CHAPTER III

RESULTS

Effects of ancistrotectorine on contactions of isolated rat aorta induced by KCl, NE, 5-HT and histamine.

KCl,NE,5-HT and histamine evoked contractile responses in rat aorta in a concentration-dependent manner. The concentration-response curves in this study are shown in Figure.6-9. In each experiment, the control cumulative concentration-response profile was reproducible when the successive experiments were separated by a recuperative time of 30-60 min. Ancistrotectorine produced a concentration- inhibition of these agent-induced contractions. The tracing of NE, 5-HT and histamine which induced contractions in the absence and presence of ancistrotectorine are presented in Figure.10-12. KCl at the concentrations ranging between 5 and 200 mM were used in an accumulative manner, KCl elicited the contractile in the presence of this agent (1.19×10^{-5} M), the induced contractile induced response was that of control, whereas with 2.37×10^{-5} M ancistrotectorine, the response was inhibited to a greater extent. The results are shown in Figure.6. By exposing the tissues to NE the contractile response were seen as well. These response were reduced in the presence of ancistrotectorine than in its absence. A dose-

dependent manner of the effects of ancistrotectorine was also revealed as suggested by a more remarkable reduction NE-evoked contraction at higher concentrations of ancistrotectorine. In case of 5-HT, 1×10^{-8} M - 1×10^{-6} M of this agonist were used in a cumulative regimen and the contractile responses were similarly obtained and the attenuation of these responses by ancistrotectorine was obtained also. By pre-incubating the tissues in 2.37×10^{-5} M ancistrotectorine, the 5-HT-induced contractile responses were lower than those caused after the pre-incubation of tissues in 1.19×10^{-5} M ancistrotectorine. The results are shown in Figure. 7. When 5×10^{-5} - 1.5×10^{-3} M of histamine were used as an agonist in the preparation. Ancistrotectorine elicited a concentration - related inhibition on histamine-induced contraction. Evidently, 2.37×10^{-5} M of ancistrotectorine produce the inhibition more than that caused by 1.19×10^{-5} M of ancistrotectorine. The tracing are shown in Figure. 12. In addition, the concentration-response curves in this study can be seen in Figue. 9. In Ca^{+2} -free solution containning 1 mM EGTA ; KCL, NE, 5-HT and histamine failed to induce contractions in the isolated rat aorta.

Effects of $CaCl_2$ on the rat aorta in Ca^{+2} -free high potassium depolarizing solution and effects of ancistrotectorine (1.19×10^{-5} M or 2.37×10^{-5} M) on

$CaCl_2$-induced contractions were shown in Figure 13. In this study, 1×10^{-4} -15×10^{-3} M of $CaCl_2$ were administered. This stimulatory agent caused the contractile response in the rat aorta. In the presence of ancistrotectorine, the contractile response was reduced and it was found that further reduction of the evoked contraction was observed with 2.37×10^{-5} M ancistrotectorine. The dose-response curves can be seen in Figure. 14. In the case of 5 - HT - induced contraction, methysergide 1×10^{-7} M, ancistrotectorine 2.37×10^{-5} M, diltiazem 1×10^{-7} M and verapamil 1×10^{-7} M were used. The calculated EC_{50} for this study are shown in Table. 1. We compared the inhibitory effect of ancistrotectorine, diltiazem and verapamil on aorta induced contractions.

For NE, $CaCl_2$ and KCl-induced contraction, only three agents were studied (ancistrotectorine, diltiazem and verapamil). The EC_{50} was shown in Table. 2, 3, 4.

From this study, it was found that the inhibitory effect of verapamil was the most potent on contractions of isolated rat aorta induced by 5-HT, histamine, NE, $CaCl_2$ and KCl.

In addition we studied the effect of methysergide, 5-HT-receptor antagonist on the contractile response of the rat aorta to 5-HT and the

interaction with ancistrotectorine. which inhibit the contractile response of 5-HT. After applying combination of methysergide ($1X10^{-7}$ M) and ancistrotectorine ($1.19X10^{-5}$ M), the contractile response to 5-HT was lower than those with methysergide ($1 X 10^{-7}$ M) alone. In this experiment, it was found that the application of $2.37 X 10^{-5}$ M ancistrotectorine and methysergide ($1 X 10^{-7}$ M) braught about the inhibition of the concentration to a higher degree than using ancistrotectorine $1.19 X 10^{-5}$ M. The dose-response curves are shown in Figure. 15 Moreover, we found that diltiazem and verapamil inhibited the contractions which induced by NE, KCl, 5-HT, histamine and $CaCl_2$ and applying the combination of these agents (diltiazem or verapamil) with ancistrotectorine, the inhibitory effect was obtained greater than applying only diltiazem or verapamil. The tracing are illustrated in Figure. 16, 17.

