5}$	$1.22 \pm 0.72 \times 10^{-5}$

(* $p < 0.05$, ** $p < 0.01$ as compared to cromakalim alone (Dunnett's test))

Table 18. Comparison of IC_{50} values of cromakalim-induced relaxation in the presence or absence of glibenclamide (10^{-7} , 10^{-6} and 10^{-5} M) in isolated rat uterus precontracted with oxytocin (1mU/ml).

Contraction	IC_{50} (Mean \pm S.E.M)			
	cromakalim alone	Glibenclamide-treated (M)		
		10^{-7}	10^{-6}	10^{-5}
Force	$2.49 \pm 0.83 \times 10^{-6}$	$5.29 \pm 2.05 \times 10^{-6}$	** $3.01 \pm 1.06 \times 10^{-5}$	ND
frequency	$7.40 \pm 2.72 \times 10^{-6}$	$3.71 \pm 1.82 \times 10^{-5}$	$0.48 \pm 2.26 \times 10^{-5}$	ND

(* $p < 0.05$, ** $p < 0.01$ as compared to cromakalim alone (Dunnett's test))

ND = not determined, maximum contraction was less than 50% of maximum response

2.5 The effect of carpaine on the cAMP-dependent relaxation of uterine smooth muscle

2.5.1 Determination of optimum concentration of Rp-cAMPS

KCl (56.3 mM) induced tonic contraction of rat uterus incubated in K⁺-free Jalon Ringer solution. This contraction was maintained during the time of the experiment (Figure 51) and for at least 3 hours. Papaverine (10^{-7} - 10^{-4} M) and forskolin (10^{-7} - 3×10^{-5} M) relaxed the tonic contraction induced by 56.3 mM KCl in a concentration-dependent manner (Figure 52 and 53). Their respective IC₅₀ values were $8.08 \pm 0.53 \times 10^{-6}$ M and $4.57 \pm 0.49 \times 10^{-6}$ M. On the other hand, the final concentration of vehicle for forskolin (DMSO) in the organ bath did not modify the KCl (56.3 mM) -induced tonic contraction (Figure 51). In the presence of the inhibitor of cAMP-dependent protein kinase, Rp-cAMPS (3×10^{-6} , 10^{-5} and 3×10^{-5} M), added 30 min prior to the addition of KCl, did not modify the amplitude of tonic contraction induced by KCl 56.3 mM. Rp-cAMPS antagonized the relaxation elicited by forskolin and papaverine (Figure 54) which significantly increased the IC₅₀ values of both drugs. However, the concentration of Rp-cAMPS that modified the IC₅₀ of forskolin (10^{-5} and 3×10^{-5} M) and papaverine (3×10^{-5} M) appeared only at the highest concentration used (3×10^{-5} M) This concentration of Rp-cAMPS was chosen for use in the next experiments. The cumulative inhibitory concentration-response relationship of forskolin and papaverine on uterine contraction induced by KCl (56.3 mM) was illustrated in Figure 54 and IC₅₀ values were presented in Table 19 and 20. The representative tracing of forskolin and papaverine in the presence of various concentrations of Rp-cAMPS were illustrated in Figure 52 and 53, respectively.

2.5.2 Modification on the relaxing effect of carpaine, forskolin and papaverine with Rp-cAMPS

To study the possible contribution of cAMP to the relaxant effect of carpaine, forskolin and papaverine, their effects were tested in the presence of the inhibitor of cAMP-

dependent protein kinase, Rp-cAMPS (3×10^{-5} M), added 30 minutes prior to the addition of KCl. The representative tracings of carpaine, forskolin and papaverine in the presence of Rp-cAMPS (3×10^{-5} M) were illustrated in Figure 55. Carpaine (10^{-6} - 3×10^{-3} M), forskolin (10^{-7} - 3×10^{-5} M) and papaverine (10^{-7} - 10^{-4} M) produced relaxing of the tonic contraction of rat uterus induced by KCl (56.3 mM) in concentration-dependent manner. Their respective IC_{50} values were $5.95 \pm 0.67 \times 10^{-5}$ M, $4.53 \pm 0.78 \times 10^{-6}$ M and $6.14 \pm 0.39 \times 10^{-6}$ M. Rp-cAMPS displaced the dose-relaxation curve to right which increased significantly the IC_{50} of carpaine ($p < 0.05$), forskolin ($p < 0.05$) and papaverine ($p < 0.05$) (Table 21). The log concentration-response relationship of carpaine, forskolin and papaverine was demonstrated in Figure 56.

2.5.3 Modification of the relaxant effect of carpaine by Rp-cAMPS

The representative tracing of carpaine in the presence of various concentrations of Rp-cAMPS was illustrated in Figure 57. In the same experiment condition, Rp-cAMPS (10^{-5} and 3×10^{-5} M) added 30 minutes prior to addition of KCl (56.3 mM), caused the rightward of the concentration-response curves of relaxation elicited by carpaine (10^{-6} - 3×10^{-3} M) (Figure 58) with a significant increase in their respective IC_{50} values ($p < 0.05$) (Table 22). However, Rp-cAMPS (3×10^{-6} M) did not significantly ($p > 0.05$) modify the relaxation elicited by carpaine.

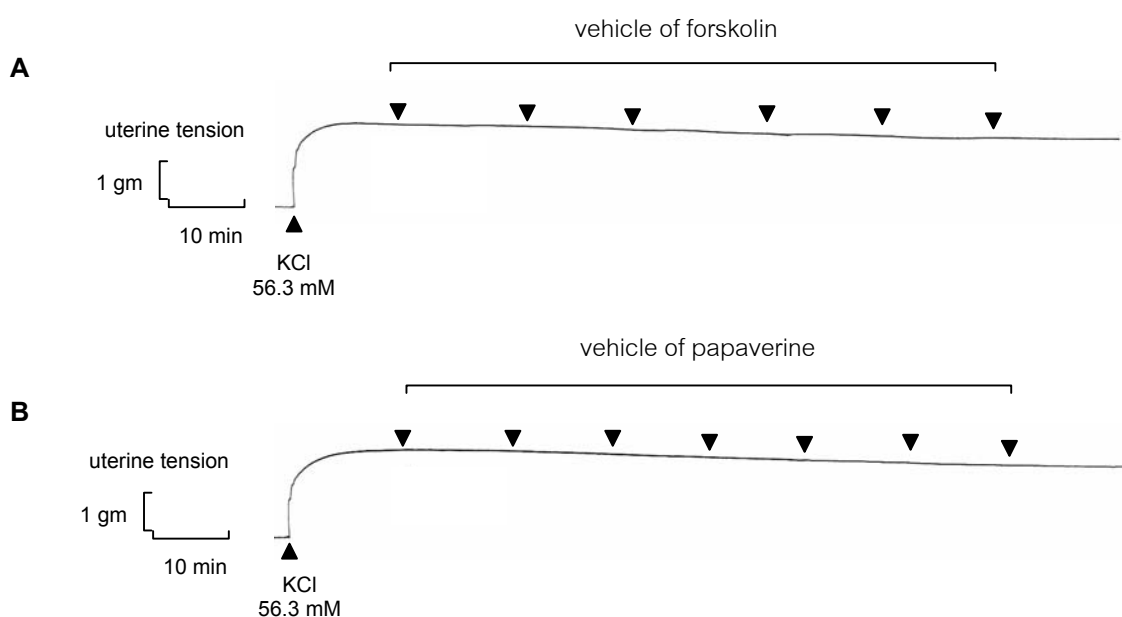


Figure 51. The representative tracings of the effect of vehicle for (A) forskolin (DMSO) and (B) papaverine (distilled water) on isolated rat uterus precontracted with 56.3 mM

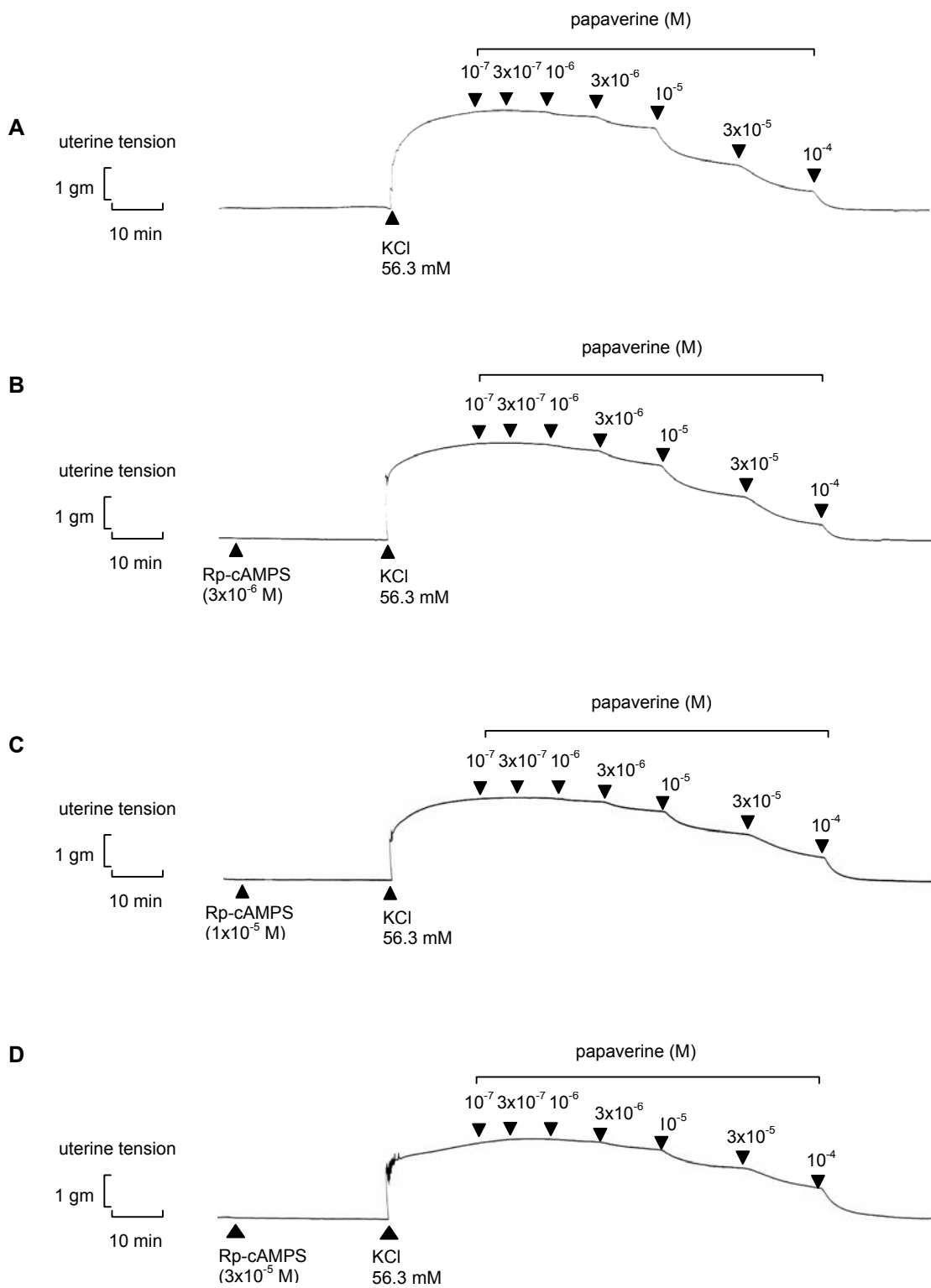


Figure 52. The representative tracings of the inhibitory effect of Rp-cAMPS (30 min) on relaxing effect of papaverine on isolated rat uterus precontracted with 56.3 mM KCl. A = papaverine alone and B, C, D = 3×10^{-6} , 10^{-5} , 3×10^{-5} Rp-cAMPS-

