CHAPTER III

RESULTS

1. Effects of ancistrotectorine and verapamil on KCl induced contraction.

Primary screening of the actions of ancistrotectorine and verapamil were performed on KCl induced contraction which are caused mainly by increasing membrane permeability to Ca^{2+} as a result of membrane deporalization. The contractile responses of rat vas deferens to K^{+} 100 mM are composed of the phasic components and later component, steady level of tension, that persists and from which it is separated by an inflection in the tension recorded is refered to as the tonic component. It was found that the phasic response was faster and larger in the prostatic half than in the epididymal half. So the prostatic half was used in this study. The effects of 15-min exposure to verapamil and ancistrotectorine are shown in Fig. 8, 9. Verapamil antagonized both phases of the responses to 100 mM KCl and had some selectivity for tonic response indicated by the percentage of inhibition of tonic response which was higher than that of the phasic response. Ancistrotectorine also reduced both phases of contraction, but at higher concentration (4.6×10^{-5}M) the alkaloid has the opposite profile to verapamil, being more active against the phasic than the tonic response (Fig. 10). The ID_{50} values from these experiments which were calculated by intrapolation of linear-regression line of log dose-response curve were compared in Table 1. The ID_{50} values of ancistrotectorine was 5 times higher than the ID_{50} value of verapamil in the phasic contraction and 72 times in the tonic contraction.

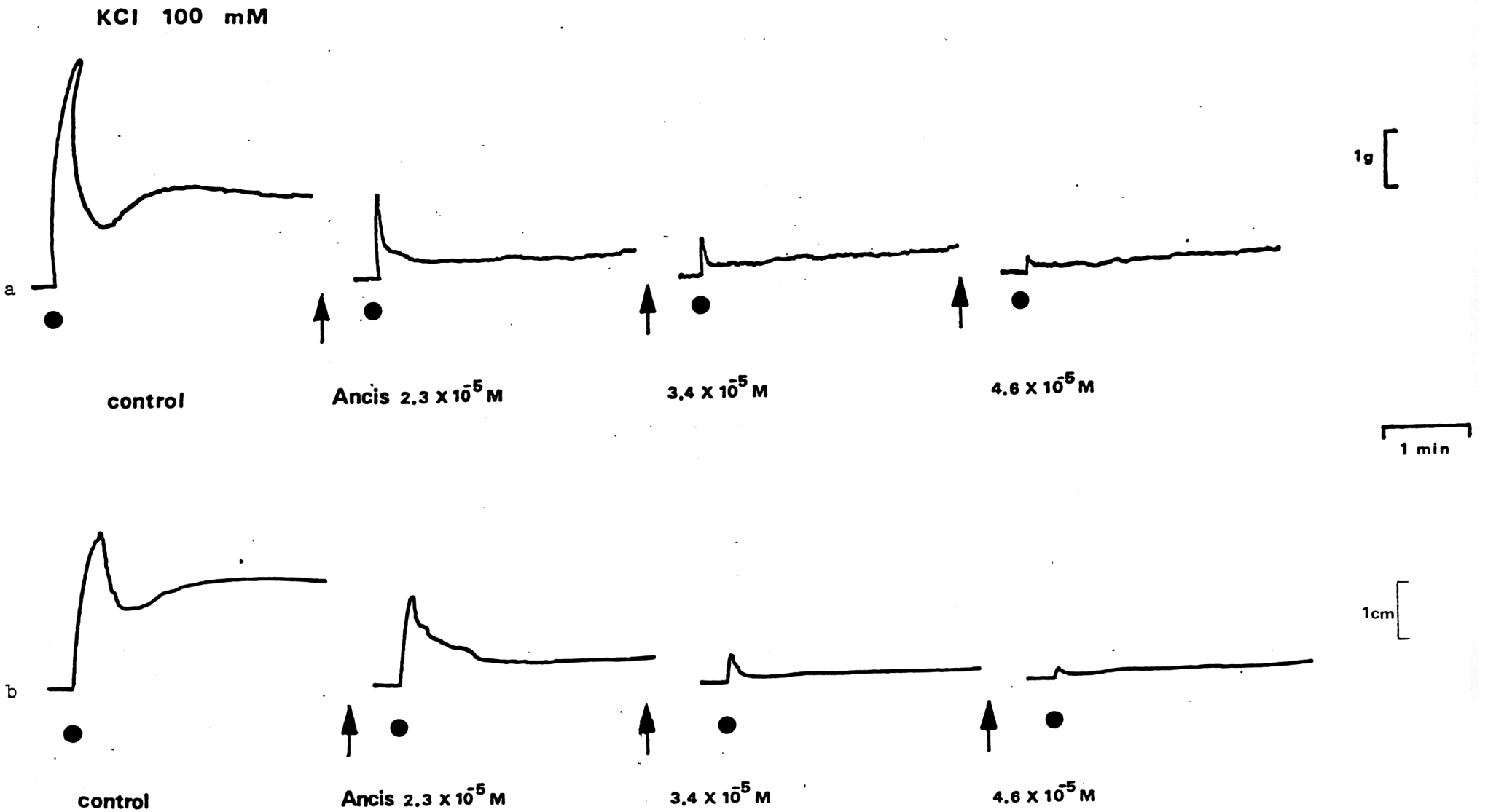

Figure 8. The effects of ancistrotectorine on responses to KCl 100 mM in the rat vas deferens. In each part, the control is repeated in the presence of increasing concentration of ancistrotectorine : (a) isometric recording (b) isotonic recording ● = addition of KCl.

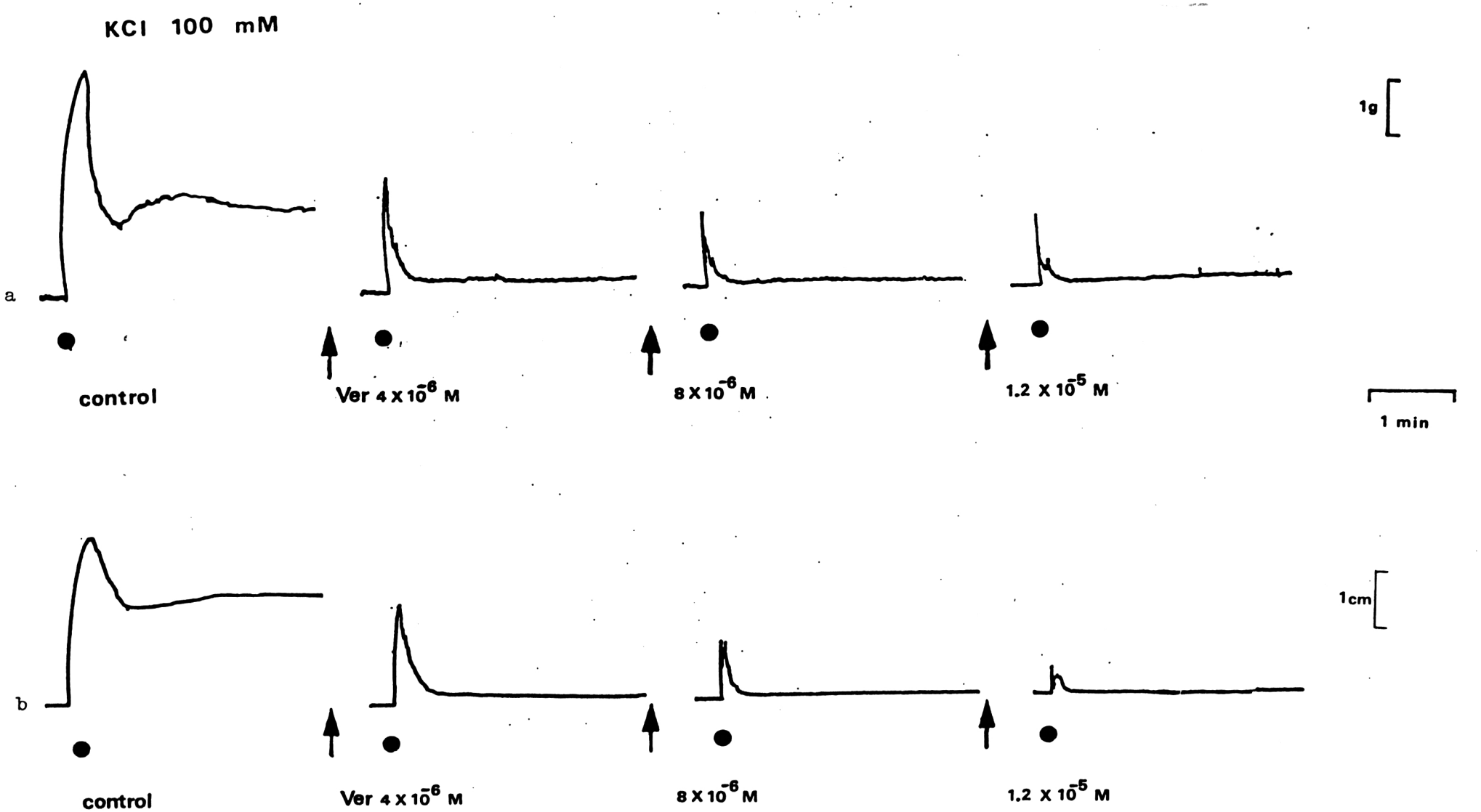

Figure 9. The effects of verapamil on responses to KCl 100 mM in the rat vas deferens. In each part the control is repeated in the presence of increasing concentration of verapamil : (a) isometric recording (b) isotonic recording. ● = addition of KCl.