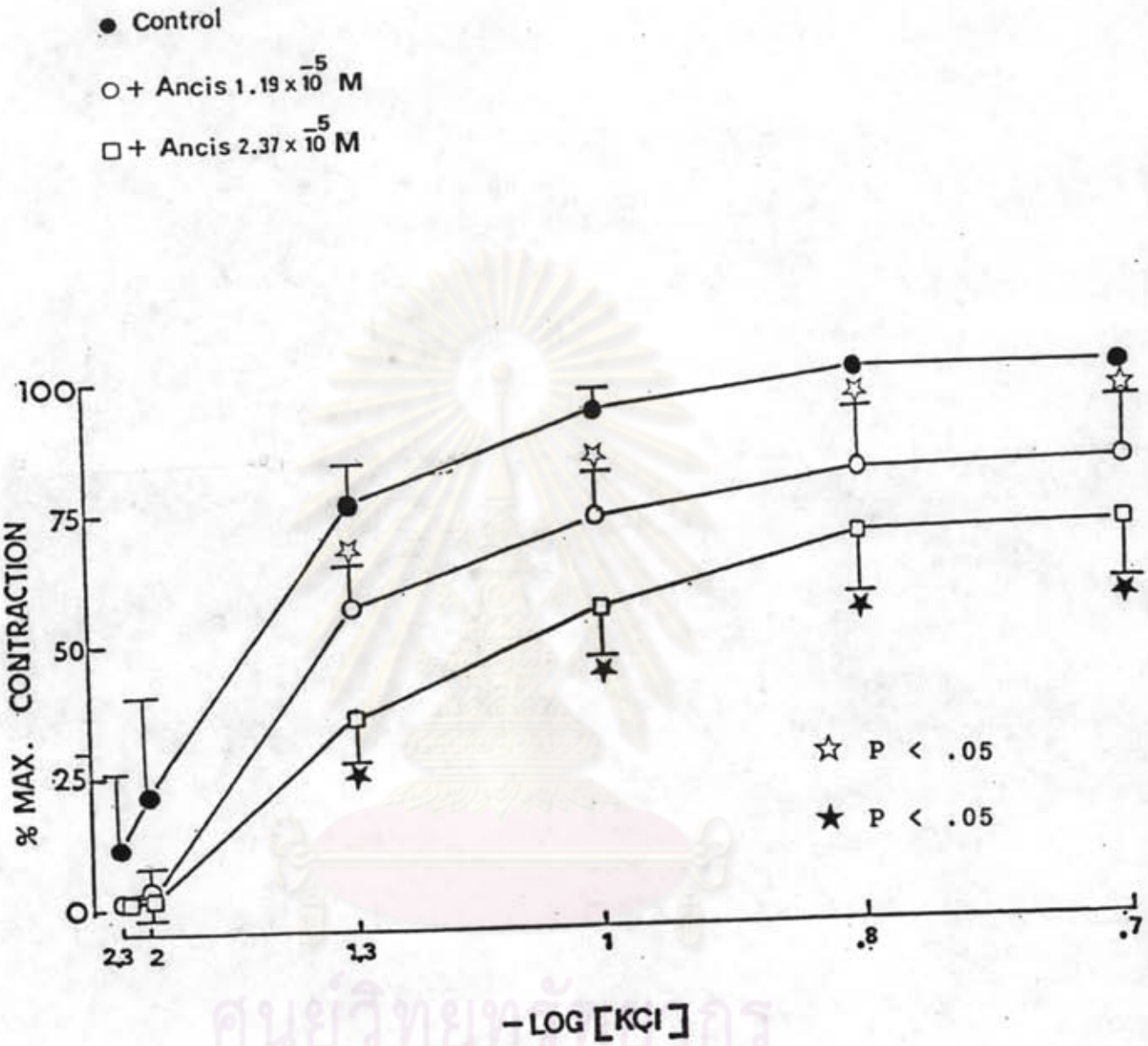

Figure. 6 The cumulative concentration-response curves for KCl-induced contractions of rat aorta in the presence of two concentrations of ancistrotectoine (after a pre-incubation time of 15 min) Graph was represented of the mean S.E.M. of % maximum contraction.