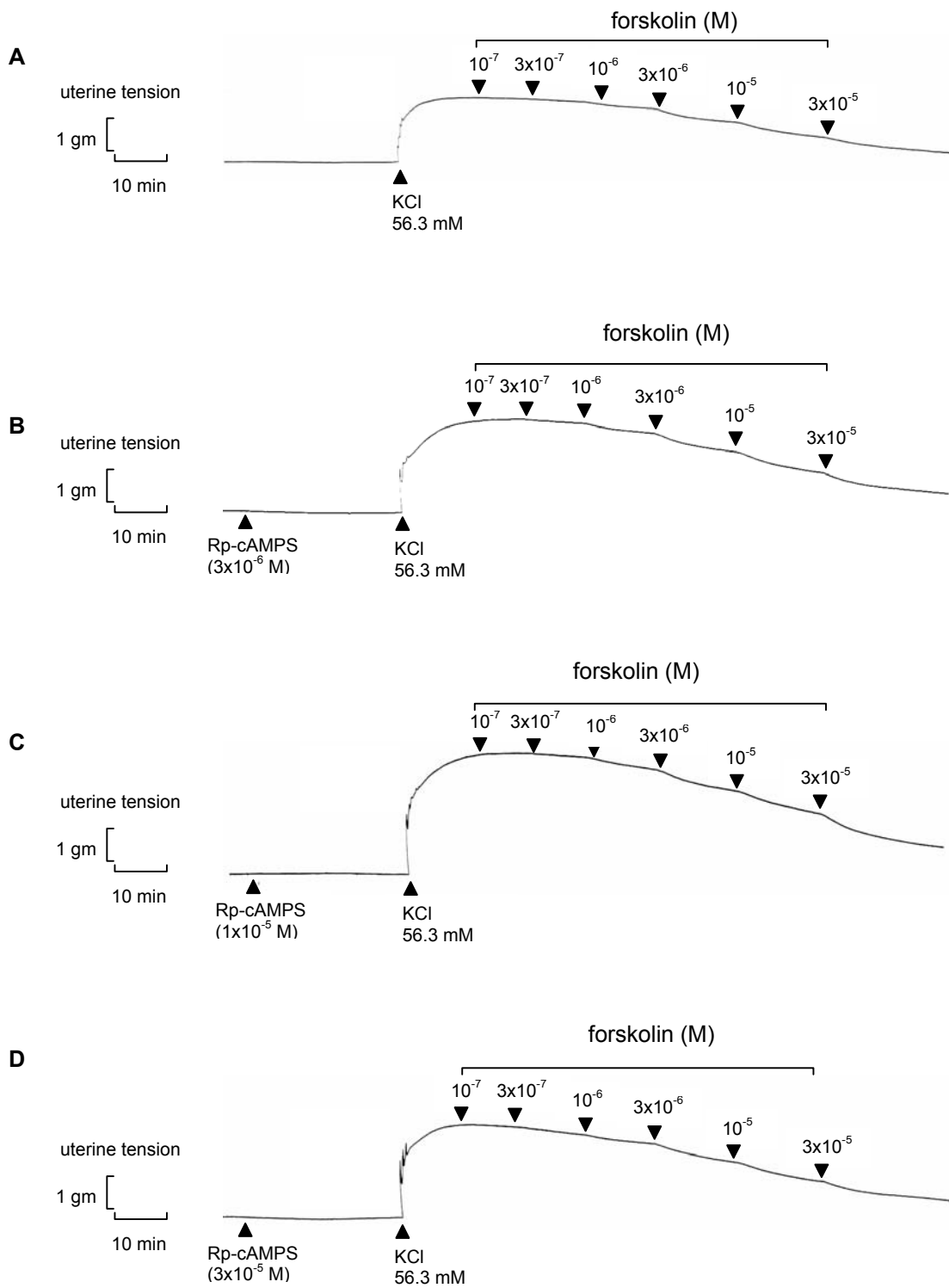
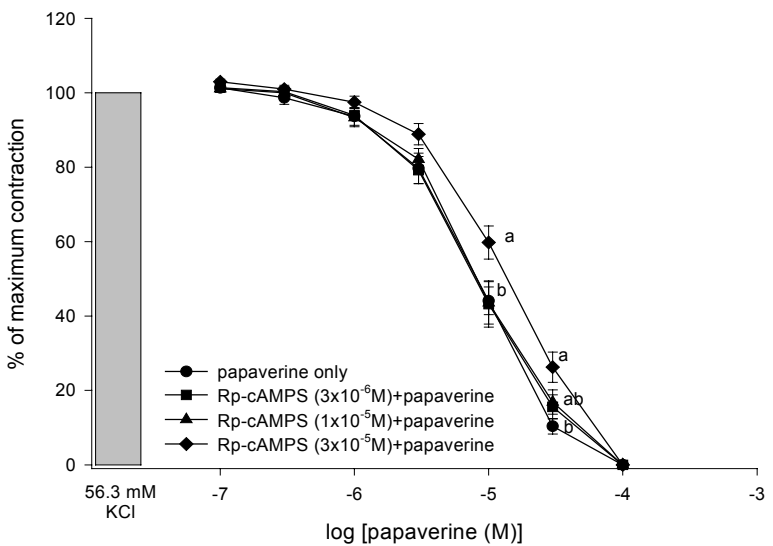
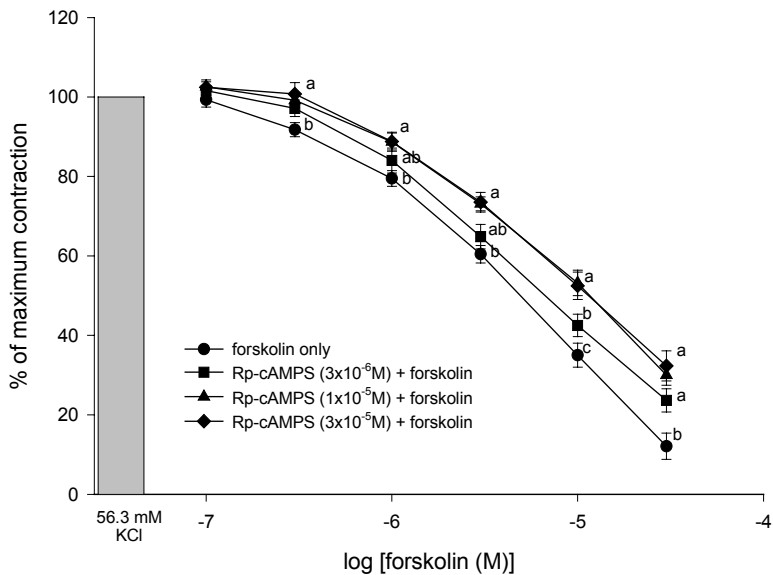


Figure 53. The representative tracings of the inhibitory effect of Rp-cAMPS (30 min) on relaxing effect of forskolin on isolated rat uterus precontracted with 56.3 mM KCl.

A =



forskolin alone and B, C, D = 3x10⁻⁶, 10⁻⁵, 3x10⁻⁵ Rp-cAMPS-pretreated respectively.

A

B

Figure 54. The concentration-response relationship of inhibitory effect of Rp-cAMPS on relaxing effect of forskolin or papaverine on isolated rat uterus precontracted with 56.3 mM KCl. Effect of various concentrations of Rp-cAMPS (3×10^{-6} , 1×10^{-5} and 3×10^{-5} M, 30 minutes) on (A) the effect of forskolin and (B) the effect of papaverine on force contraction. Each datum point represents the mean \pm S.E.M of ten experiments. Different characters (a, b and c) mean significant difference among the comparing means which in the same treatment group (forskolin or papaverine concentration) at $p < 0.05$ (Duncan's multiple range test).

Table 19. Comparison of IC₅₀ values of forskolin-induced relaxation in the presence or absence of Rp-cAMPS (3x10⁻⁶, 10⁻⁵ and 3x10⁻⁵ M) on isolated rat uterus precontracted with 56.3 mM KCl

IC ₅₀ (Mean ± S.E.M)			
forskolin alone	Rp-cAMPS-treated (M)		
	3x10 ⁻⁶	1x10 ⁻⁵	3x10 ⁻⁵
4.57 ± 0.49 x10 ⁻⁶	5.51 ± 0.78 x10 ⁻⁶	**0.92 ± 0.10 x10 ⁻⁵	**0.91 ± 0.13 x10 ⁻⁵

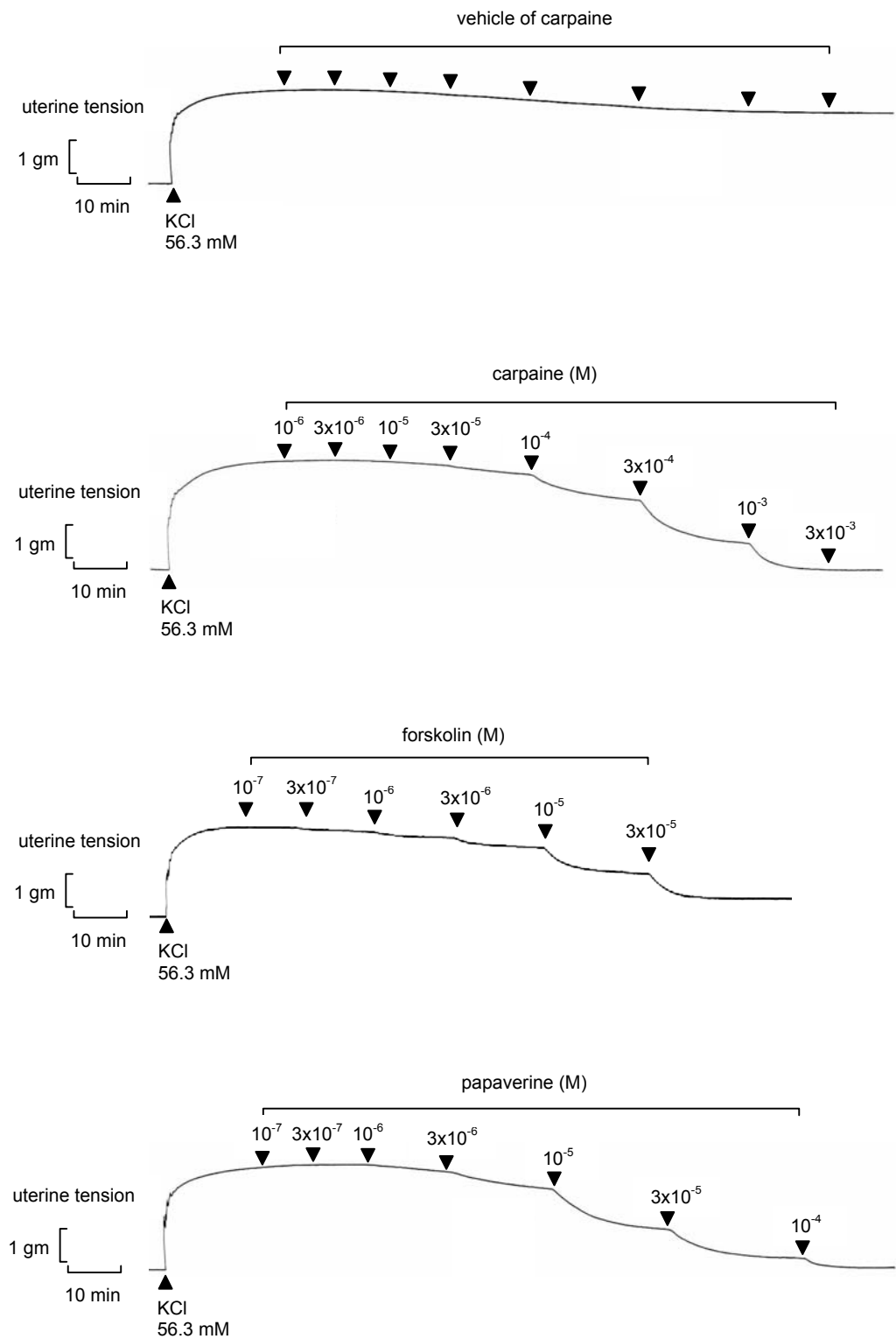
(** $p < 0.01$ as compared to forskolin alone (Dunnett's test))

Table 20. Comparison of IC₅₀ values of papaverine -induced relaxation in the presence or absence of Rp-cAMPS (3x10⁻⁶, 10⁻⁵ and 3x10⁻⁵ M) on isolated rat uterus precontracted with 56.3 mM KCl

IC ₅₀ (Mean ± S.E.)			
papaverine alone	Rp-cAMPS-treated (M)		
	3x10 ⁻⁶	1x10 ⁻⁵	3x10 ⁻⁵
8.08 ± 0.53 x10 ⁻⁶	7.94 ± 0.63 x10 ⁻⁶	8.44 ± 0.66 x10 ⁻⁶	**1.30 ± 0.09 x10 ⁻⁵

(** $p < 0.01$ as compared to papaverine alone (Dunnett's test))