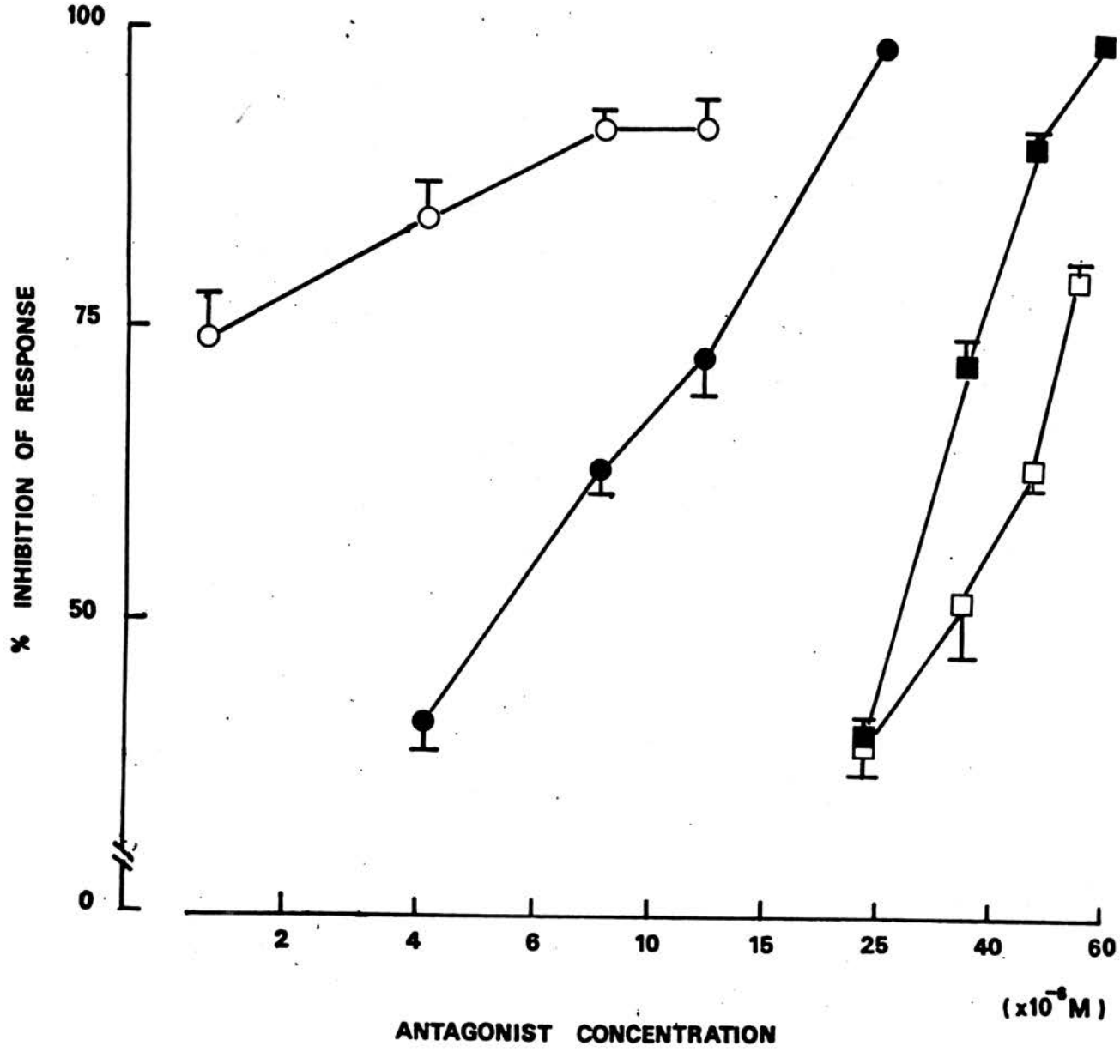

Figure 10. Effects of 15 min incubation with ancistrotectorine (○, n = 13) and verapamil (□, n = 7) responses to KCl 100 mM. Verapamil inhibition of phasic (●) and tonic (○); Ancistrotectorine inhibition of phasic (■) and tonic (□). The vertical bars represent the mean ± S.E.

agonist	component	Verapamil	Ancistrotectorine
KCl	phasic	4.8×10^{-6}	2.5×10^{-5}
	tonic	0.5×10^{-6}	3.6×10^{-5}
$BaCl_2$	phasic	1.2×10^{-6}	1.2×10^{-5}
NE	phasic	1.1×10^{-5}	3.5×10^{-5}
	tonic	1.4×10^{-6}	2.8×10^{-5}
5-HT	phasic	1×10^{-6}	2.2×10^{-5}

Table 1. Concentrations of ancistrotectorine and verapamil (M) which produced 50 % inhibition (ID_{50}) of maximal responses to agonists. The ID_{50} values were calculated from linear regression lines which intrapolate or extrapolate from the scales. (see appendix)

The results obtained during the beginning of this investigation showed that the responses obtained from both isometric and isotonic contraction were not significantly different (Fig. 11).

2. Effects of ancistrotectorine and verapamil on $BaCl_2$ induced contraction.

Ancistrotectorine and verapamil were tested in prostatic half of rat vas deferens for their further effects on $BaCl_2$ induced contraction. The concentration of $BaCl_2$ 2 mM produced a two component responses : an initial marked increase in phasic component followed by rhythmic contraction. Sample traces showing the effects of ancistrotectorine and verapamil are given in Fig. 12. Ancistrotectorine 2.3×10^{-5}M and verapamil 3×10^{-6}M abolished phasic component. Both verapamil and ancistrotectorine produced dose-related reduction in amplitude of the phasic contraction (Fig. 13). The frequency of rhythmic contraction was uneffected by verapamil at $0.5 - 1 \times 10^{-6}$M whereas 2×10^{-6}M and higher concentration (3.8×10^{-5}M) reduced the frequency of rhythmic contraction significantly. In contrast to the effect of verapamil, the frequency of rhythmic contraction was increased significantly by ancistrotectorine $1.2 - 2.3 \times 10^{-5}$M (Fig. 14). High concentration of ancistrotectorine (8.9×10^{-5}M) inhibited the amplitude more than the frequency of rhythmic contraction (Fig. 15). The inhibitory effect of verapamil 8.9×10^{-5}M on rhythmic response was reversed by increasing $(Ca^{2+})_o$. $CaCl_2$ 1.05×10^{-4}M almost reversed the inhibitory effect of verapamil on rhythmic response to the control, whereas this concentration of $CaCl_2$ reversed the amplitude of the inhibition of rhythmic contraction of ancistrotectorine only slightly. The ID_{50} value of verapamil