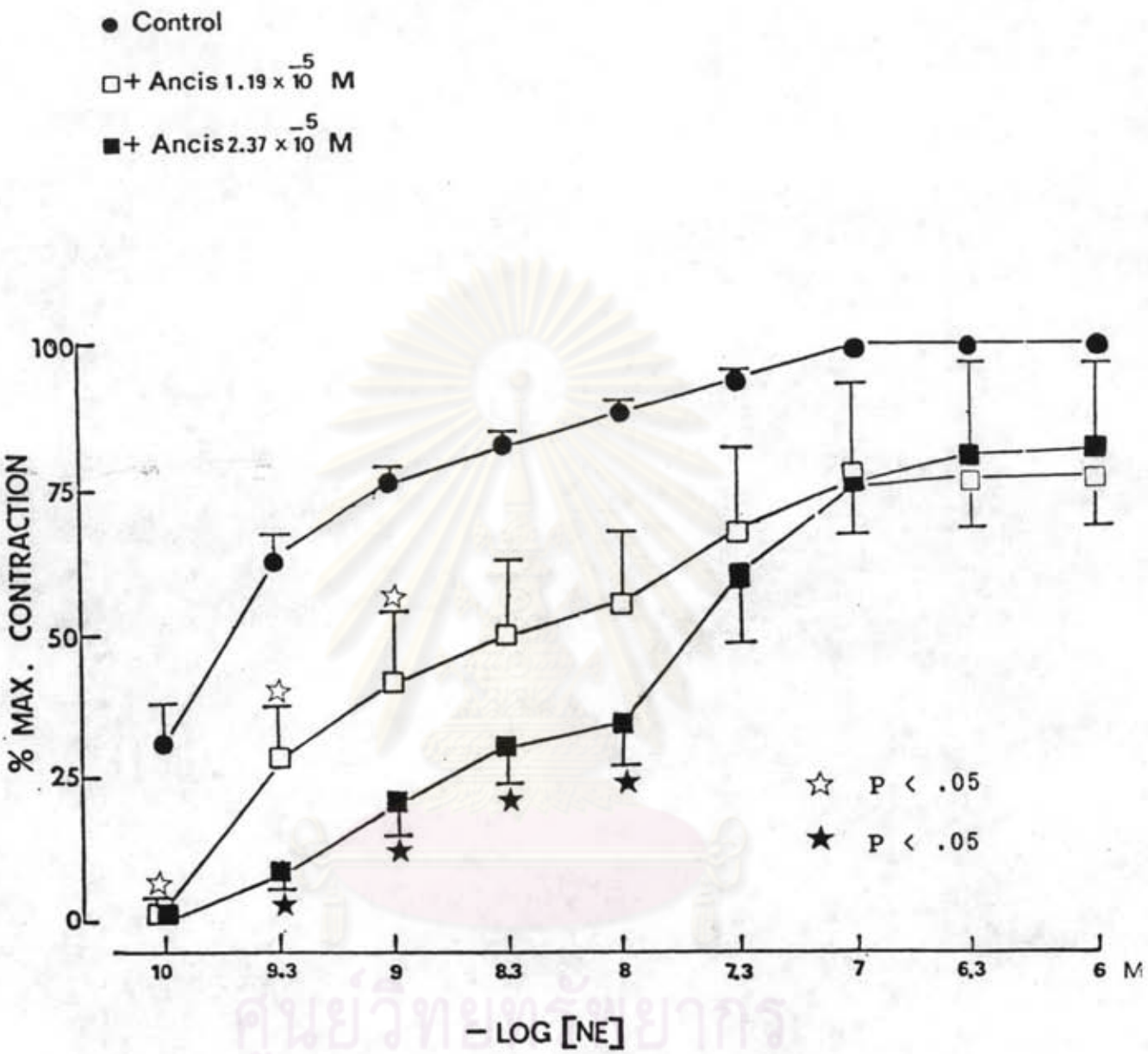

Figure. 7 The cumulative concentration-response curves for NE-induced contractions of rat aorta in the presence of two concentrations of ancistrotectoine (after a pre-incubation time of 15 min) Graph was represented of the mean S.E.M. of % maximum contraction.