A



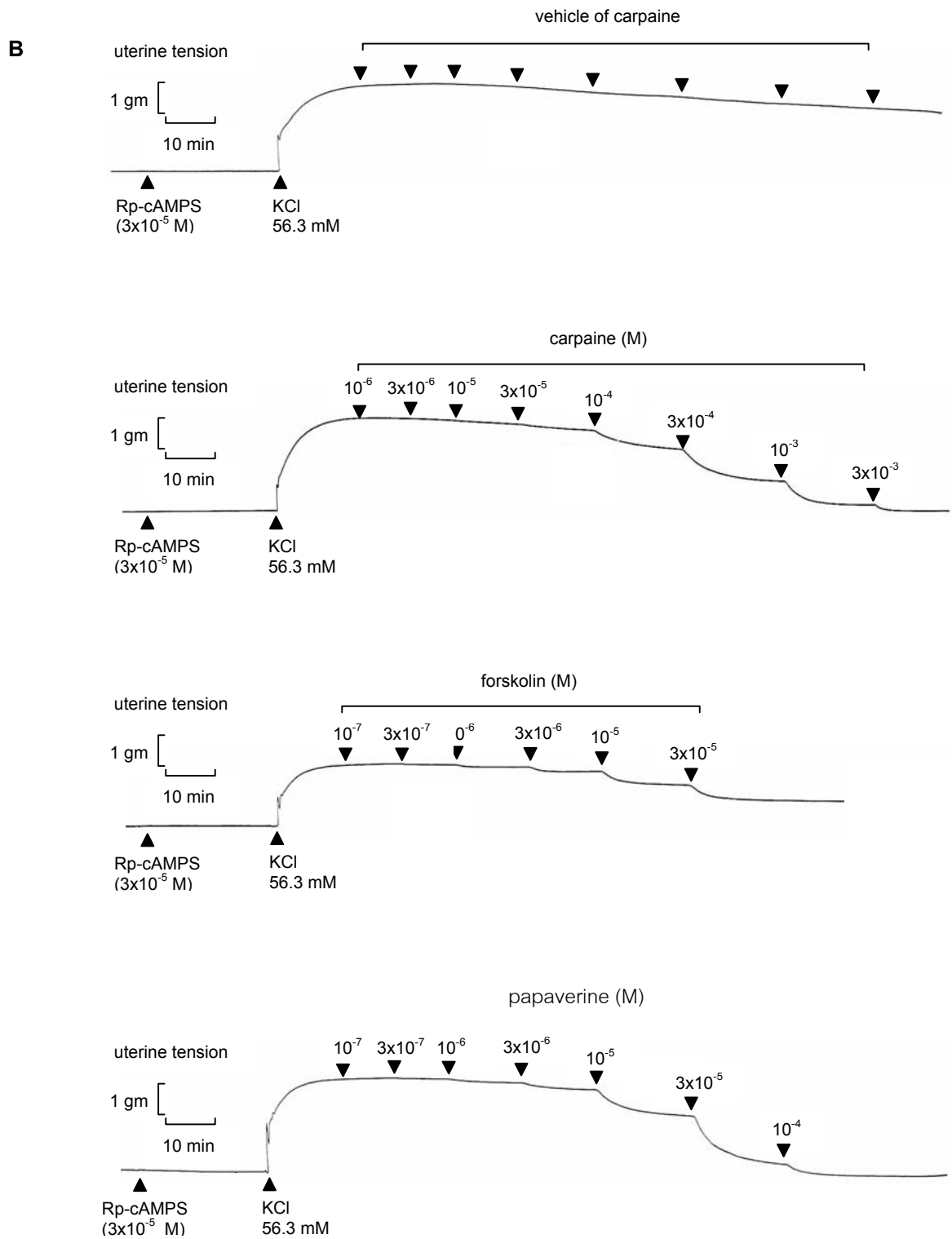


Figure 55. The representative tracings of the relaxing effect of carpaine, forskolin, papaverine and its time control (A), or pretreated with Rp-cAMPS (3×10^{-5} M) for 30 minutes and its time control (B) on isolated rat uterus precontracted with 56.3 mM KCl.

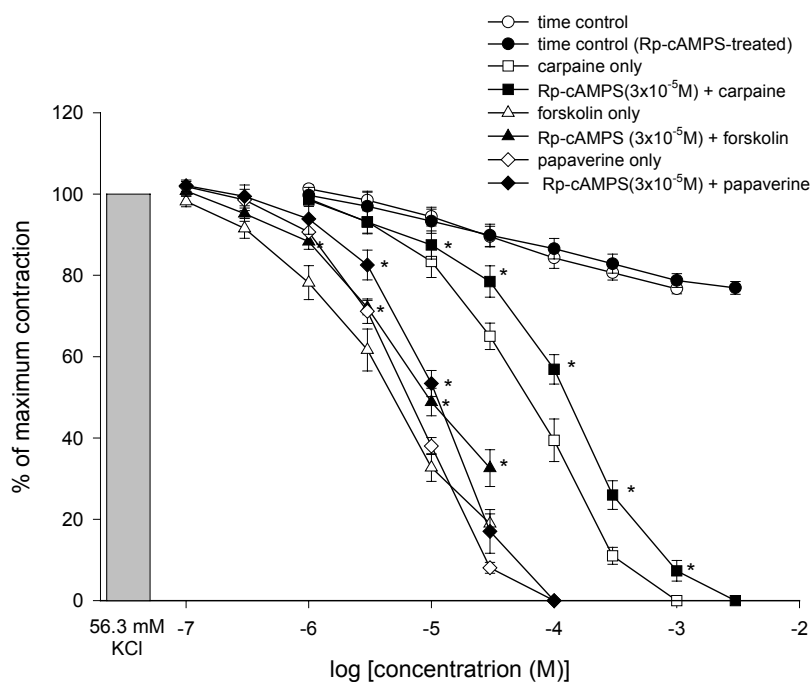


Figure 56. The concentration-response relationship of inhibitory effect of Rp-cAMPS (3×10^{-5} M, 30 minutes) on relaxing effect of forskolin, papaverine and carpaine on isolated rat uterus, the uteri were precontracted with 56.3 mM KCl. (** $p < 0.01$ as compared to each compounds alone (independent sample t -test)

Table 21. Comparison of EC_{50} values of carpaine, forskolin or papaverine-induced relaxation in the presence or absence of Rp-cAMPS (3×10^{-5} M) on isolated rat uterus precontracted with 56.3 mM KCl.

Compounds	EC_{50} (Mean \pm S.E.M)	
	EC_{50} of compounds	EC_{50} of Rp-cAMPS-treated (3×10^{-5} M)
carpaine	$5.95 \pm 0.67 \times 10^{-5}$	* $1.29 \pm 0.12 \times 10^{-4}$
forskolin	$4.53 \pm 0.78 \times 10^{-6}$	* $9.06 \pm 1.32 \times 10^{-6}$
papaverine	$6.14 \pm 0.39 \times 10^{-6}$	* $1.03 \pm 0.09 \times 10^{-5}$

(* $p < 0.05$ as compared to each compound alone)

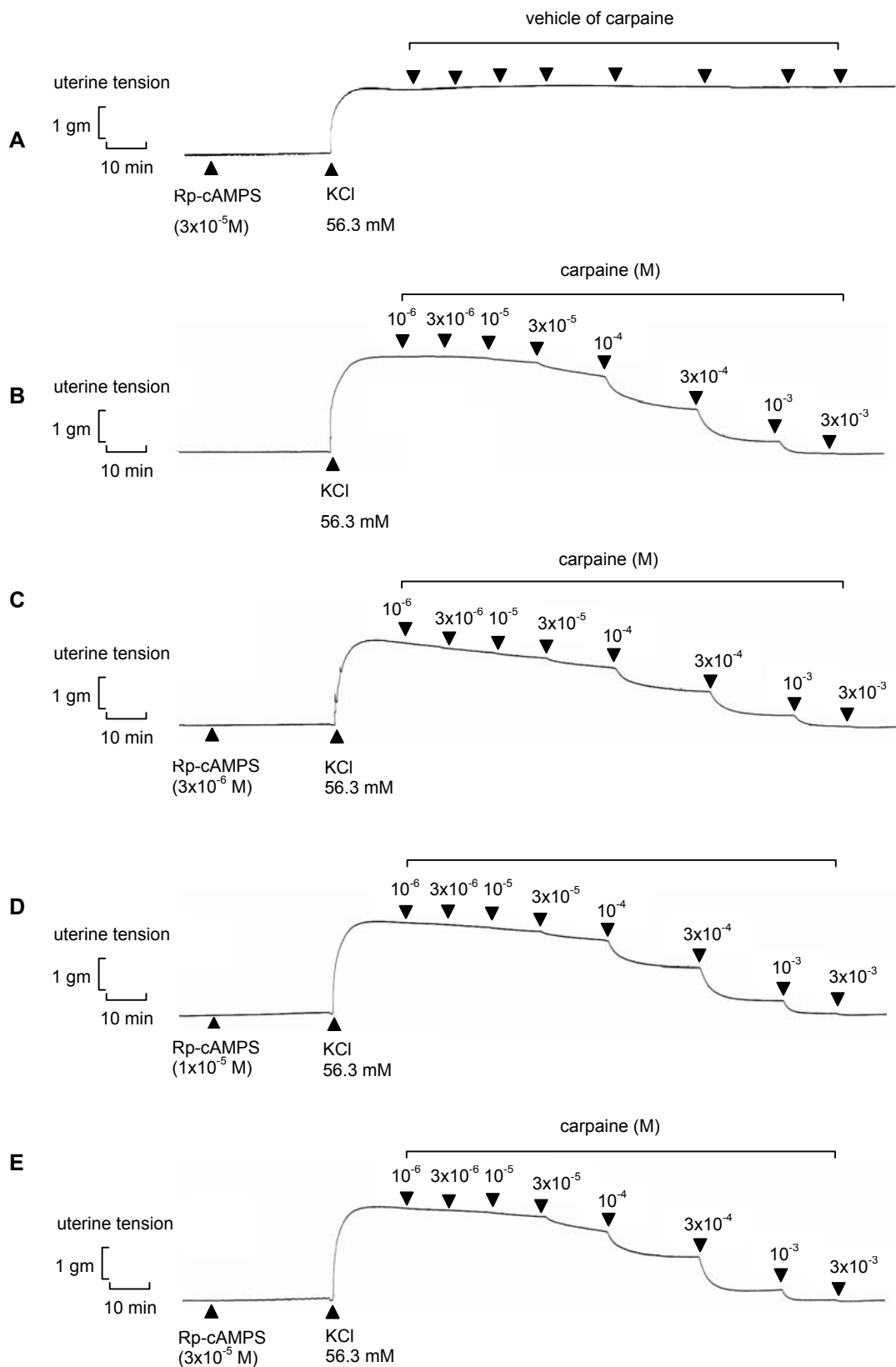


Figure 57. The representative tracings of the inhibitory effect of Rp-cAMPS (30 min) on relaxing effect of carpaine on isolated rat uterus precontracted with 56.3 mM KCl. A = its vehicle, B = carpaine alone and C, D, E = 3×10^{-6} , 10^{-5} , 3×10^{-5} Rp-cAMPS-pretreated respectively.

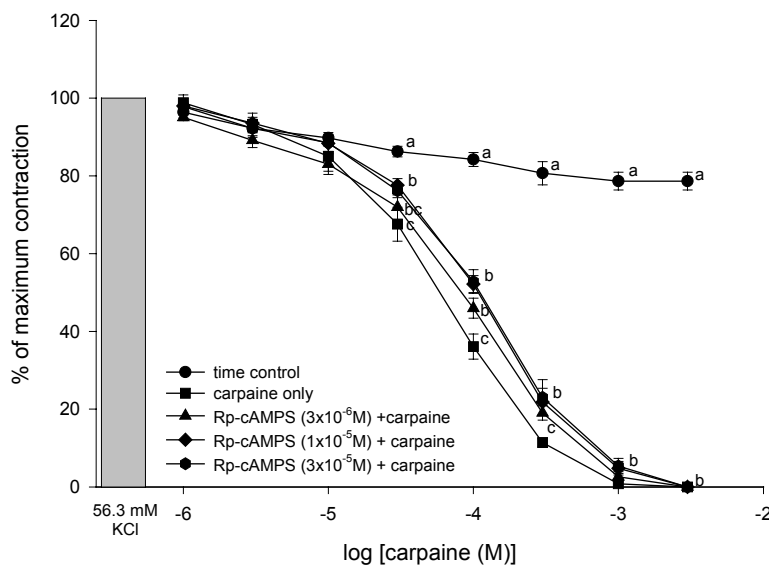


Figure 58. The concentration-response relationship of inhibitory effect of various concentrations of Rp-cAMPS (3×10^{-6} , 1×10^{-5} and 3×10^{-5} M, 30 minutes) on relaxing effect of carpine on isolated rat uterus, the uteri were precontracted with 56.3 mM KCl. Each datum point represents the mean \pm S.E.M of ten experiments. Different characters (a, b and c) mean significant difference among the comparing means which in the same treatment group (carpine concentration) at $p < 0.05$ (Duncan's multiple range test).