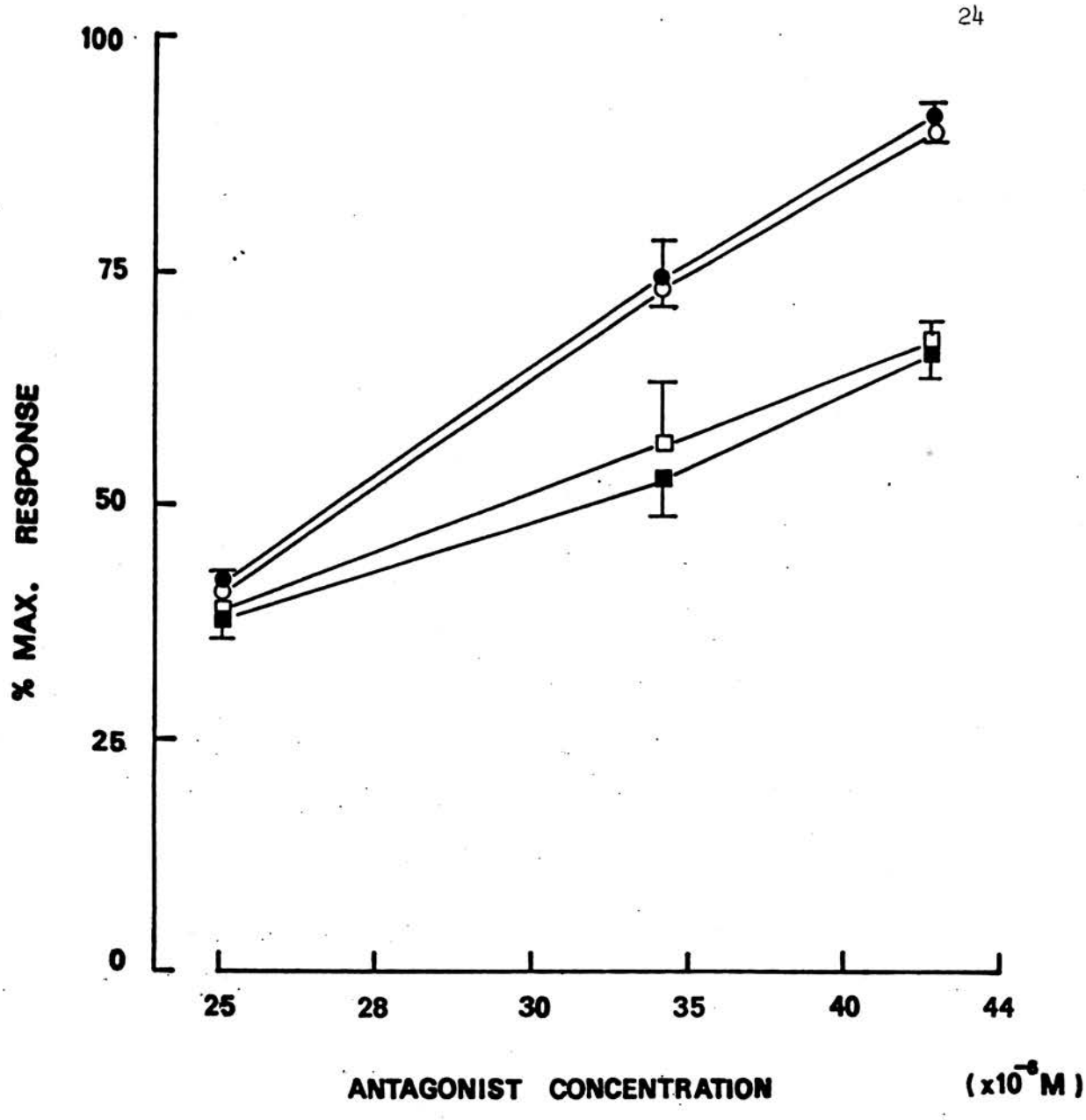

Figure 11. Effect of ancistrotectorine on KCl induce phasic and tonic contraction as recorded by isotonic and isometric transducers; ● phasic response by isotonic recording; O phasic response by isometric recording; ■ tonic response by isotonic recording; □ tonic response by isometric recording. Vertical bars represent S.E. Results are the mean of 6 preparations.

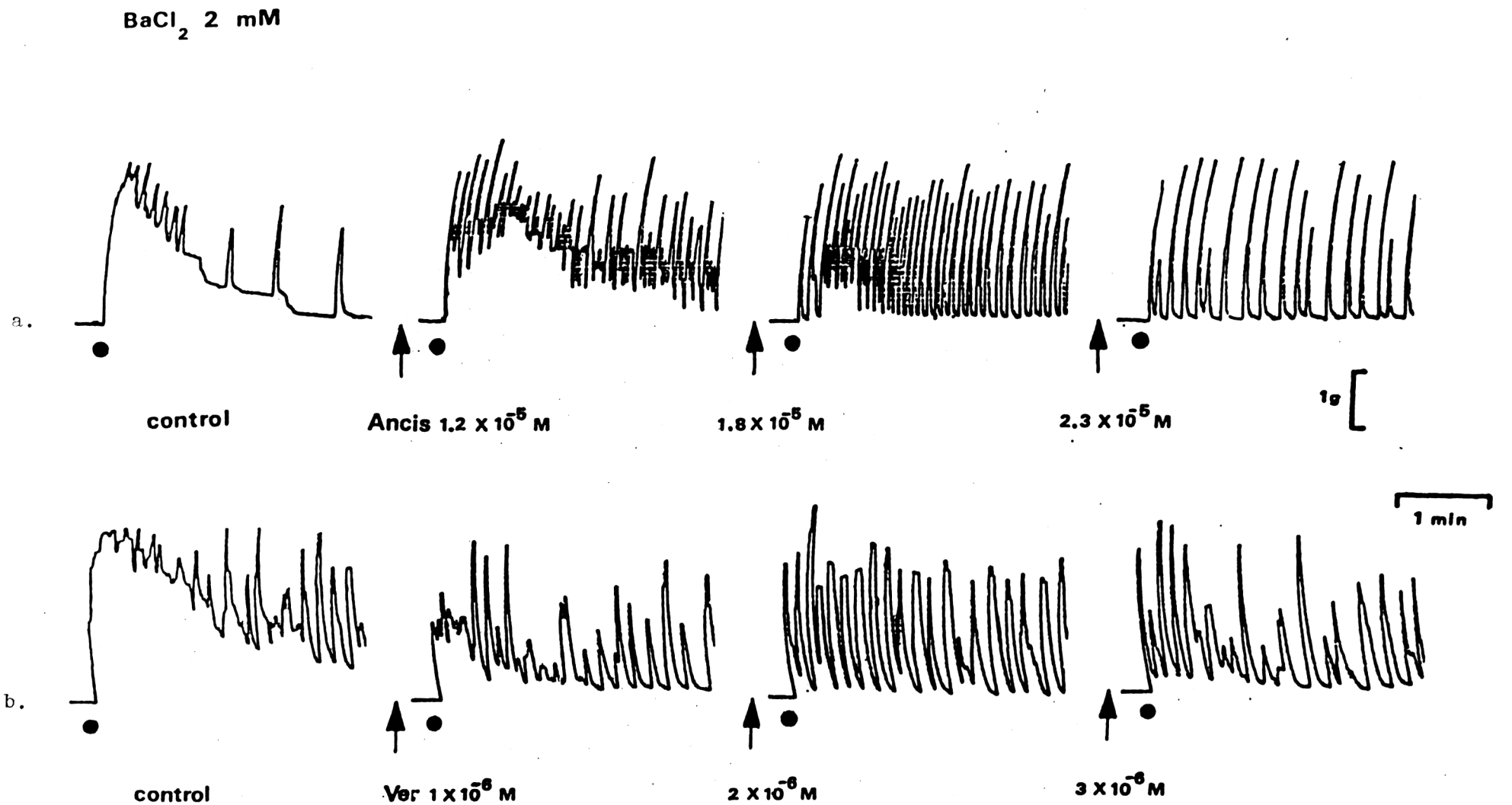

Figure 12. Effects of ancistrotectorine (a) and verapamil (b) on the phasic and rhythmic contractions produced by $BaCl_2$ 2 mM. In each part, the control is repeated in the presence of increasing concentration of antagonists. ● = addition of $BaCl_2$.

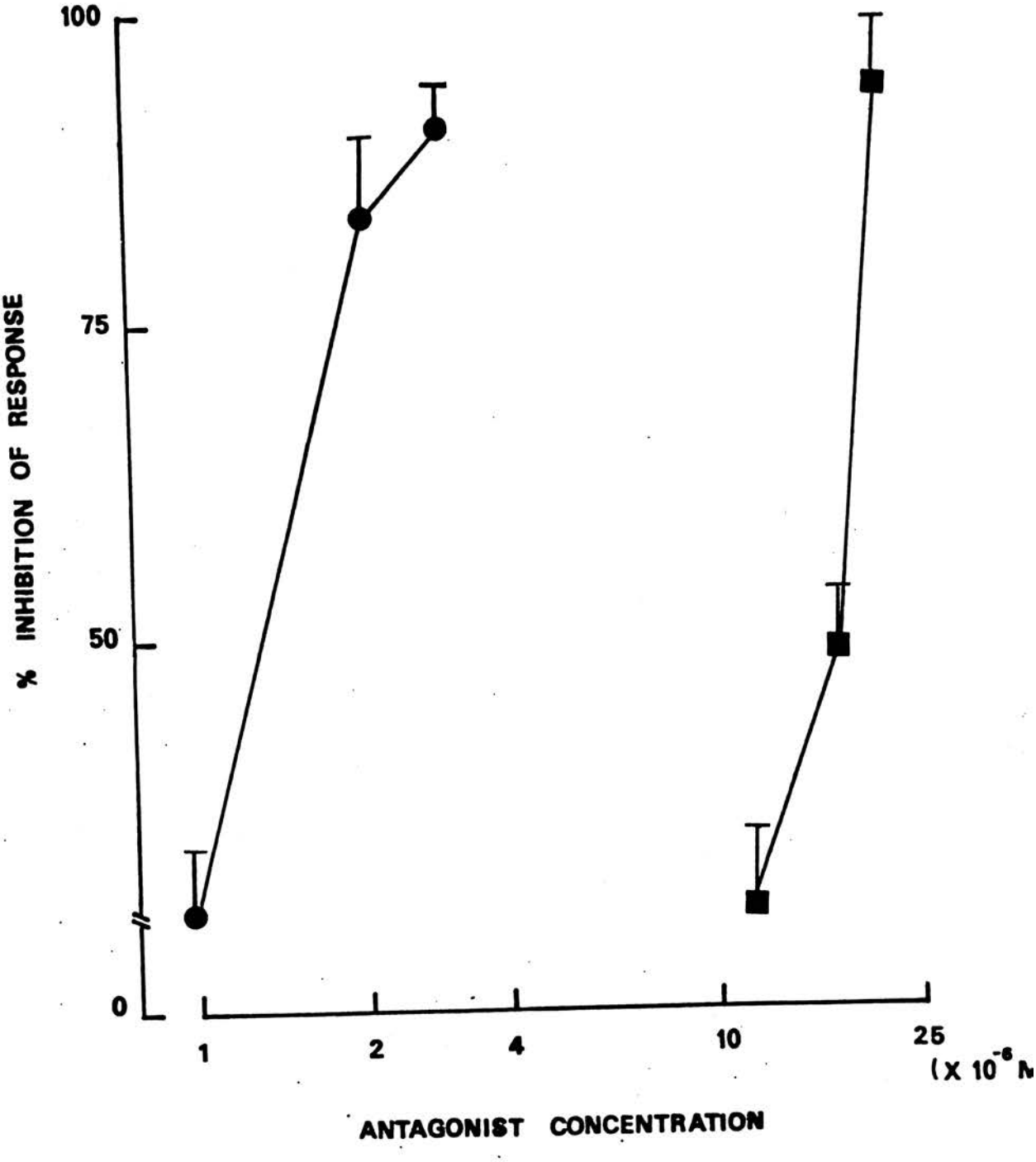

Figure 13. Effects of ancistrotectorine (■) and verapamil (●) on contraction produced by $BaCl_2$ 2 mM on the phasic response. Vertical bars indicate S.E. Each point is the mean of 6 preparations.