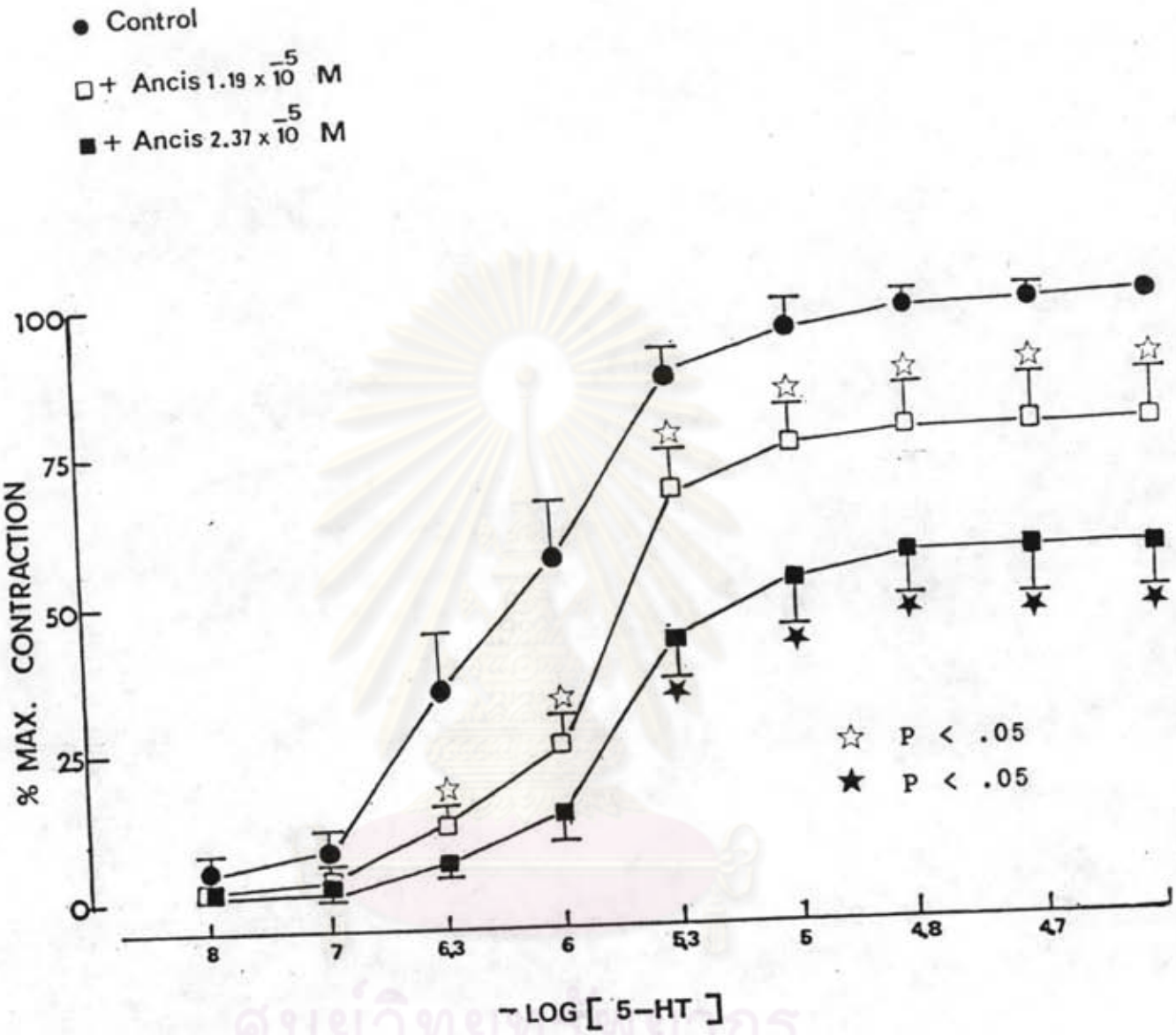

Figure. 8 The cumulative concentration-response curves for 5-HT-induced contractions of rat aorta in the presence of two concentrations of ancistrotectoine (after a pre-incubation time of 15 min) Graph was represented of the mean S.E.M. of % maximum contraction.