Table 22. Comparison of IC_{50} values of carpine -induced relaxation in the presence or absence of Rp-cAMPS (3×10^{-6} , 10^{-5} and 3×10^{-5} M) on isolated rat uterus precontracted with 56.3 mM KCl.

IC_{50} (Mean \pm S.E.M)			
carpine alone	Rp-cAMPS-treated (M)		
	3×10^{-6}	1×10^{-5}	3×10^{-5}
$5.99 \pm 0.52 \times 10^{-5}$	$9.29 \pm 0.67 \times 10^{-5}$	$*1.10 \pm 0.07 \times 10^{-4}$	$*1.12 \pm 0.09 \times 10^{-4}$

(* $p < 0.05$ as compared to carpine alone (Dunnett's test))

2.6 The effect of caripaine on the role of NO-induced relaxation on isolated rat uterus.

2.6.1 Effect of NO on uterine contraction induced by depolarizing solution

The representative tracing of uterine relaxing effect of L-arginine and SNP were demonstrated in Figure 59. Concentration-relaxation curves for NOS substrate L-arginine and NO donor SNP are shown in Figure 60 and the pharmacodynamic parameters (E_{max}) derived are shown in Table 23. L-arginine and SNP were slightly effective to induce relaxation on depolarizing solution-induced contraction. The addition of vehicle of L-arginine or sodium nitroprusside (SNP) had no effects on uterine contraction induced by depolarizing solution.

Table 23. Comparison of % E_{max} values of sodium nitroprusside and L-arginine induced relaxation on the isolated rat uterus precontracted with depolarizing solution (KCl 56.3 mM).

Compounds	% E_{max} (Mean \pm S.E.M)
sodium nitroprusside	61.66 \pm 4.18
L-arginine	70.67 \pm 4.09

2.6.2 Effect of NOS substrate on uterine contraction induced by depolarizing solution

2.6.2.1. Determination of optimal concentration of L-NOARG

The representative tracing of the blocking activity of L-NOARG on uterine relaxing effect of L-arginine at various concentrations was demonstrated in Figure 61 and the log concentration-response relationship was demonstrated in Figure 62. Cumulative increase in concentration of L-arginine (10^{-5} - 3×10^{-3} M) produced relaxing effect on uterine contraction precontracted with depolarizing solution, E_{max} value of L-arginine was 64.70 ± 3.52 M. In the presence of 10^{-4} , 3×10^{-4} and 10^{-3} M of L-NOARG, thus inhibition was antagonized, as observed by a shift of the log concentration-response curve of L-arginine to the right. E_{max} values of L-arginine in the presence of 10^{-4} , 3×10^{-4} and 10^{-3} M of L-NOARG were $76.52 \pm$

3.25, 75.83 ± 3.16 and 79.41 ± 11.39 , respectively (Table 24). The concentration of L-NOARG at 10^{-3} M was chosen to use in the next experiment.

2.6.1.2 Effect of L-NOARG on uterine relaxation induced by carpine or L-arginine

The representative tracings of carpine and L-arginine induced uterine relaxation precontracted with depolarizing solution were illustrated in Figure 63 and 64, respectively. The log concentration-response relationship of carpine and L-arginine in the presence of L-NORAG (10^{-3} M) was demonstrated in figure 65. IC_{50} values were presented in Table 25. 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 carpine or L-arginine had no effects on uterine contraction induced by depolarizing solution. The addition of cumulative concentration of L-arginine inhibited the uterine contraction in a concentration-dependent manner with E_{max} of 68.77 ± 2.14 %. In the presence of 10^{-3} M L-NOARG, the relaxing effect of L-arginine was significantly inhibited ($p < 0.05$) as observed by the shift of log concentration-response curve of L-arginine to the right with an increase in E_{max} of 78.33 ± 1.95 %. On the other hand, the addition of cumulative concentration of carpine completely inhibited this contraction with IC_{50} value of $4.99 \pm 0.35 \times 10^{-5}$ M and E_{max} of 100 %. In the presence of 10^{-3} M L-NOARG, carpine also inhibited this contraction with IC_{50} value of $6.84 \pm 0.53 \times 10^{-5}$ M and E_{max} of 100 % which was not significantly different from that obtained in the absence of L-NOARG ($P > 0.05$).

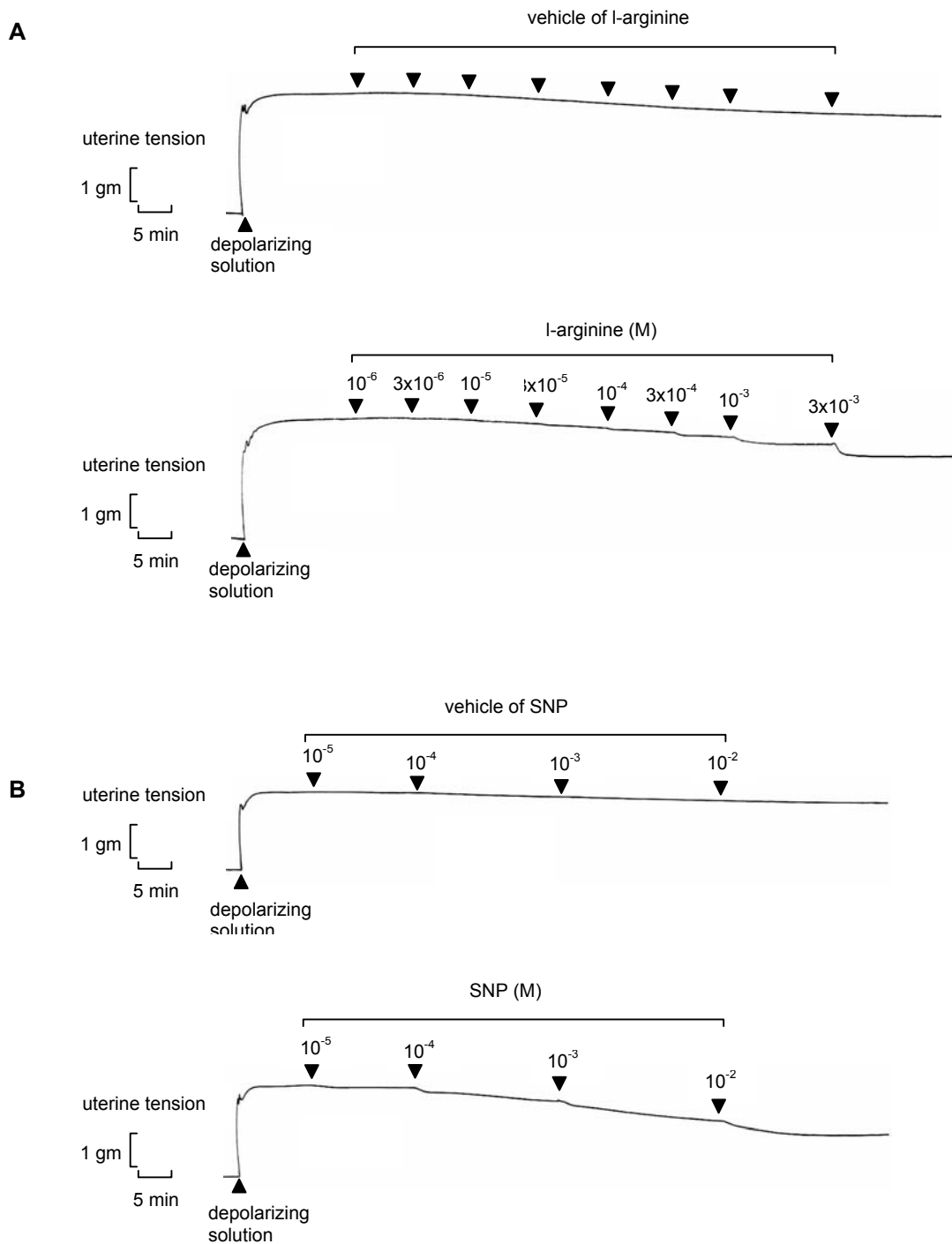


Figure 59. The representative tracings of the relaxing effect of L-arginine and its time control (A) and sodium nitroprusside (SNP) and its time control (B) on isolated rat uterus precontracted with depolarizing solution (KCl 56.3 mM)

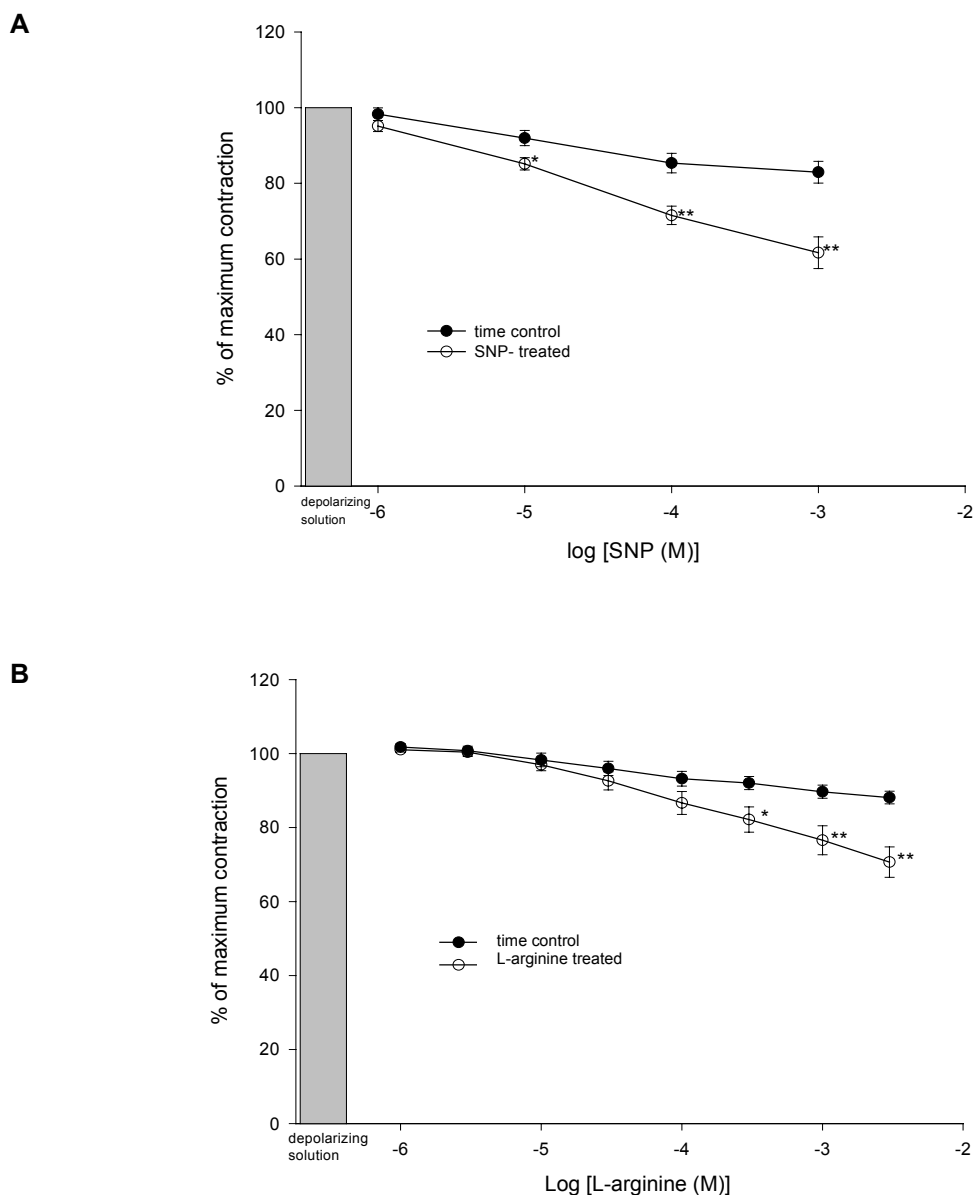
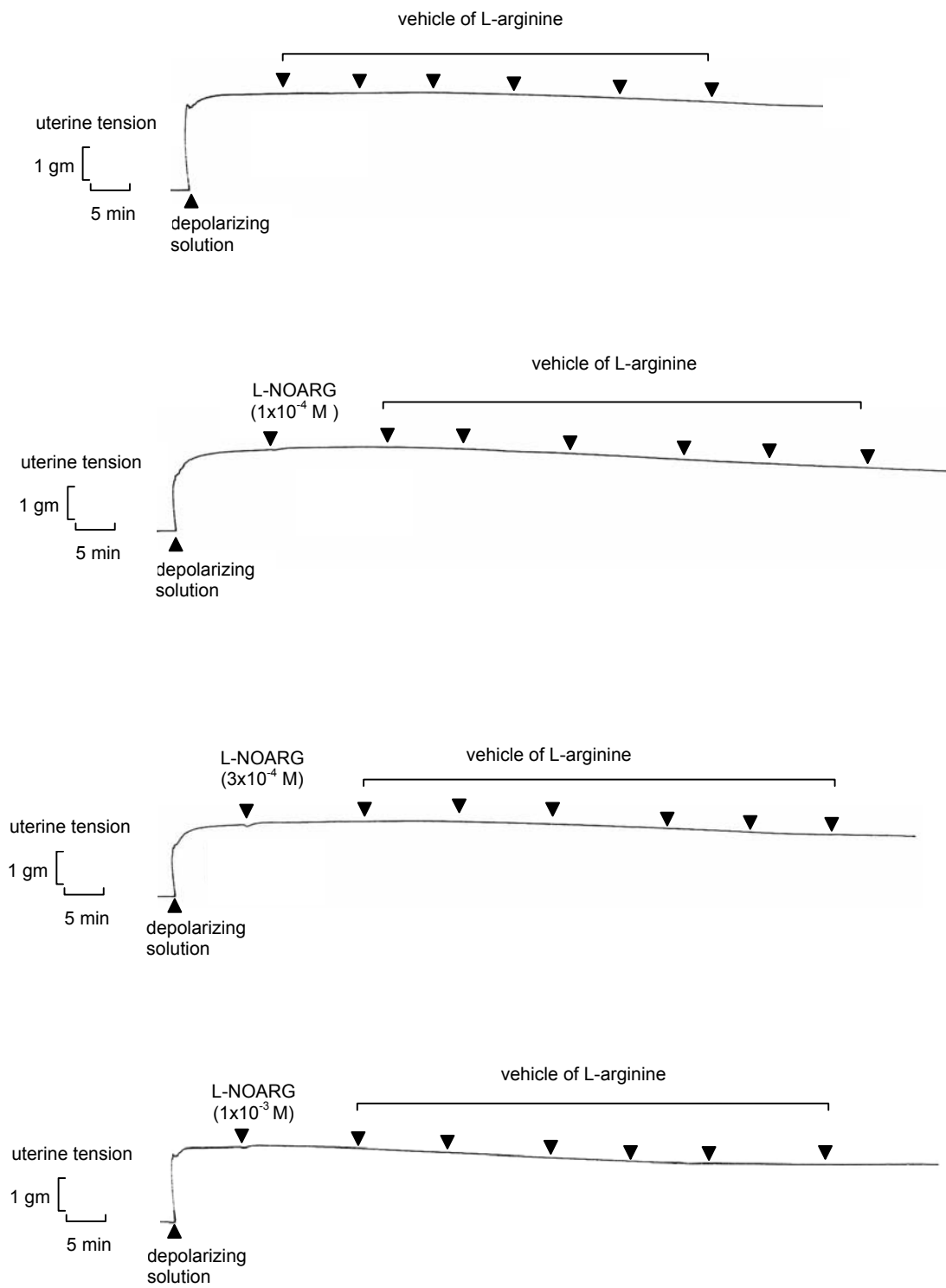