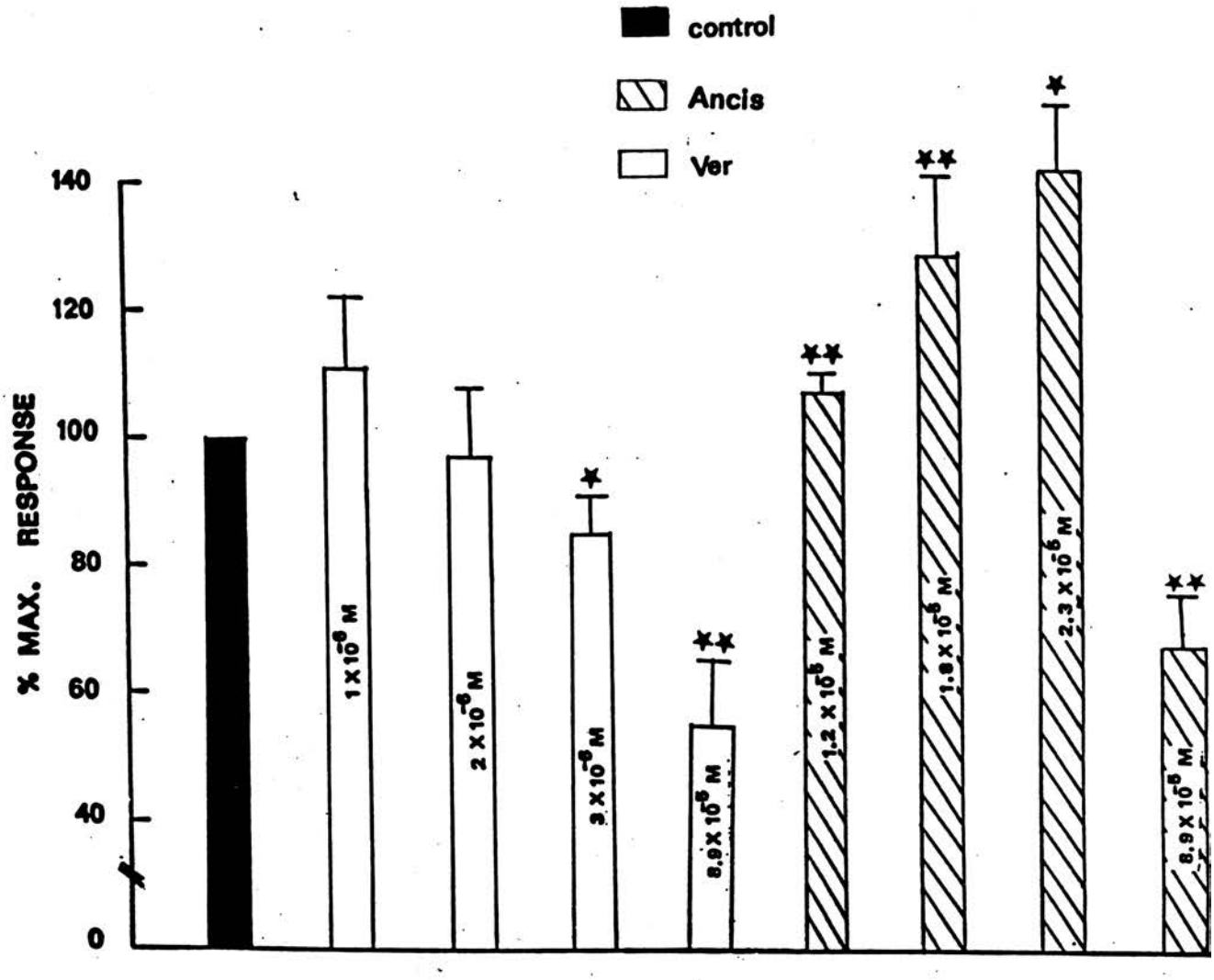

Figure 14. Effects of ancistrotectorine and verapamil on rhythmic responses produced by $BaCl_2$ 2 mM. Columns are means value ± S.E. mean, n = 4 - 6. Significant differences compare to percentage of maximal control response, ★ $P < 0.05$; ★★ $P < 0.01$.

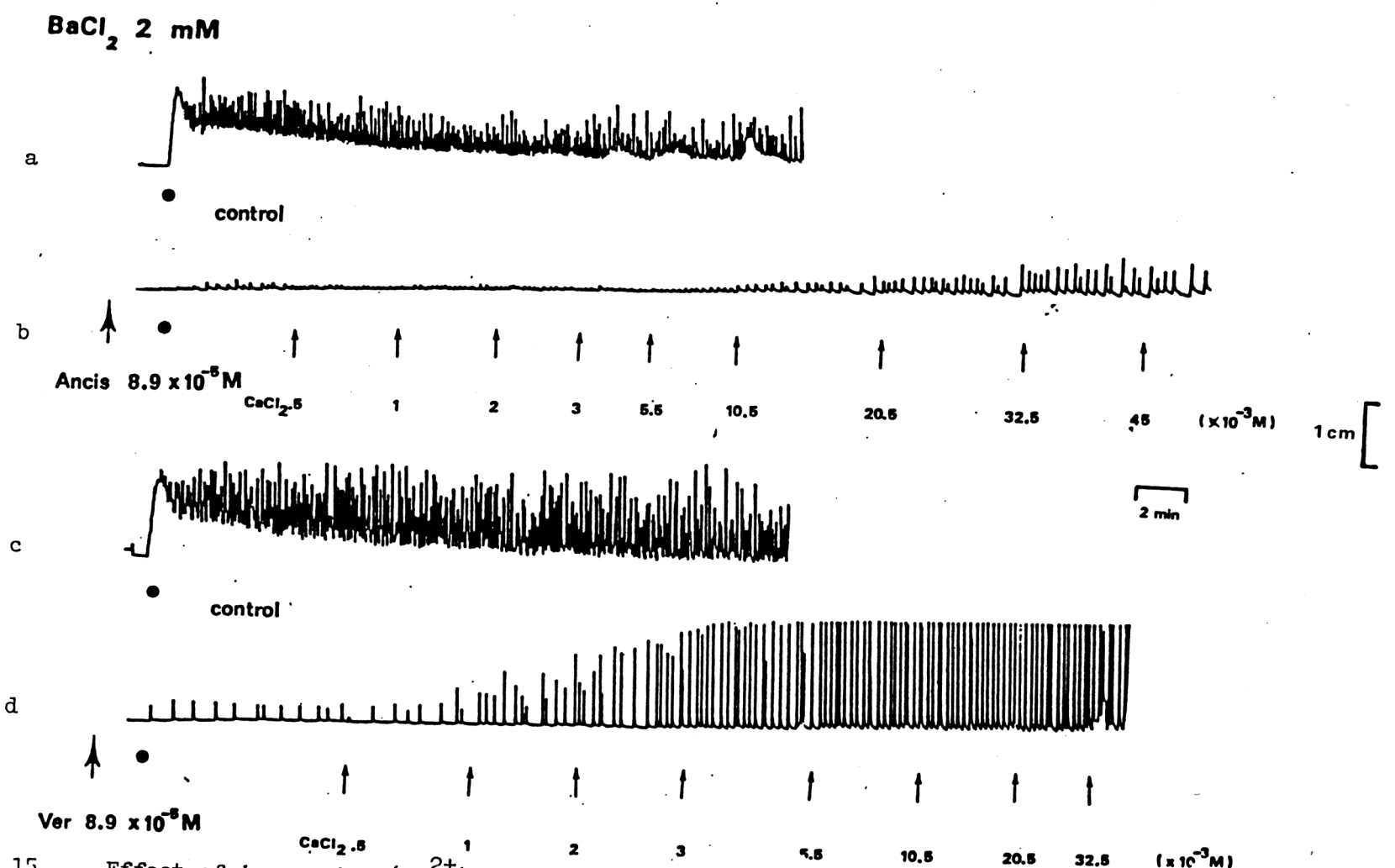

Figure 15. Effect of increasing $(Ca^{2+})_o$ on the inhibitory actions of ancistrotectorine and verapamil on rhythmic contractions produced by $BaCl_2$. Control response of ancistrotectorine (a) and verapamil (c) to $BaCl_2$ were inhibited by ancistrotectorine (b) and verapamil (d) 3.9×10^{-5} M, and in the continuous presence of antagonists, $(Ca^{2+})_o$ was added cumulatively to give total concentration 45 mM. ● = addition of $BaCl_2$.

on phasic contraction induced by $BaCl_2$ was more potent than ancistrotectorine.