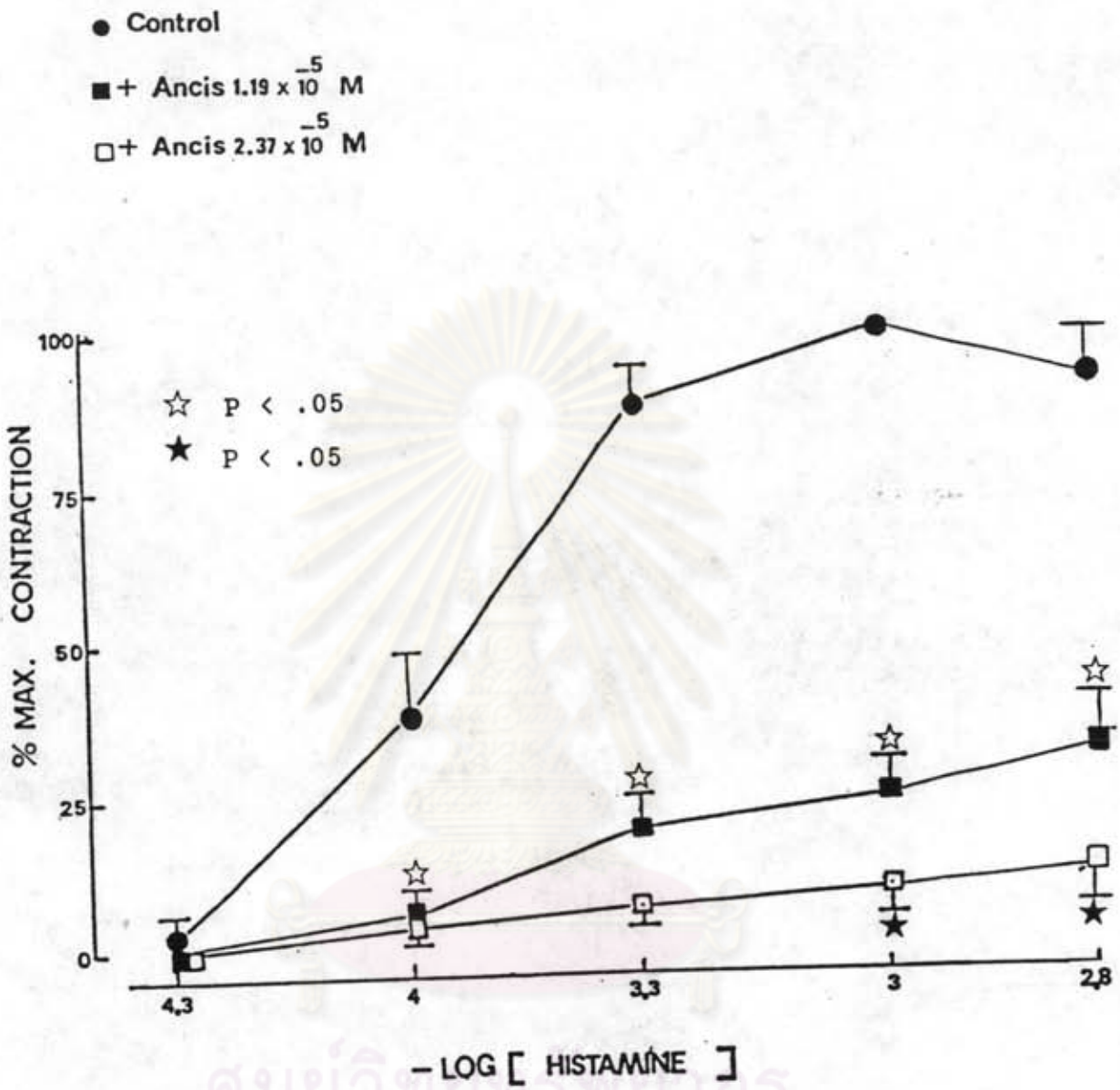

Figure. 9 The cumulative concentration-response curves for histamine -induced contractions of rat aorta in the presence of two concentrations of ancistrotectorine (after a pre-incubation time of 15 min) Graph was represented of the mean S.E.M. of % maximum contraction.

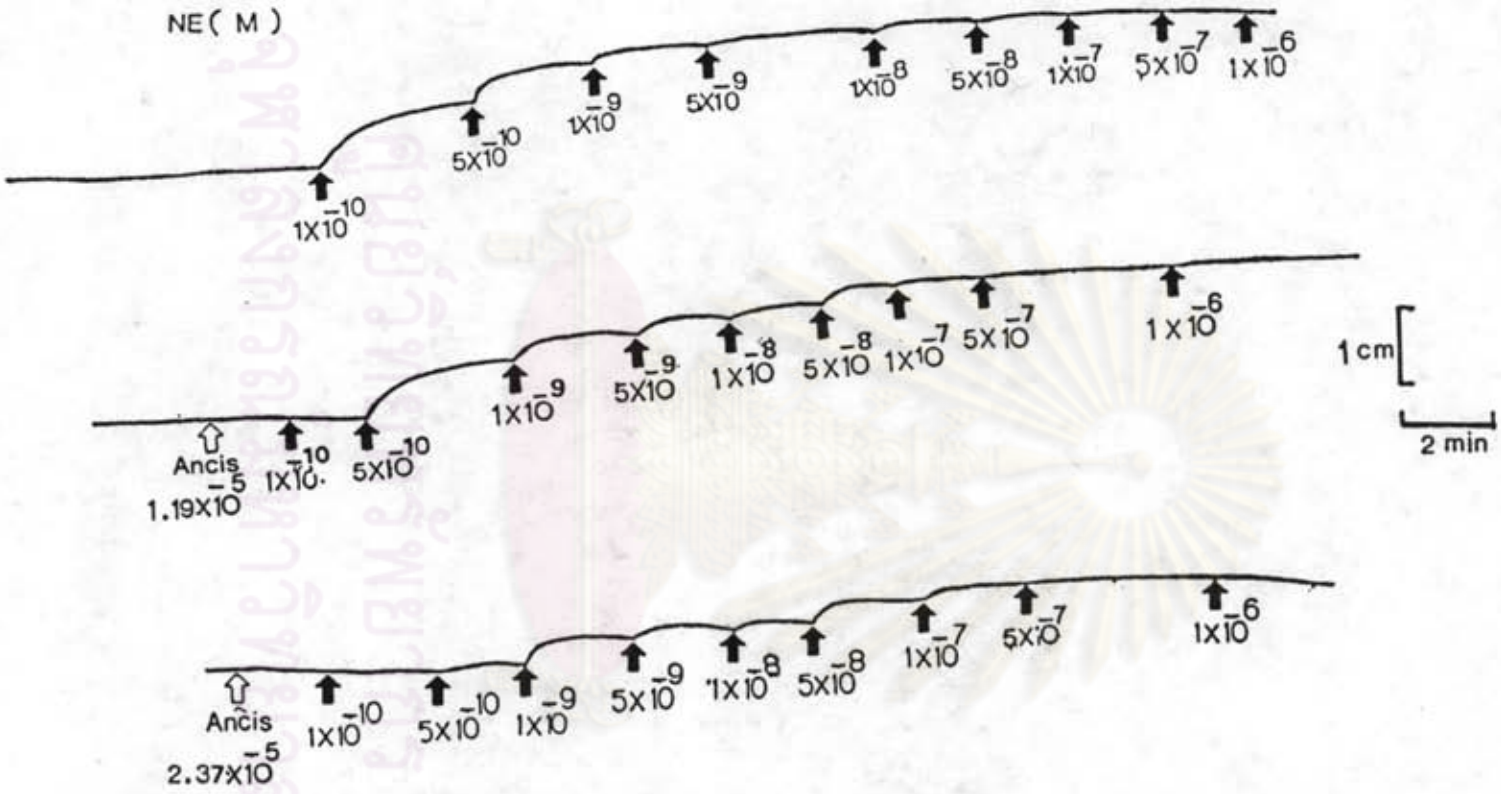

Figure. 10 Typical tracings illustrating the effects of ancistrotectorine on the contractile responses of the rat aorta to NE.