Figure 60. The concentration-response relationship of (A) sodium nitroprusside and (B) L-arginine-induced uterine relaxation on isolated rat uterus precontracted with depolarizing solution. Each value is expressed as a percentage of the initial maximal contraction in response to depolarizing solution. Each datum point represents the mean \pm S.E.M of ten experiments. (* $p < 0.05$ as compared to L-arginine or SNP alone (independent sample t -test)).

A



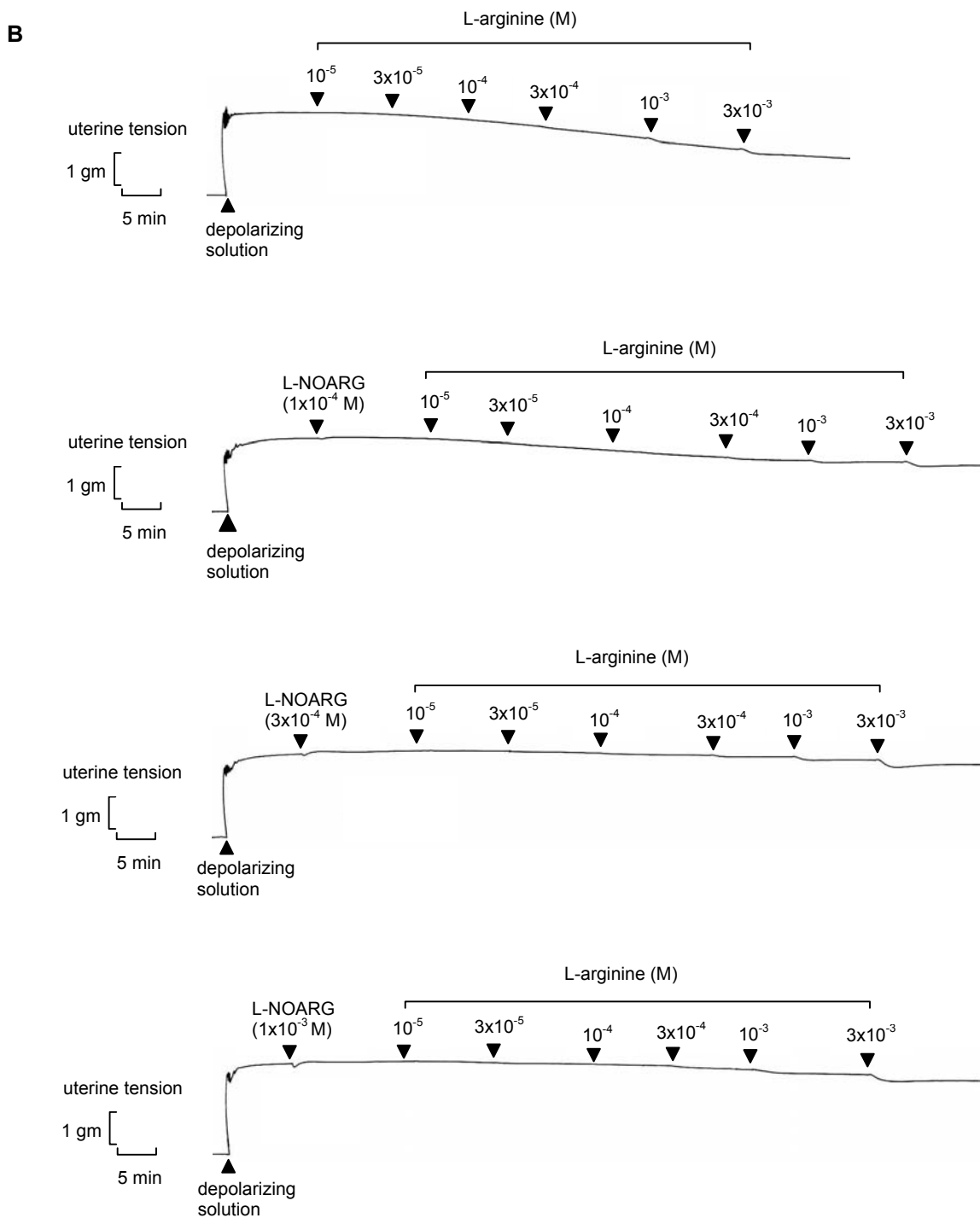


Figure 61. The representative tracing of the inhibitory effect of L-NOARG on relaxing effect of L-arginine on isolated rat uterus precontracted with the depolarizing solution (KCl 56.3 mM). A = time control of each experiment, B = L-arginine alone or in the presence of 10^{-4} , 3×10^{-4} , 10^{-3} M L-NOARG respectively.

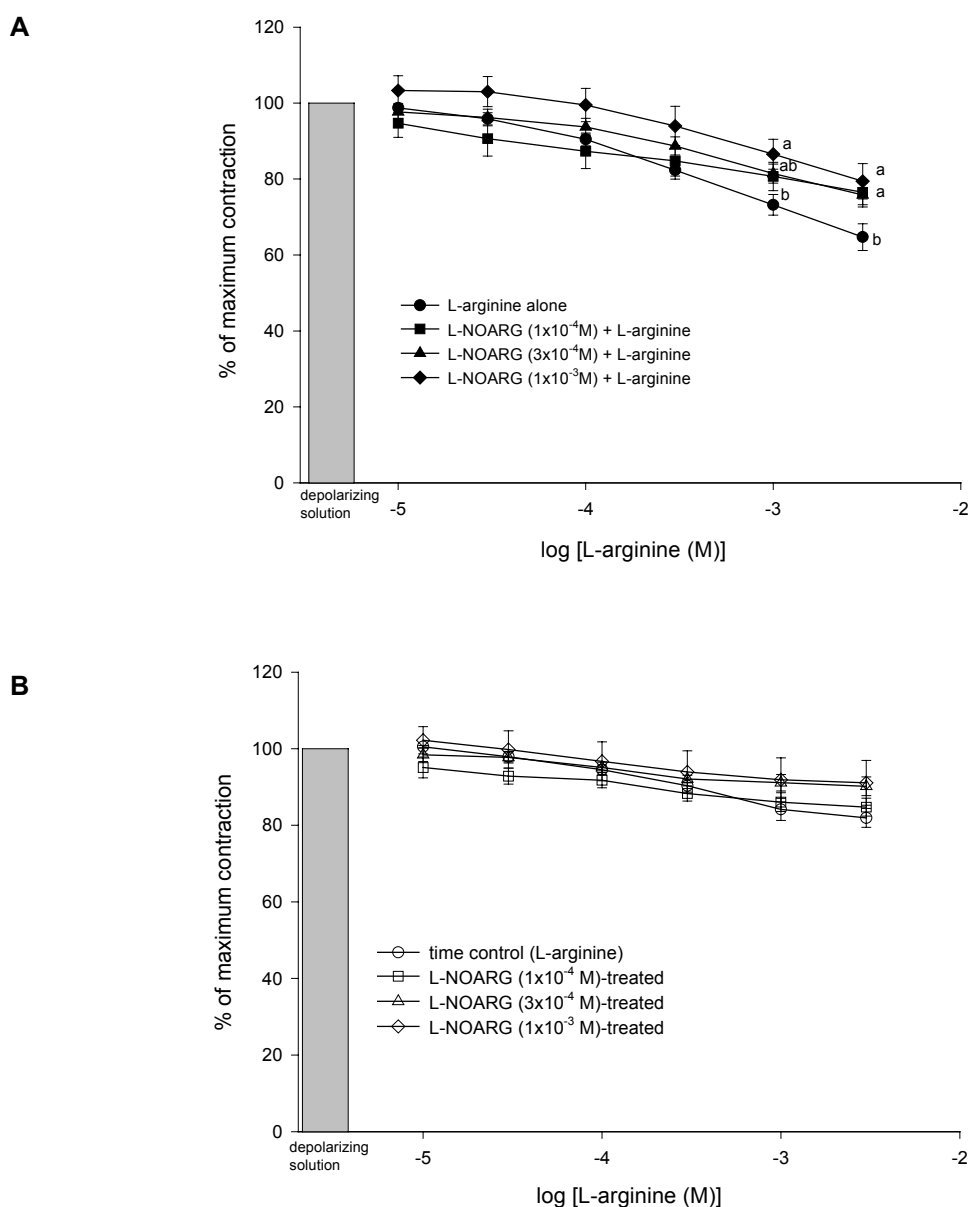


Figure 62. The concentration-response relationship of inhibitory effect of L-NOARG on relaxing effect of L-arginine on isolated rat uterus, the uteri were precontracted with depolarizing solution. (A) Effect of various concentrations of L-NOARG (10^{-4} , 3×10^{-4} and 10^{-3} M) on the force contraction of L-arginine and (B) its time control. Each datum point represents the mean \pm S.E.M of ten experiments. Different characters (a and b) mean significant difference among the comparing means which in the same treatment group (L-arginine concentration) at $p < 0.05$ (Duncan's multiple range test).

Table 24. Comparison of % E_{max} values of L-arginine-induced relaxation in the presence or absence of L-NOARG (10⁻⁴, 3x10⁻⁴ and 10⁻³ M) on isolated rat uterus precontracted with depolarizing solution (KCl 56.3 mM).