3. Effects of ancistrotectorine and verapamil on 5-HT induced contraction.

Administration of 5-HT produces phasic contraction followed by rhythmic contraction. The effect of 5-HT was studied on the epididymal half because the initial phasic response was larger, and the rhythmic contractions were also larger and much more frequent than the prostatic half (Hay & Wadsworth, 1982d). Ancistrotectorine and verapamil produced a dose-dependent reduction in the phasic contraction (Fig. 16 and Fig. 17). Verapamil 8 x 10^{-6}M abolished the initial phasic contraction. The ID_{50} value of ancistrotectorine in phasic contraction was 22 times higher than of verapamil Ancistrotectorine 1.2 x 10^{-5}M had no significant effect on the frequency but higher dose of ancistrotectorine (2.3 x 10^{-5}M and 3.5 x 10^{-5}M) produced augmentation. The frequency of rhythmic contraction was substantially reduced but the amplitude were slightly increased by verapamil 2 x 10^{-6} to 8 x 10^{-6}M (Fig. 18). The frequency of rhythmic contraction was noticeably enhanced by ancistrotectorine (1.2 x 10^{-5}M - 3.5 x 10^{-5}M). Ancistrotectorine and verapamil 8.9 x 10^{-5}M abolished phasic and rhythmic contractions. Increasing the $(Ca^{2+})_o$ reversed the inhibitory effect of verapamil on frequency and amplitude of contraction. Increasing $(Ca^{2+})_o$ from .5 - 45 mM was without effect on the rhythmic inhibition of ancistrotectorine (Fig. 19).

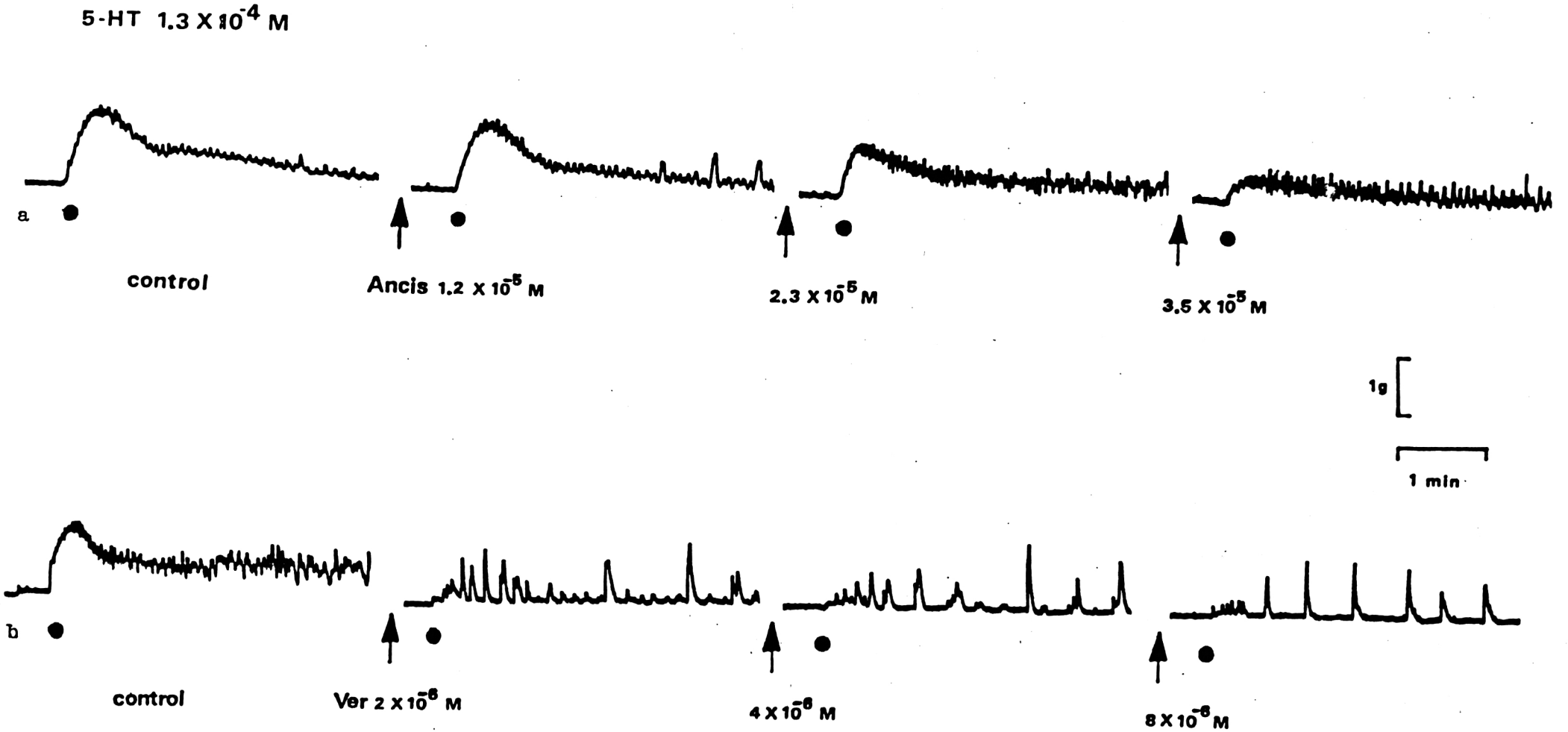

Figure 16. Effects of ancistrotectorine (a) and verapamil (b) on responses to 5-HT 1.3 x 10^{-4} M in the rat vas deferens. In each part, the control is repeated in the presence of increasing concentration of antagonists. ● = addition of 5-HT.

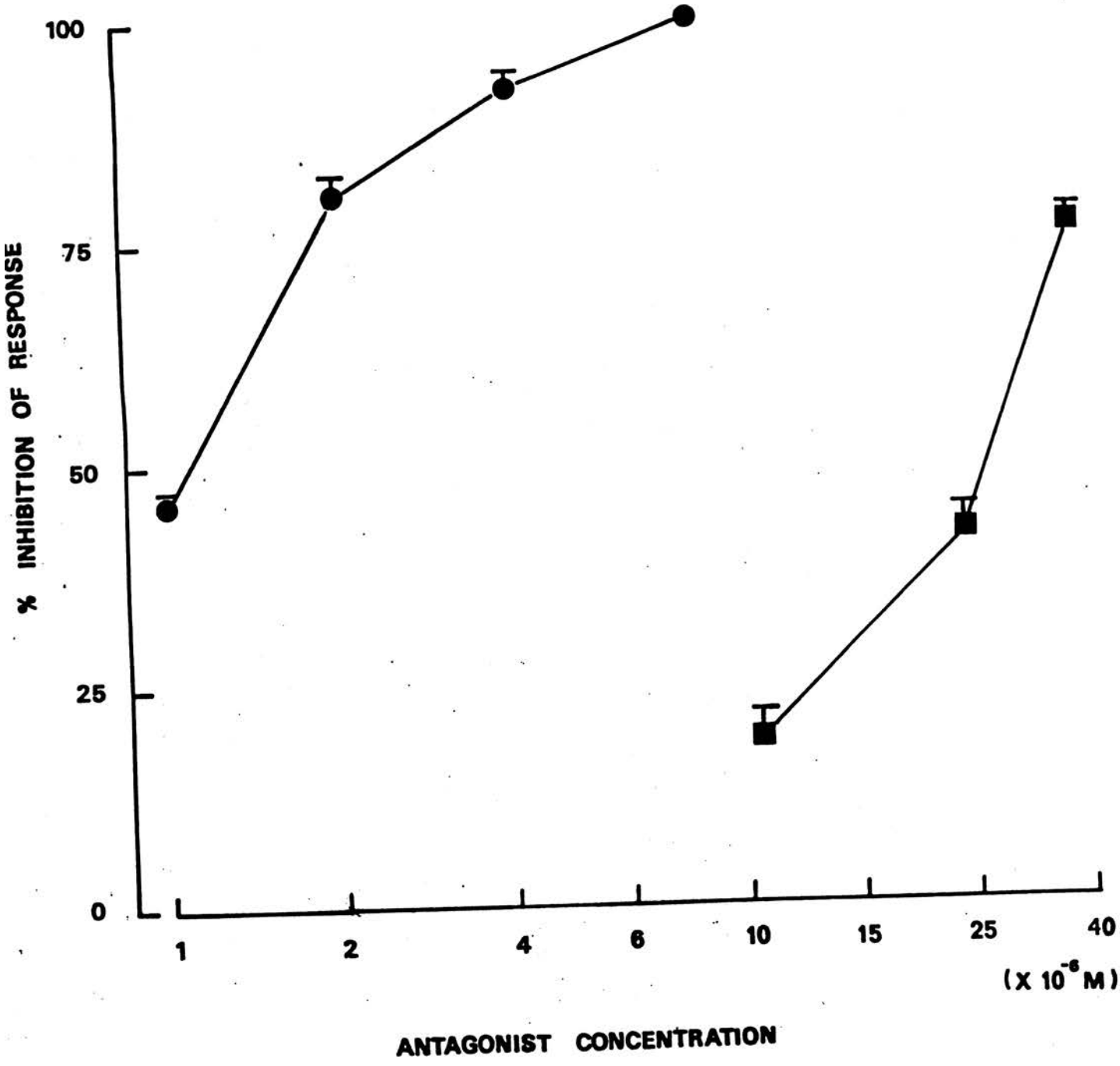

Figure 17. The effects of ancistrotectorine (■) and verapamil (●) on phasic contraction produced by 5-HT 1.3×10^{-4} M n = 7.