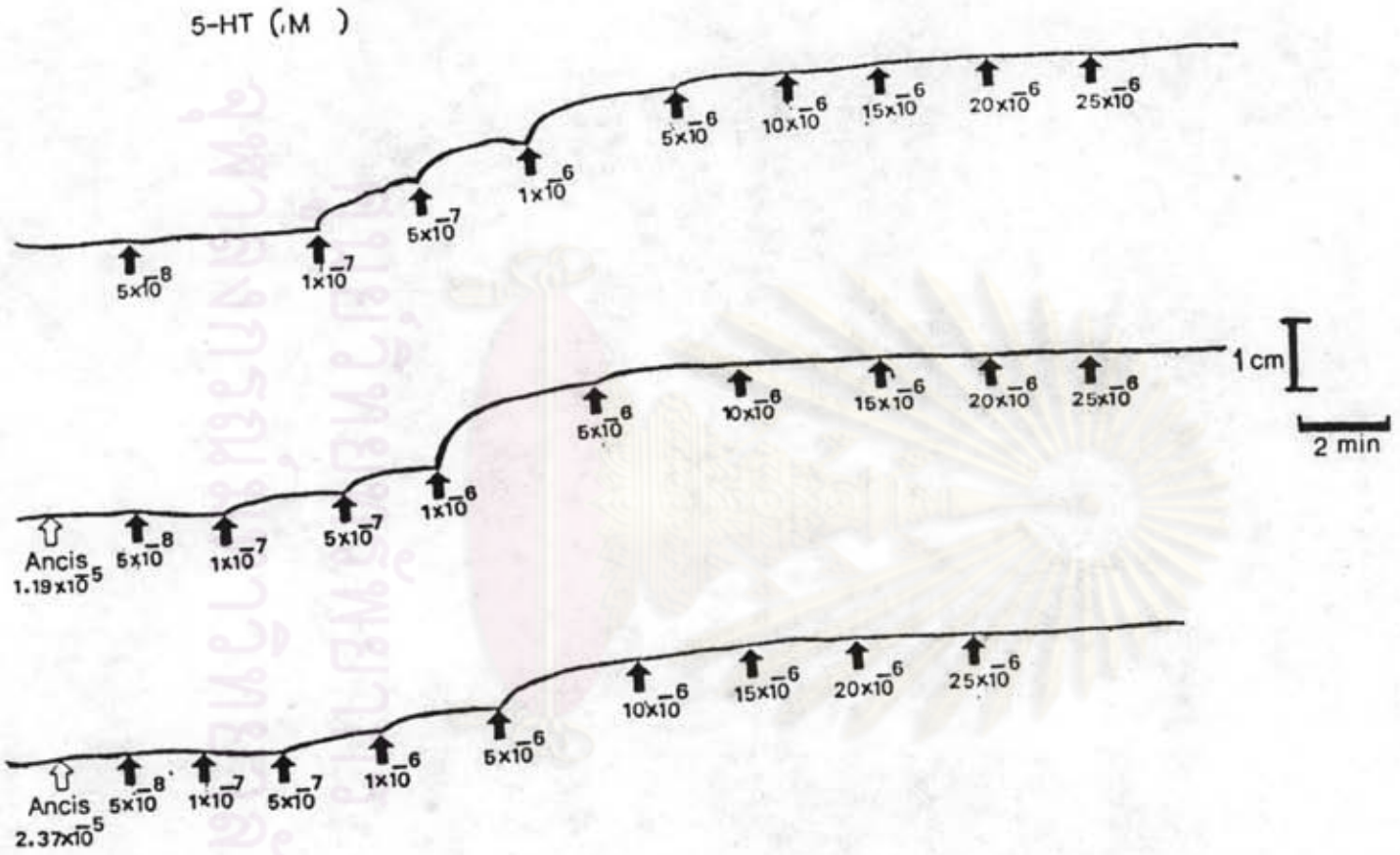

Figure. 11 Typical tracings illustrating the effects of ancistrotectorine on the contractile responses of the rat aorta to 5-HT.