% E _{max} (Mean ± S.E.M)			
L-arginine alone	L-NOARG -treated (M)		
	10 ⁻⁴	3x10 ⁻⁴	10 ⁻³
64.70 ± 3.52	*76.52 ± 3.25	*75.83 ± 3.16	**79.41 ± 11.39

(* $p < 0.05$, ** $p < 0.01$ as compared to L-arginine alone (Dunnett's test))

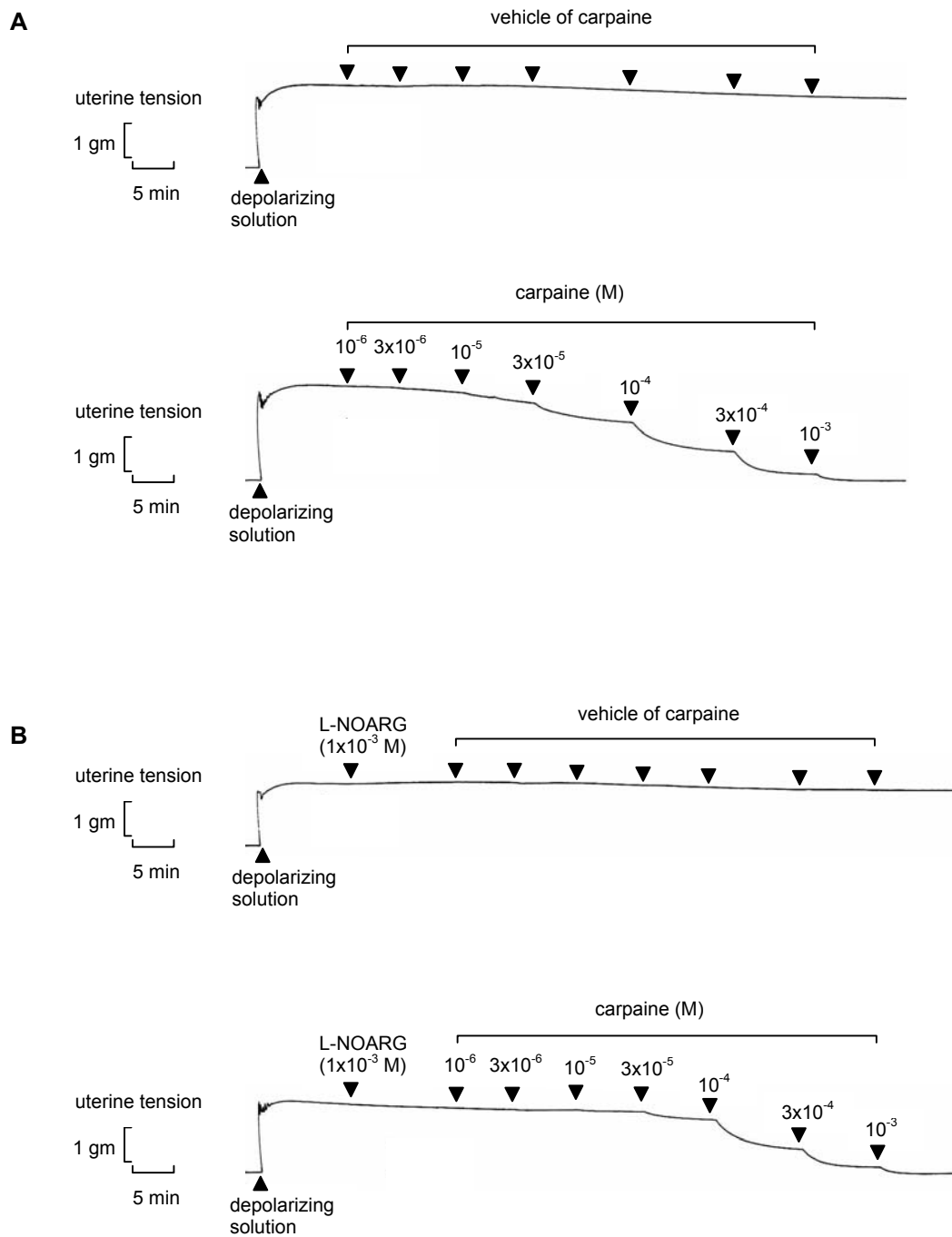


Figure 63. The representative tracings of the relaxing effect of carpaine and its time control (A), or pretreated with L-NOARG (10^{-3} M) for 15 minutes and its time control (B) on isolated rat uterus precontracted with depolarizing solution (KCl 56.3 mM).

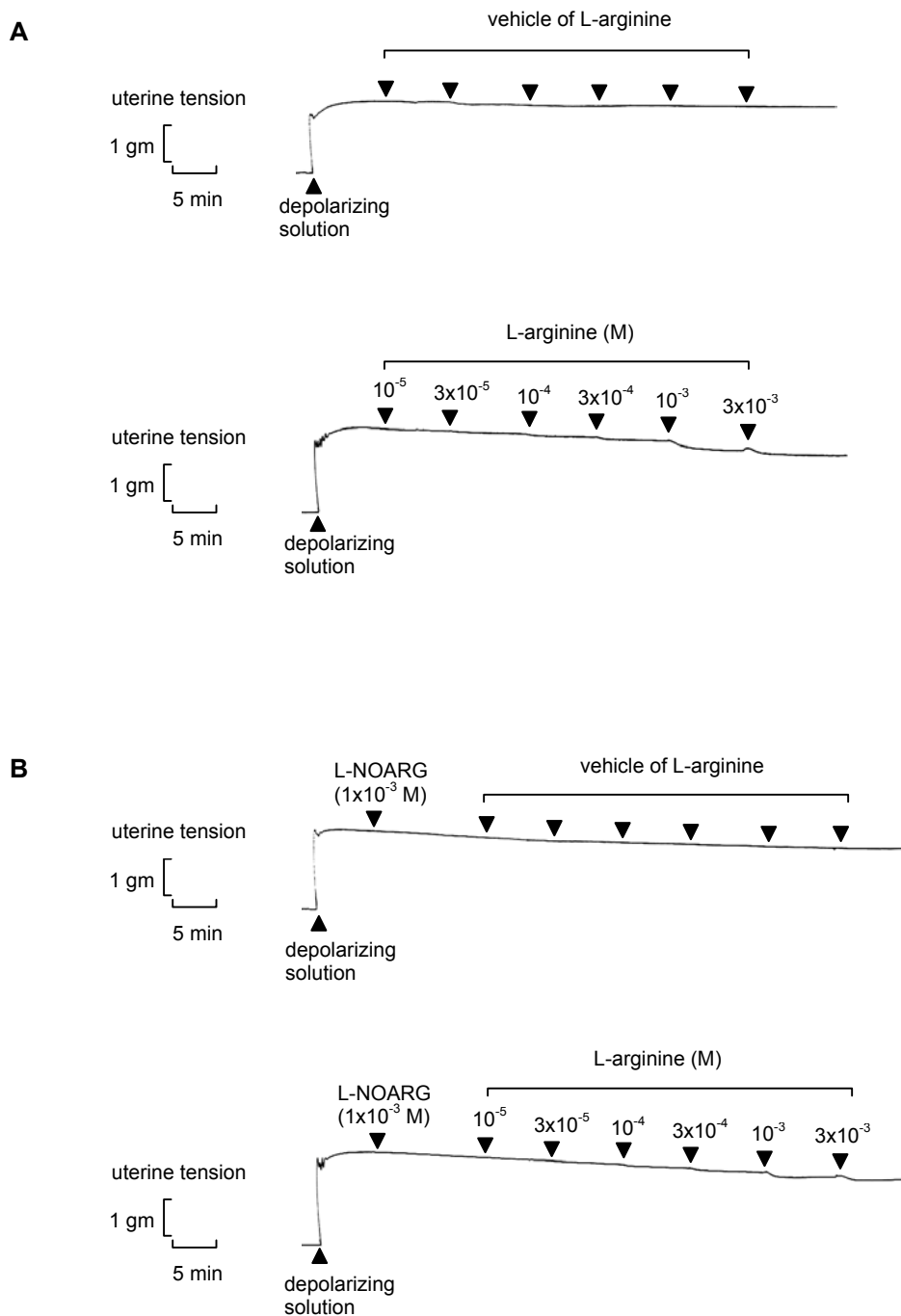


Figure 64. The representative tracings of the relaxing effect of L-arginine and its time control (A), or pretreated with L-NOARG (10^{-3} M) for 15 minutes and its time control (B) on isolated rat uterus precontracted with depolarizing solution (KCl 56.3 mM).

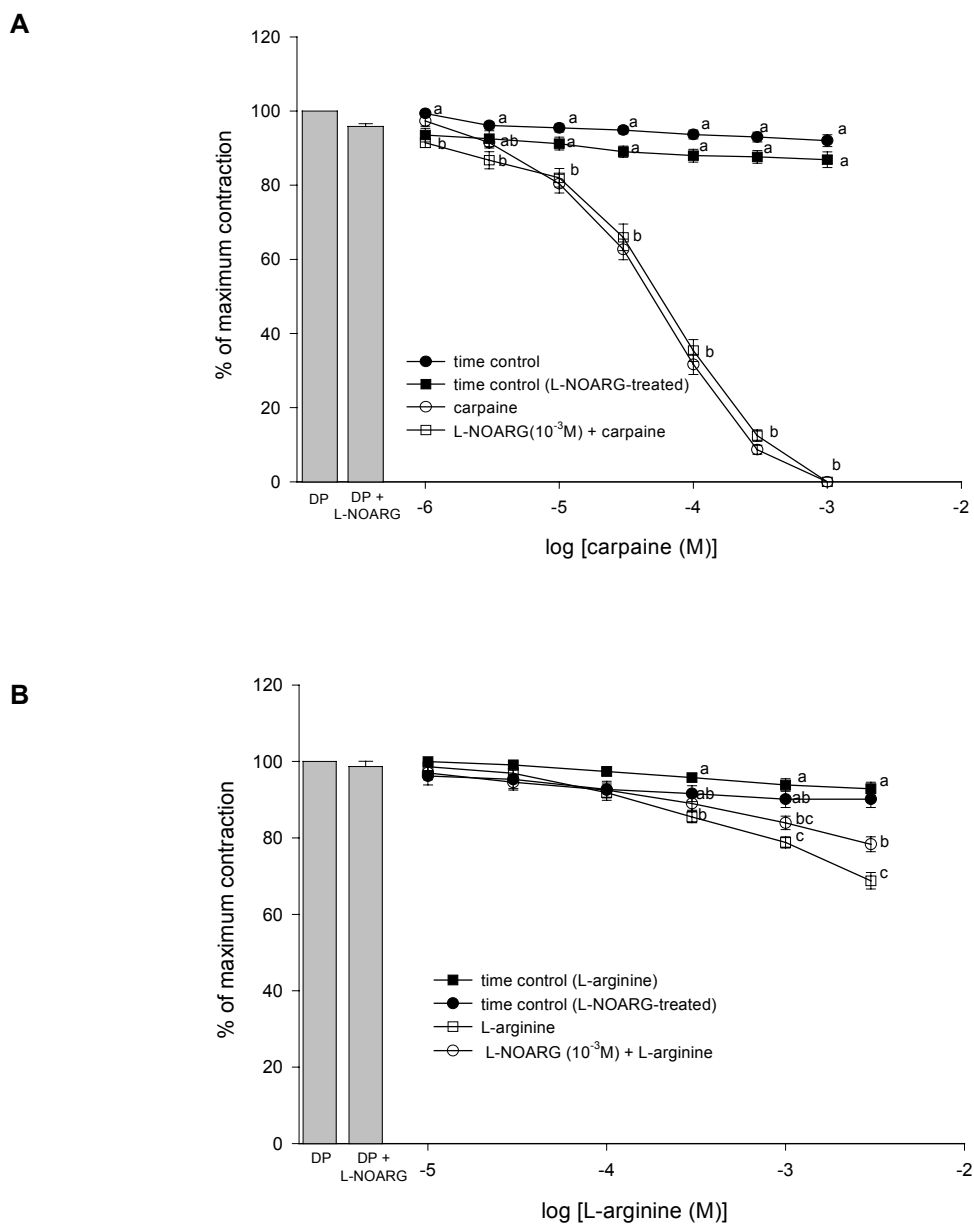


Figure 65. The concentration-response relationship of inhibitory effect of L-NOARG 10^{-3} M, 15 minutes) on relaxing effect of L-arginine (A) and carpaine (B) on isolated rat uterus precontracted with depolarizing solution (KCl 56.3 mM). Each datum point represents the mean \pm S.E.M. of ten experiments. Different characters (a, b and cc) mean significant difference among the comparing means which in the same treatment group (carpaine or L-arginine concentration) at $p < 0.05$ (Duncan's multiple range test).

Table 25. Comparison of % E_{max} values of carpaine or L-arginine-induced relaxation in the presence or absence of L-NOARG (10^{-3} M) on isolated rat uterus precontracted with 56.3 mM KCl.