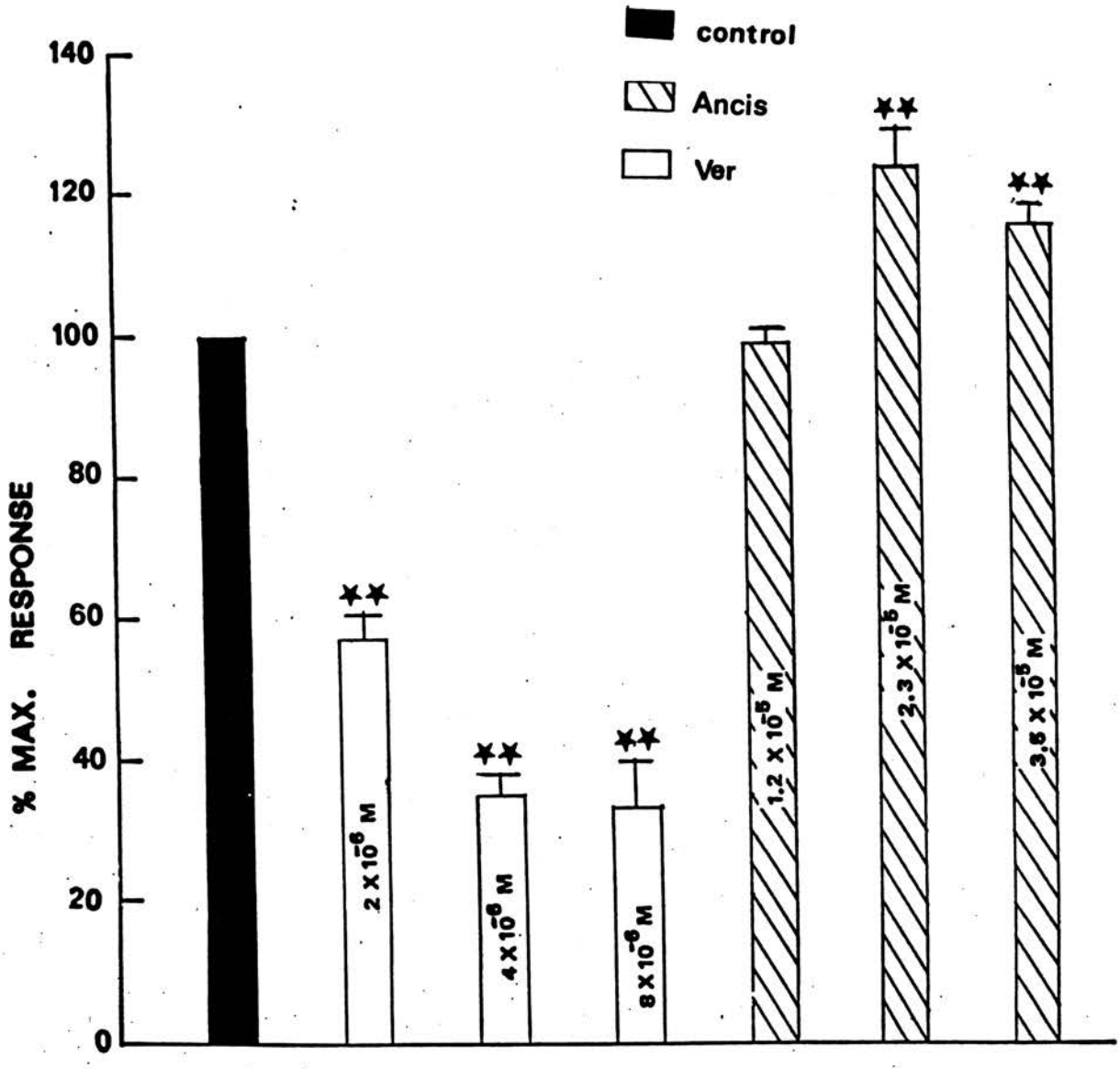

Figure 18. Effects of ancistrotectorine and verapamil on rhythmic contraction produced by 5-HT 1.3×10^{-4}M. Columns are mean values ± S.E. mean, n = 4 - 6, (Significant differences compare to percentage of maximal control response), ** $P < 0.01$.

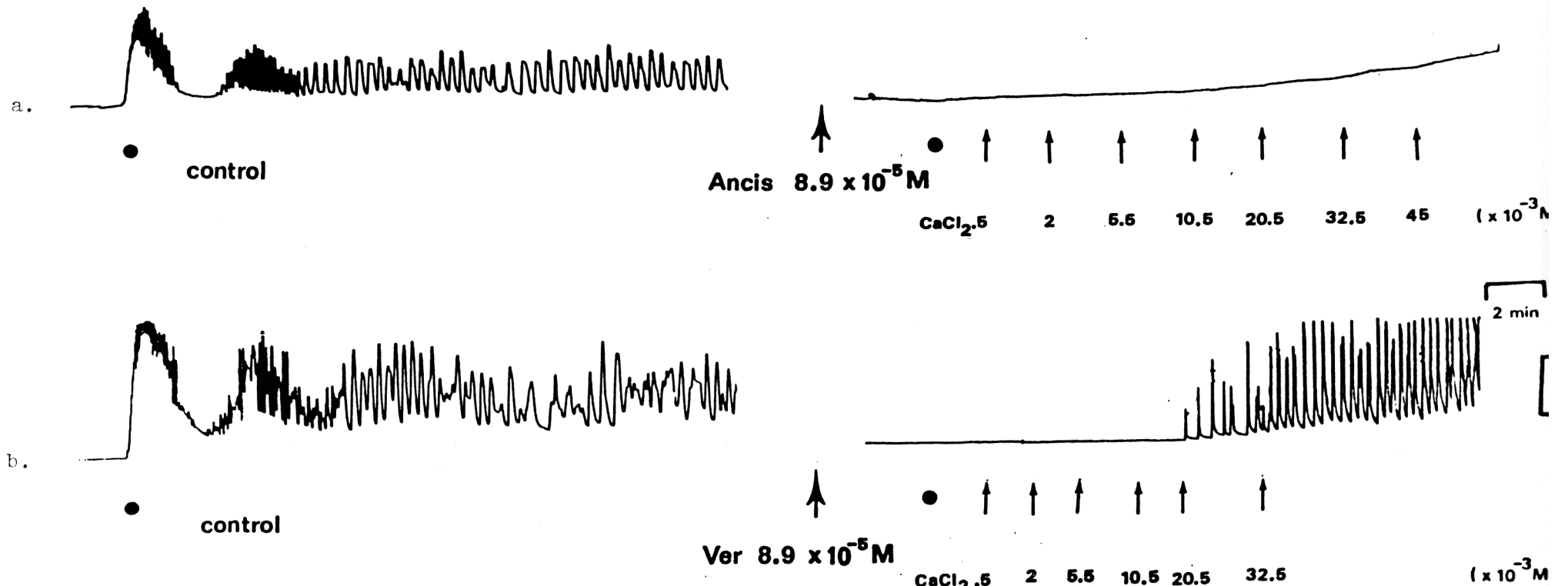

Figure 19. Effect of increasing $(Ca^{2+})_o$ on the inhibitory actions of ancistrotectorine and verapamil on rhythmic contractions produced by 5-HT. Control responses to 5-HT are shown in the first panel of each section. (a and b). Phasic response and rhythmic response were abolished by ancistrotectorine and verapamil 8.9×10^{-5}M, and in the continuous presence of antagonists, $(Ca^{2+})_o$ was added cumulatively to give the total of 45mM ● = addition of 5-HT.

4. Effects of ancistrotectorine and verapamil on NA induced contration.

Contraction produced by NA 3 x 10^{-5}M was biphasic consisting of an initial rapid phasic and a slower sustained tonic component in the prostatic half. This concentration of NA produced a contraction in the epididymal half that is approximately 50 % of maximum contraction of the prostatic half. The phasic contraction was depressed by either ancistrotectorine or verapamil. As shown in Fig. 20 and 21, the tonic component was more sensitive to inhibition than was the phasic component by both antagonists. The ID_{50} values of verapamil and ancistrotectorine were expressed in Table 1. Both verapamil 1.2 x 10^{-5}M and ancistrotectorine 1.6 x 10^{-5}M almost abolished tonic contraction. In some experiments NA 3 x 10^{-5}M show slight rhythmic contraction (Fig. 20). By observation, administration of ancistrotectorine 1.2 x 10^{-5}M and 2.3 x 10^{-5}M showed more potentiating rhythmic contraction in all preparations than control. However, at a dose of ancistrotectorine 3.4 x 10^{-5}M most of the rhythmic contraction were abolished. The slight rhythmic contraction could be observed with verapamil too. Verapamil 2 x 10^{-5}M to 5 x 10^{-5}M were used in some preparation. It was found that the phasic contraction could not be abolished.

5. Effects of ancistrotectorine and verapamil on cumulative $CaCl_2$ induced contraction of rat vas deferens in depolarizing solution.