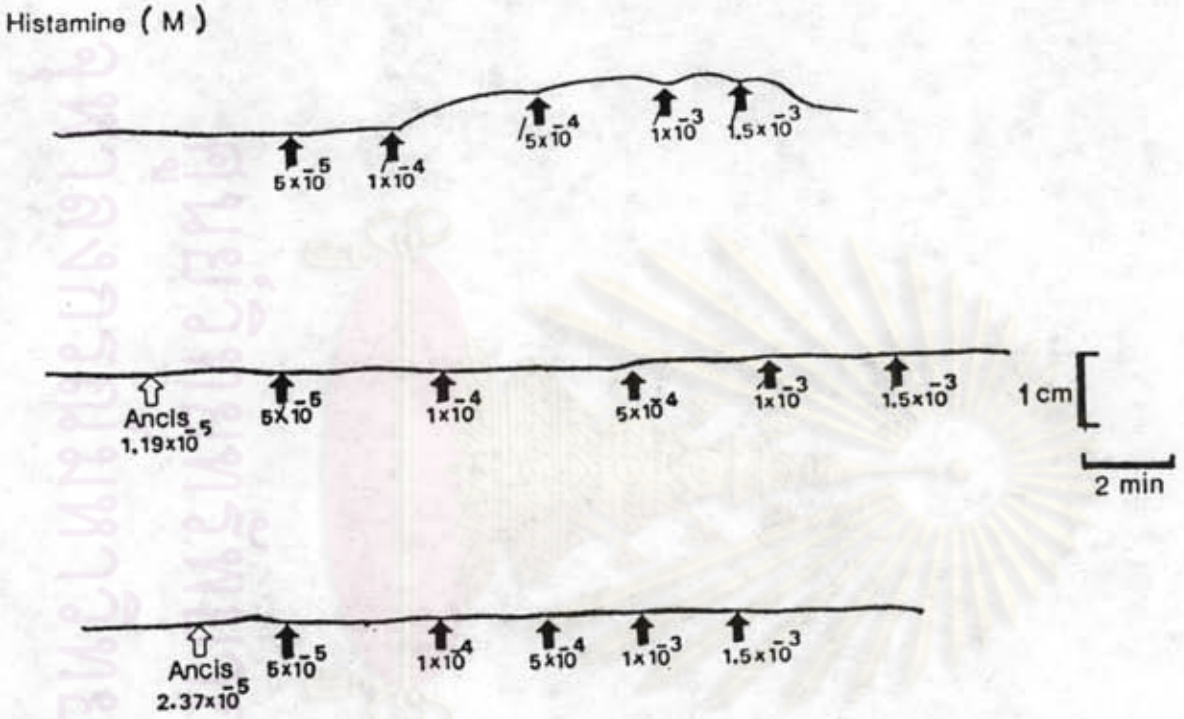

Figure. 12 Typical tracings illustrating the effects of ancistrotectorine on the contractile responses of the rat aorta to histamine.