Treatment	% E_{max} (Mean \pm S.E.M)	EC_{50} (M)(Mean \pm S.E.M)
carpaine alone	0	$4.99 \pm 0.35 \times 10^{-5}$
carpaine L-NOARG-pretreated	0	$6.84 \pm 0.53 \times 10^{-5}$
L-arginine alone	68.77 ± 2.14	-
L-arginine L-NOARG-pretreated	**78.33 \pm 1.95	-

(** $p < 0.01$ as compared to carpaine or L-arginine alone (Dunnett's test))

2.6.3 Effect of NO-donor on uterine contraction induced by depolarizing solution

2.6.3.1 Determination of optimal concentration of methylene blue (MB)

Cumulative addition of SNP (10^{-5} - 3×10^{-3} M) produced relaxing effect on uterine contraction precontracted with depolarizing solution. E_{max} values of SNP was 66.35 ± 2.94 %. In the presence of 10^{-7} , 10^{-6} and 10^{-5} M of MB, the inhibition was antagonized, as observed by a shift of the log concentration response curve of SNP to right. The vehicle of MB was without effect. The E_{max} values for SNP for amplitude of contraction in the presence of MB (10^{-7} , 10^{-6} and 10^{-5} M) were shown in Table 26. MB antagonized the relaxation elicited by SNP (Figure 66) with an increase in the E_{max} values. However, the concentration of MB that significantly modified the E_{max} of SNP was 10^{-5} M ($p < 0.05$). The log concentration-response relationship was demonstrated in Figure 66 and the representative tracing of the blocking activity of MB on uterine relaxing effect of SNP at various concentrations was demonstrated in Figure 67. The concentration of MB at 10^{-5} M was then chosen to use in the next experiment.

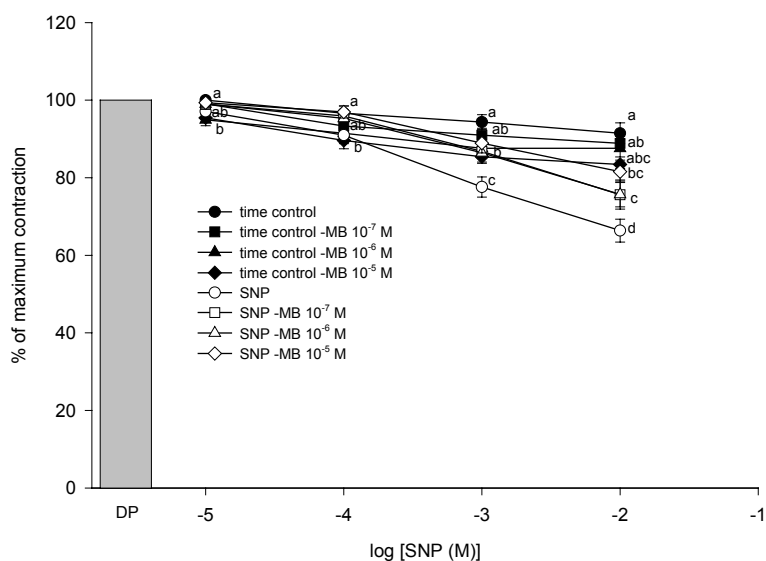


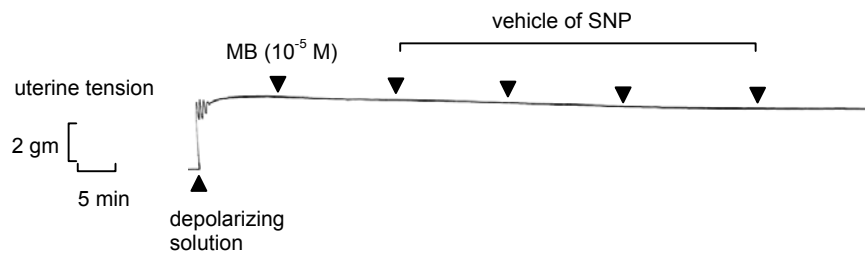
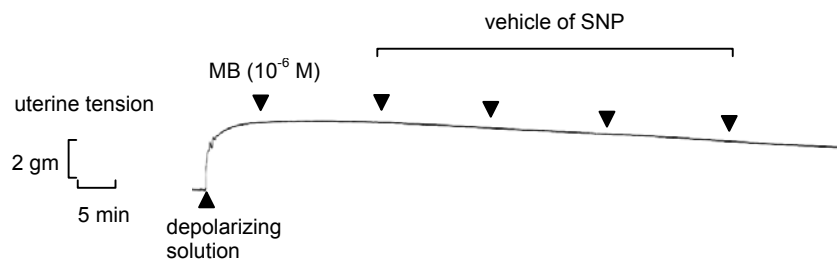
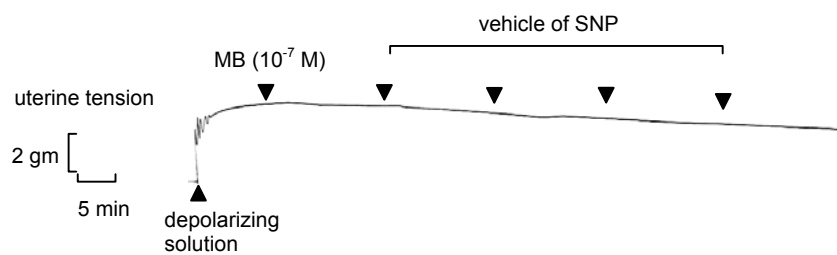
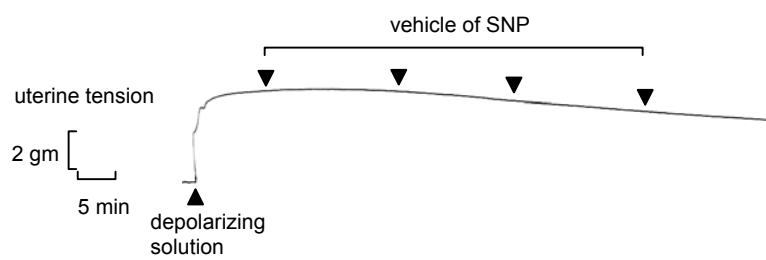
Figure 66. The concentration-response relationship of inhibitory effect of methylene blue (MB) on relaxing effect of sodium nitroprusside (SNP) on isolated rat uterus precontracted with depolarizing solution. Effect of various concentrations of MB (10^{-7} , 10^{-6} and 10^{-5} M, 15 minutes) on force contraction of SNP. Each datum point represents the mean \pm S.E.M of ten experiments. Different characters (a, b, c and d) mean significant difference among the comparing means which in the same treatment group (SNP concentration) at $p < 0.05$ (Duncan's multiple range test).

Table 26. Comparison of % E_{max} values of sodium nitroprusside-induced relaxation in the presence or absence of methylene blue (10^{-7} , 10^{-6} and 10^{-5} M) on isolated rat uterus precontracted with depolarizing solution (KC I56.3 mM).

% E_{max} (Mean \pm S.E.M)			
SNP alone	MB-treated (M)		
	10^{-7}	10^{-6}	10^{-5}
66.35 \pm 2.94	75.63 \pm 3.14	75.68 \pm 3.75	*81.56 \pm 2.43

(* $p < 0.05$, as compared to SNP alone (Dunnett's test))

A



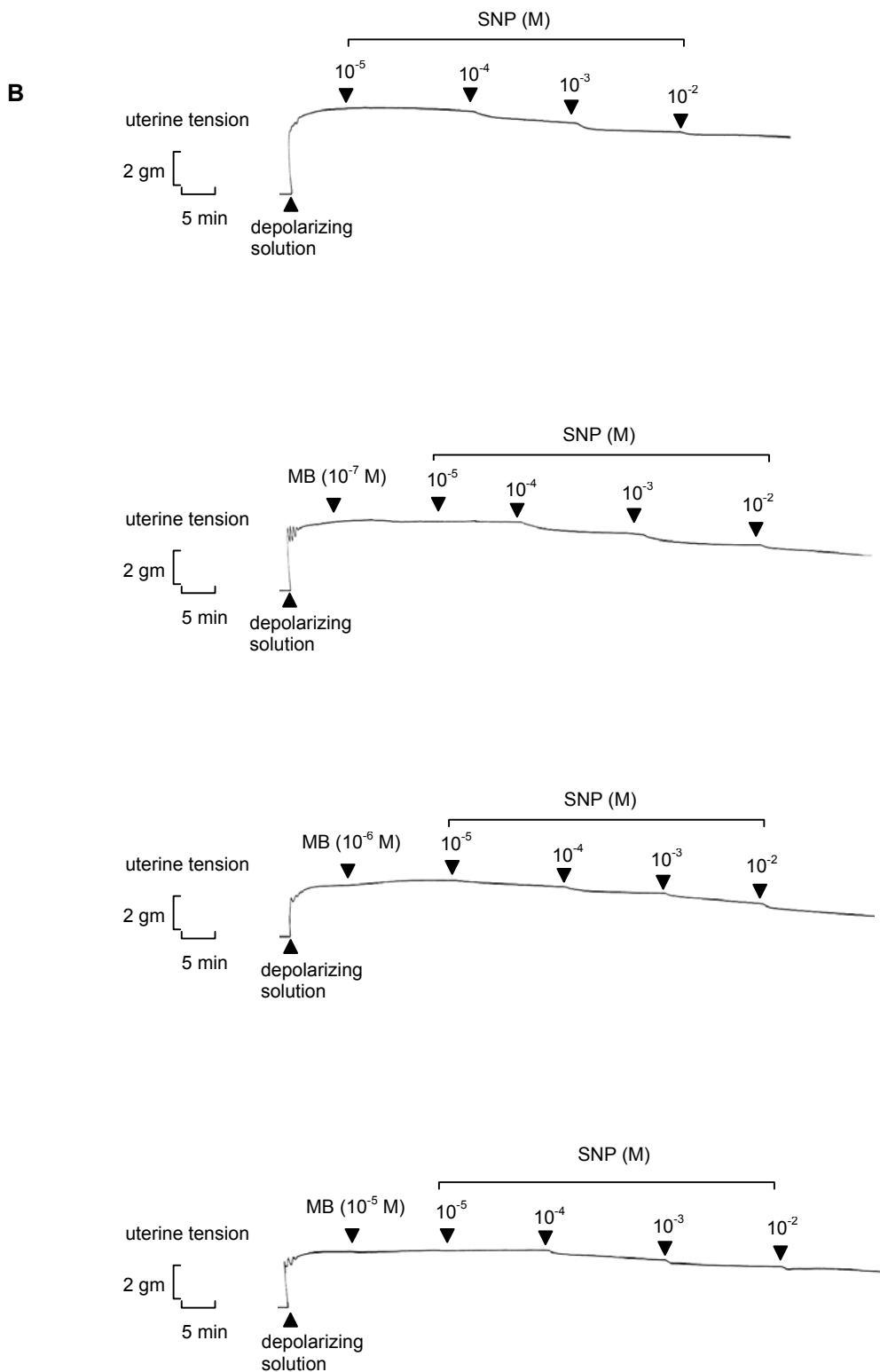


Figure 67. The representative tracing of the inhibitory effect of methylene blue on relaxing effect of sodium nitroprusside on isolated rat uterus precontracted with the depolarizing solution (KCl 56.3 mM). A = time control of each experiment, B = SNP alone or in the presence of 10^{-7} , 10^{-6} , 10^{-5} M MB respectively.

1.2 Effect of MB on uterine relaxation induced by carpaine and SNP

The representative tracing of carpaine and SNP induced uterine relaxation precontracted with depolarizing solution were illustrated in Figure 68 and 69, respectively. The addition of vehicle of carpaine or SNP had no effects on uterine contraction induced by depolarizing solution. The addition of cumulative concentration of carpaine inhibited this contraction with IC_{50} value and E_{max} of $8.55 \pm 0.55 \times 10^{-5}$ M and 100 % respectively. In the presence of 10^{-5} M of MB, carpaine also inhibited this contraction which was not significantly different from that obtained in the absence of MB ($p > 0.05$). The mean IC_{50} values and E_{max} for carpaine inhibition of amplitude contraction were $7.21 \pm 0.55 \times 10^{-5}$ M and 100 % (Table 27.) respectively. The addition of cumulative concentration of SNP inhibited the uterine contraction in a concentration-dependent manner with 59.79 ± 2.00 % E_{max} . In the presence 10^{-5} M of MB, the relaxing effect of SNP was significantly inhibited ($p < 0.05$). SNP inhibited this contraction in the presence of 10^{-5} M of MB with the mean E_{max} values of 76.65 ± 2.18 % (Table 27) which was significantly different from that obtained in the absence of MB ($p < 0.05$). The log concentration-response relationship of carpaine and SNP in the presence of MB (10^{-5} M) was demonstrated in Figure 70.