Another experiment was performed in order to determine quantitatively the antagonistic activity of ancistrotectorine and verapamil. It has been studied whether they acted under our experiments in competitive with $CaCl_2$ or not. So the effects of ancistrotectorine and

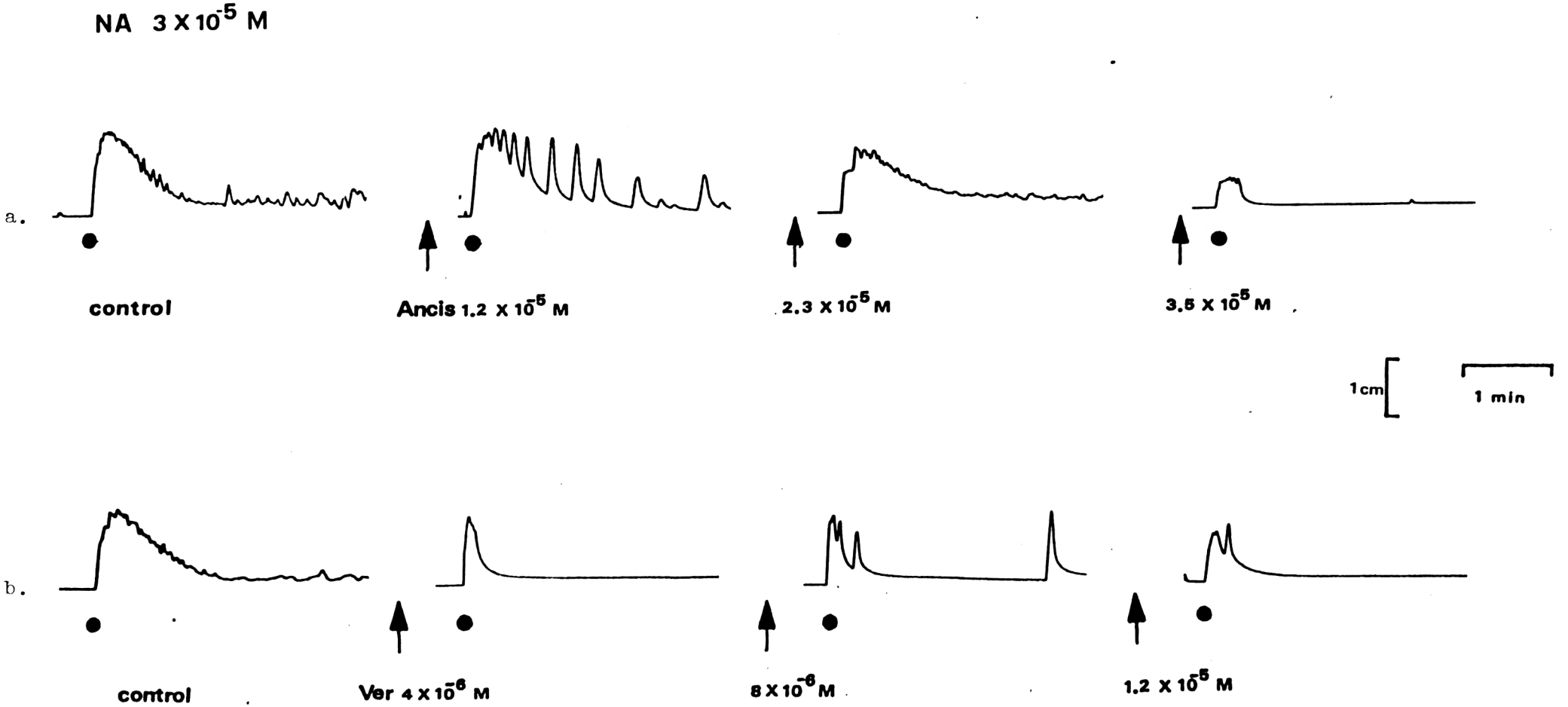

Figure 20. The effects of ancistrotectorine (a) and verapamil (b) on response of rat vas deferens to NA 3×10^{-5}M. In each part, the control is repeated in the presence of increasing concentration of antagonists. ● = addition of NA.

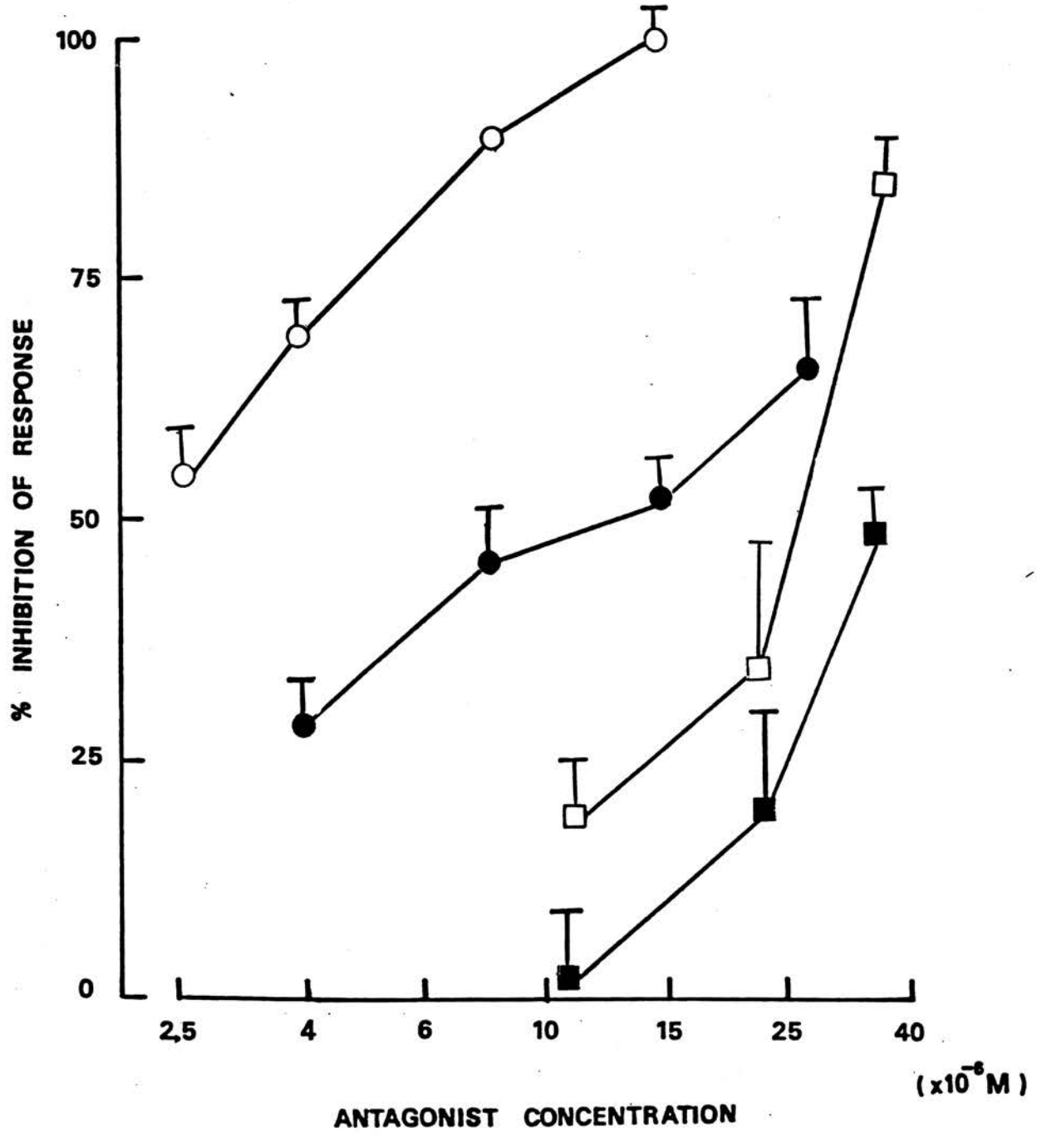

Figure 21. Effects of 15 min incubation with ancistrotectorine and verapamil responses to NA 3 x 10^{-5}M : ancistrotectorine inhibition of phasic (■) and tonic (□); verapamil inhibition of phasic (●) and tonic (○). Each point is the mean of six records. Vertical bars represent S.E.

verapamil were examined against Ca^{2+} responses elicited in K^{+} depora-lized rat vas deferens, Since under our experimental condition ancistrotectorine was acted in a non-competitive fashion except ancis-trotectorine 2.1 x 10^{-5}M antagonized the contractions caused by low $CaCl_2$ concentrations (up to 30 mM) much more than those by high $CaCl_2$ concentrations. The maximum of the dose-response curve for $CaCl_2$ was not affected by ancistrotectorine 2.1 x 10^{-5}M which produced competi-tive antagonism against $CaCl_2$ (Fig. 22). Ancistrotectorine 4.3 x 10^{-5}M caused a slight rightward parallel shift of the curve. together with depression of maximum response. Verapamil 4 x 10^{-6}M and 8 x 10^{-6}M affected the $CaCl_2$ concentration curve in a non competitive manner (Fig. 23). The maximum contraction was depressed more than fifty percent. pD_2' values were calculated by the method of Van Rossum (Van Rossum, 1963). The result of pD_2' values were summarized in Table 2. pD_2' values of verapamil 4 x 10^{-6}M and 8 x 10^{-6}M to $CaCl_2$ were not significant difference. pD_2' values of verapamil was higher than obtained from ancistrotectorine.