Table 27. Comparison of IC_{50} values of carpaine or sodium nitroprusside-induced relaxation in the presence or absence of MB (10^{-5} M) on isolated rat uterus precontracted with 56.3 mM KCl.

Treatment	% E_{max} (Mean \pm S.E.M)	EC_{50} (M)(Mean \pm S.E.M)
carpaine alone	0	$8.55 \pm 0.55 \times 10^{-5}$
carpaine- MB-pretreated	0	$7.21 \pm 0.55 \times 10^{-5}$
SNP alone	59.79 ± 2.00	-
SNP- MB-pretreated	** 76.65 ± 2.18	-

(** $p < 0.01$ as compared to carpaine or SNP alone (Dunnett's test))

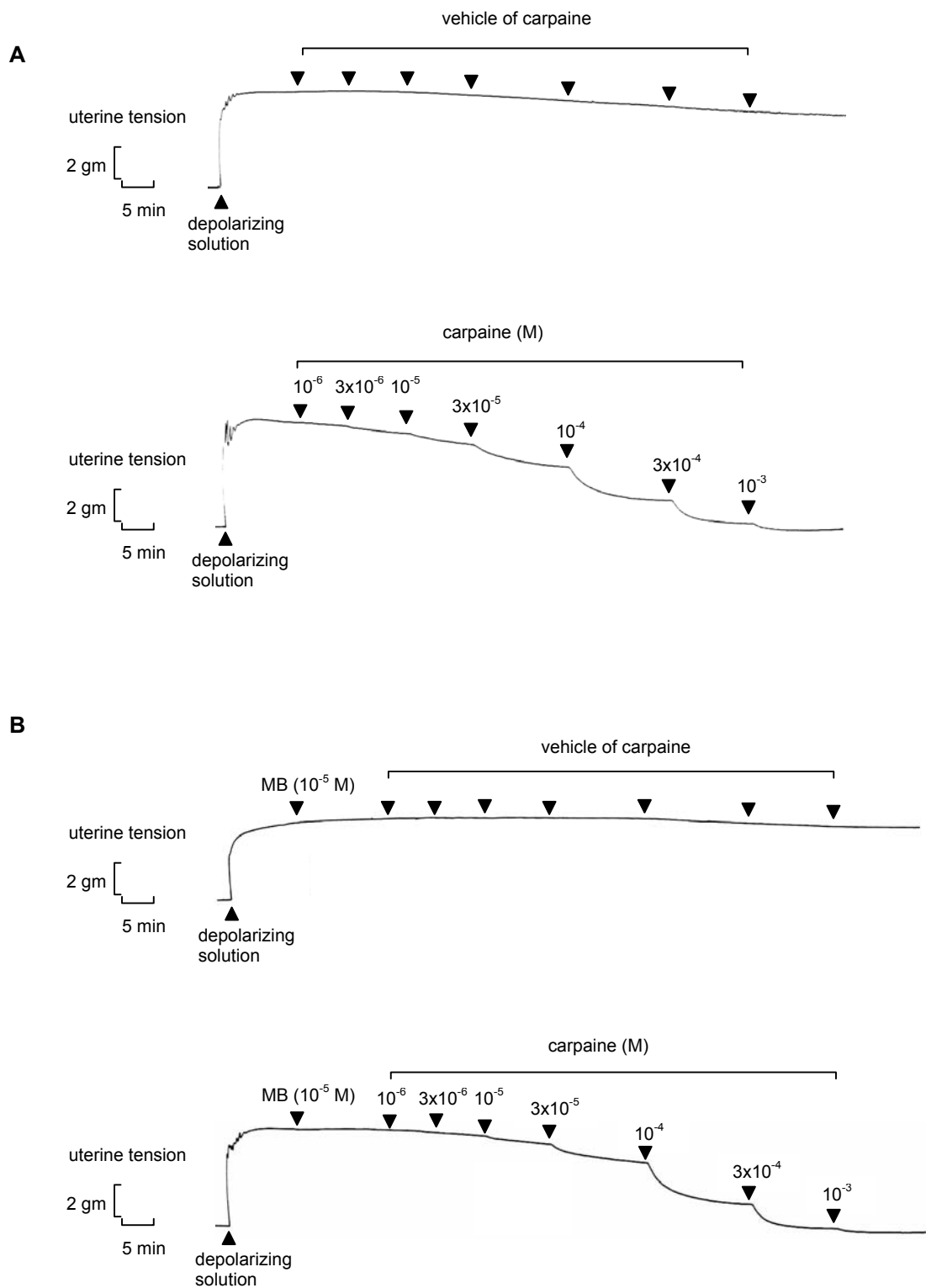


Figure 68. The representative tracings of the relaxing effect of carpaine and its time control (A), or pretreated with methylene blue (MB) (10^{-5} M) for 15 minutes and its time control (B) on isolated rat uterus precontracted with depolarizing solution (KCl 56.3 mM).

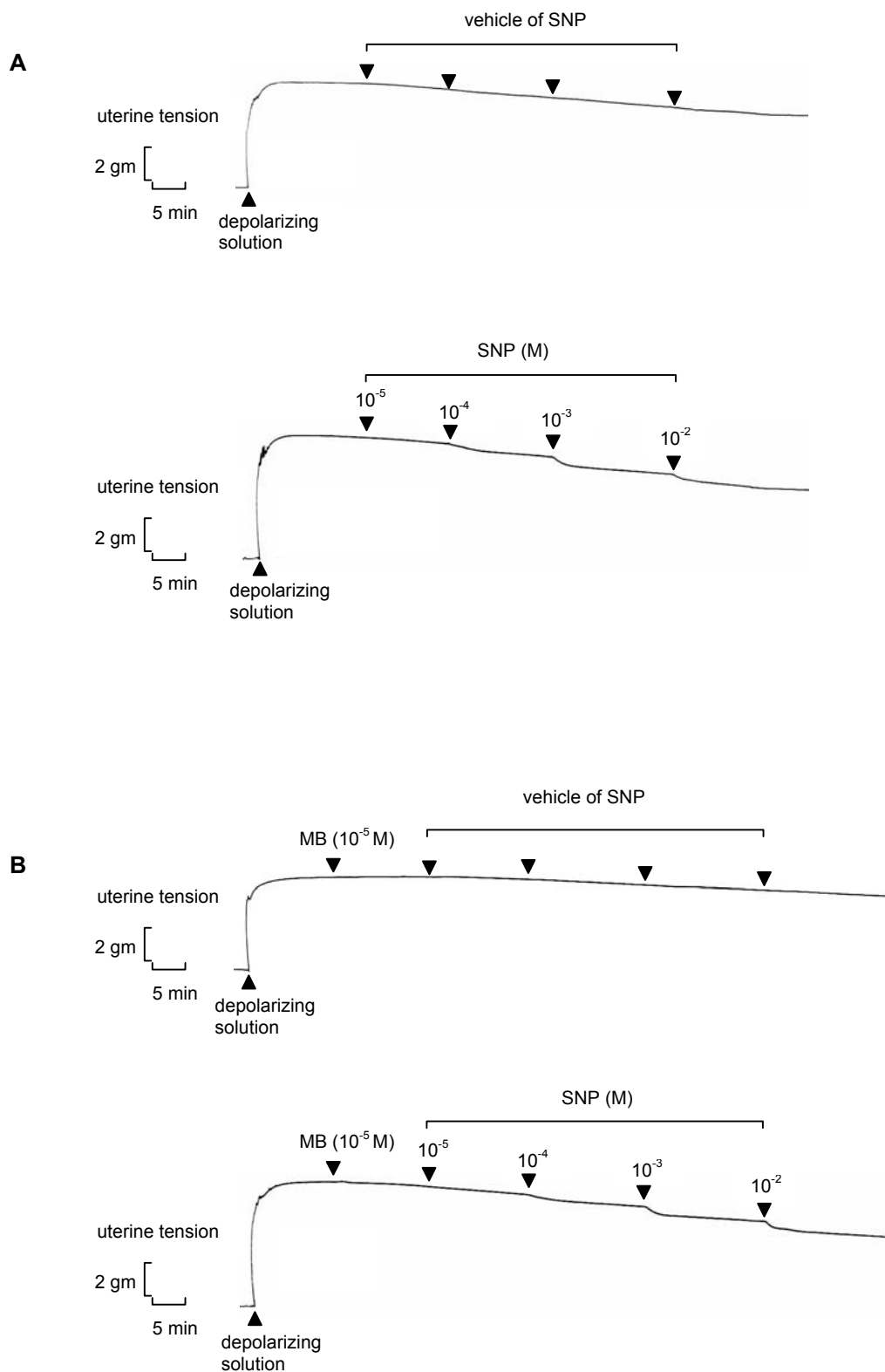
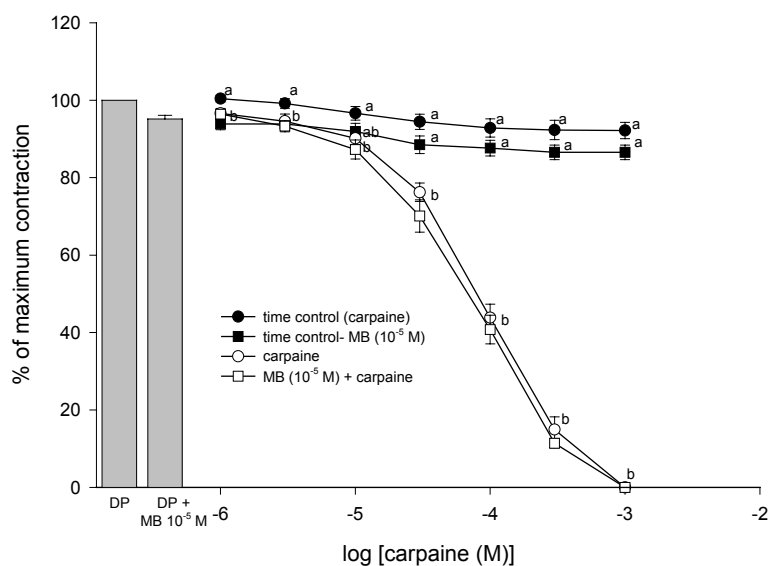


Figure 69. The representative tracings of the relaxing effect of sodium nitroprusside and its time control (A), or pretreated with methylene blue (MB) (10^{-5} M) for 15 minutes and its time control (B) on isolated rat uterus precontracted with depolarizing solution (KCl 56.3 mM).

A



B

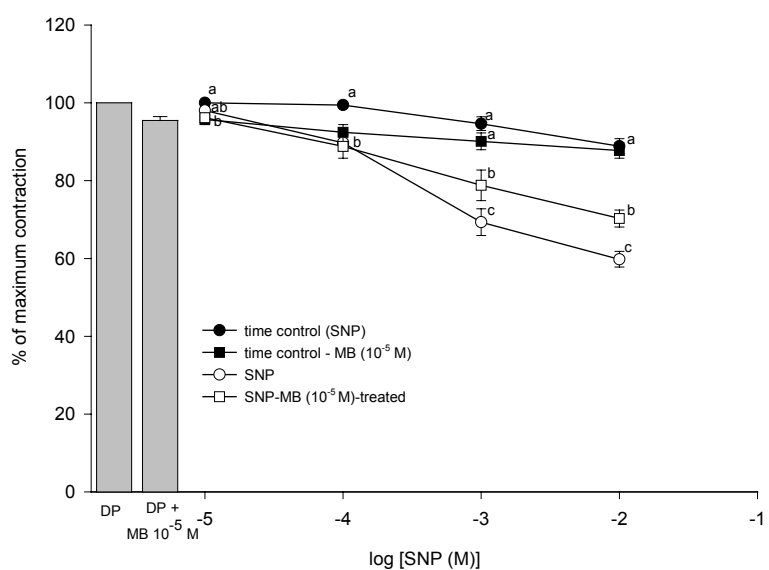


Figure 70. The concentration-response relationship of inhibitory effect of methylene blue (MB) (10⁻⁵ M, 15 minutes) on relaxing effect of carpaine (A) or SNP (B) on isolated rat uterus precontracted with depolarizing solution. Each datum point represents the mean \pm S.E.M of ten experiments. Different characters (a, b and c) mean significant difference among the comparing means which in the same treatment group (carpaine or SNP concentration) at $p < 0.05$ (Duncan's multiple range test).