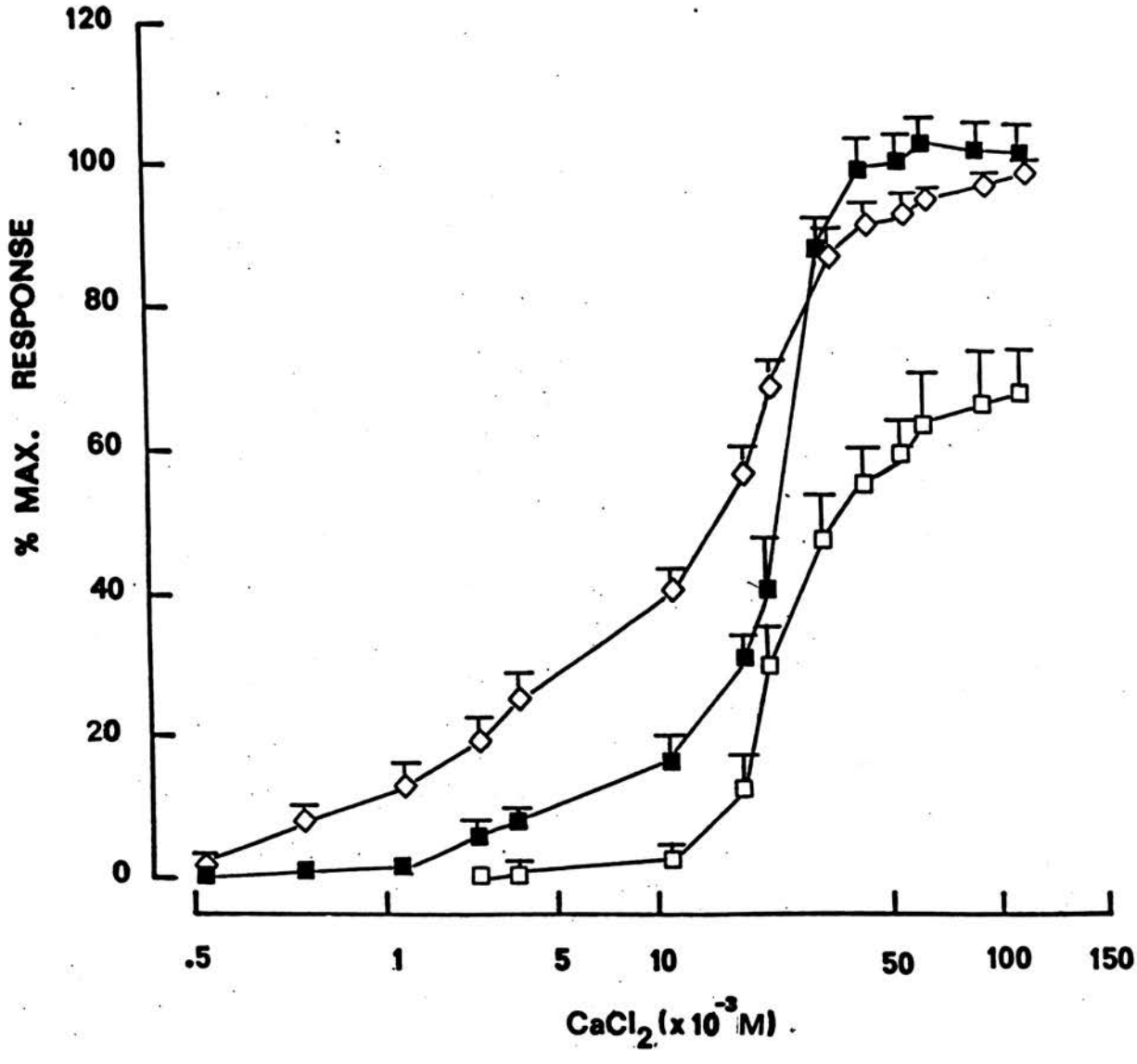

Figure 22. Effect of ancistrotectorine on concentration-response curves to $CaCl_2$ in deporalized rat vas deferens with 128 mM K^+. In each tissue, responses at each calcium concentration are expressed as of the maximal control response for that tissue before addition of ancistrotectorine. Control (◇ , n = 12) ancistrotectorine 2.1×10^{-5}M (■ , n = 8); ancistrotectorine 4.3×10^{-5}M (□ , n = 7).

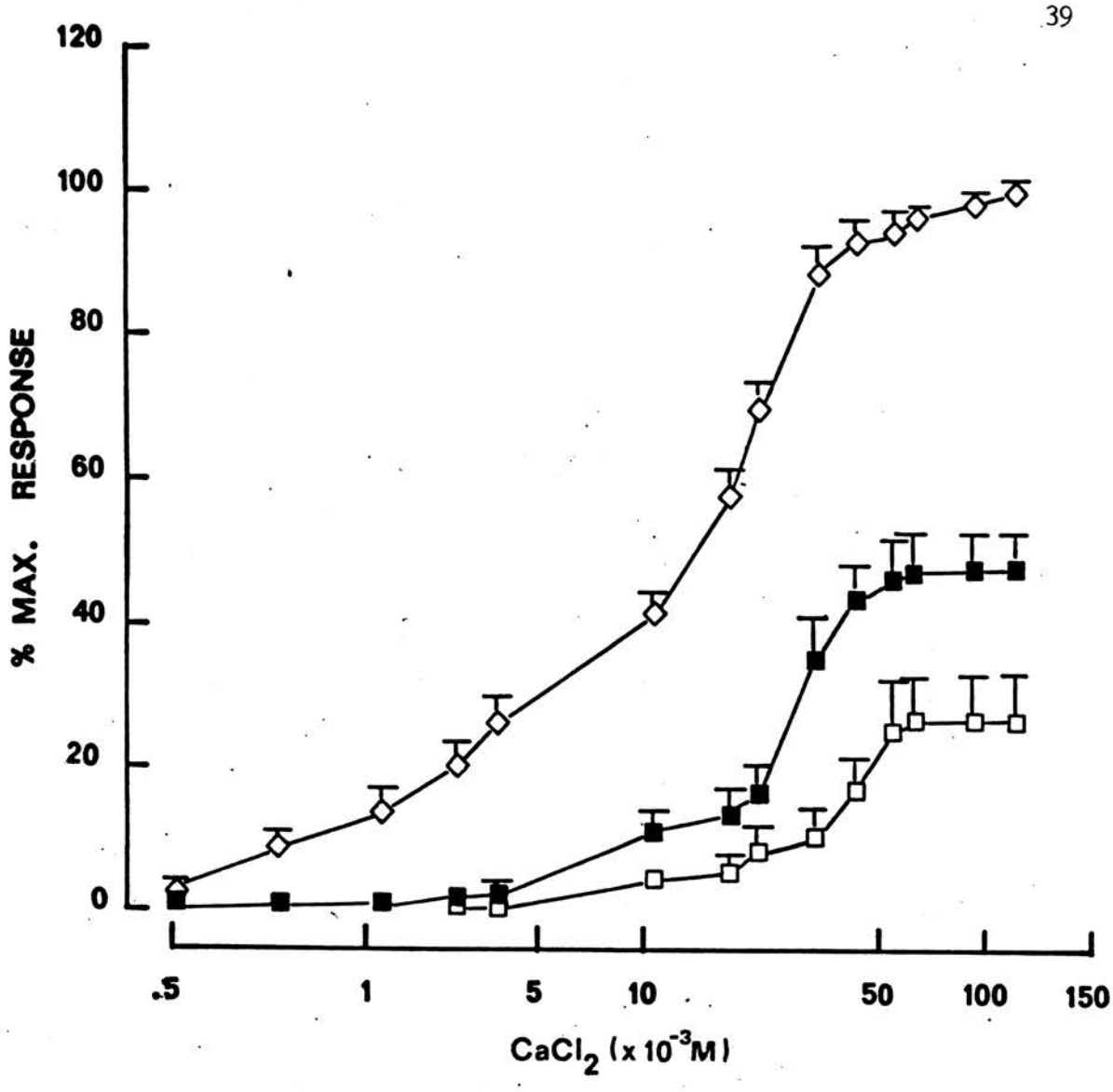

Figure 23. Effect of verapamil on concentration-response curves to $CaCl_2$ in deporalized rat vas deferens with 128 mM K^+. In each tissue, responses at each calcium concentration are expressed as a percentage of the maximal control response for that tissue before addition of verapamil. Control (◇ , n = 12); verapamil 4 x 10^{-6}M (■ , n = 9); verapamil 8 x 10^{-6}M (□ , n = 10).

Antagonist		pD_2 value
Ver	4×10^{-6} M	5.47 ± 0.03 (n = 9)
	8×10^{-6} M	5.5 ± 0.05 (n = 10)
Ancis	2.1×10^{-5} M*	-
	4.3×10^{-5} M	3.98 ± 0.16 (n = 7)

Table 2. pD_2 values of ancistrotectorine and verapamil against $CaCl_2$ in deporalized rat vas deferens. The Table shows mean pD_2 values and standard errors of means. The number of observations (n) is given in parenthesis.

* This concentration cannot block effect of $CaCl_2$ in depolarizing solution.