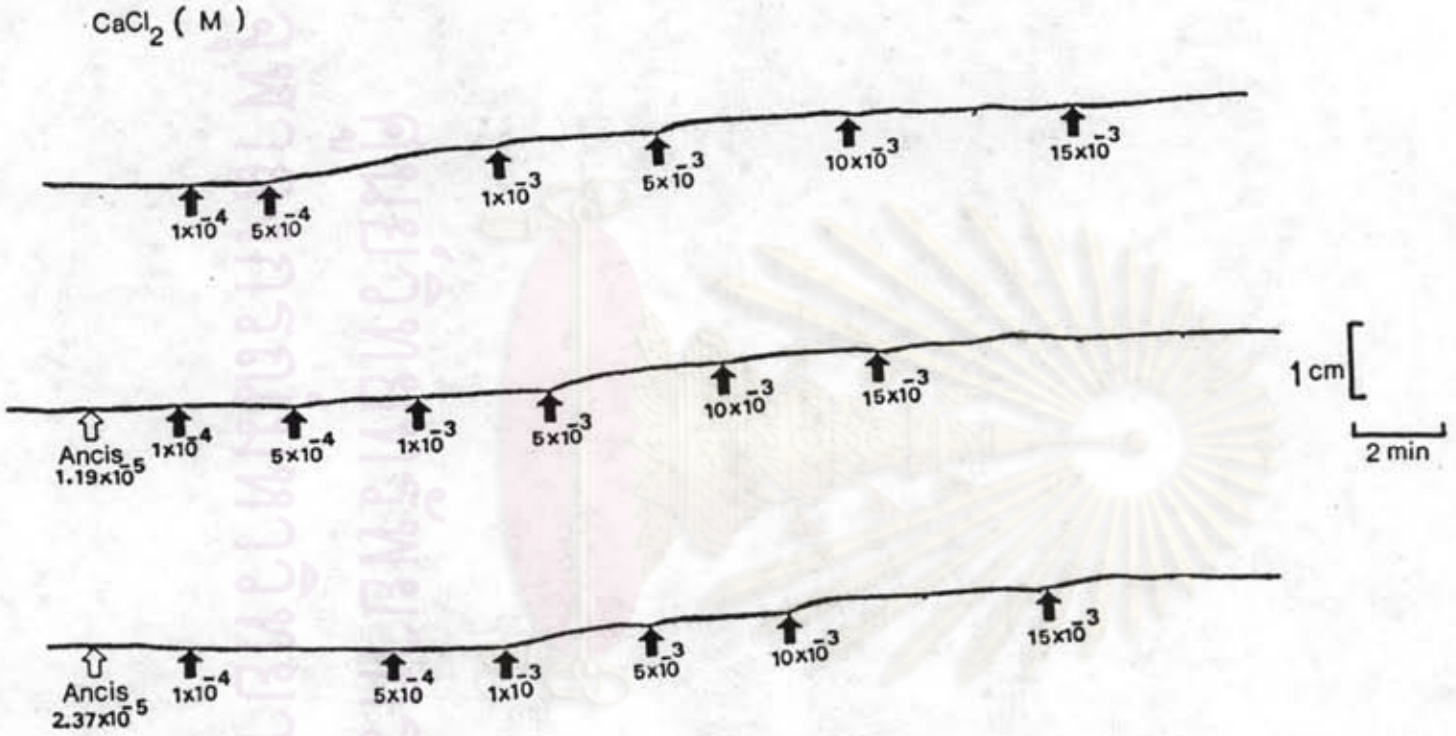

Figure. 13 Typical tracings illustrating the effects of ancistrotectorine on the contractile responses of the rat aorta to $CaCl_2$

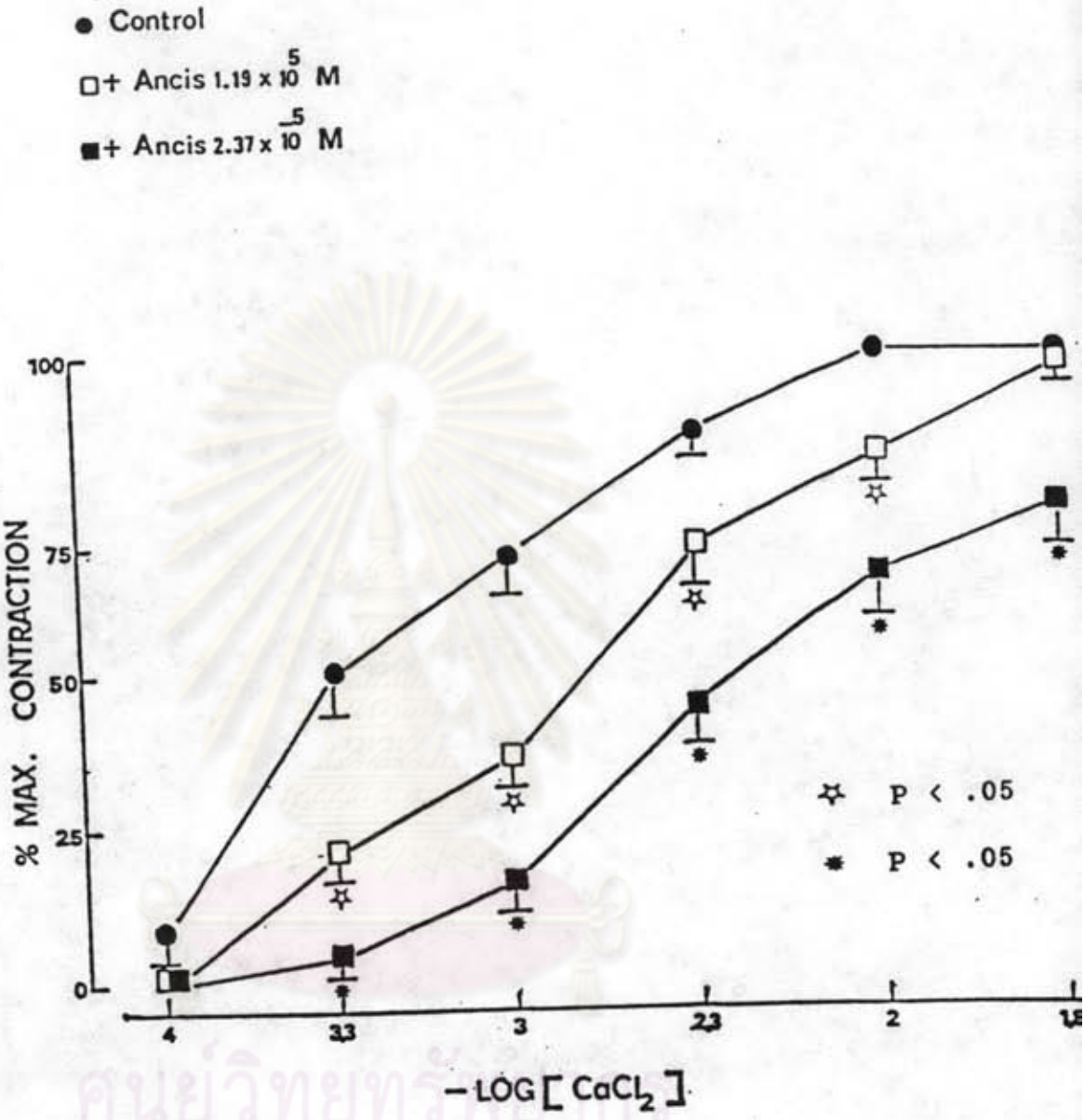

Figure. 14 The cumulative concentration-response curves for $CaCl_2$-induced contractions of rat aorta in the presence of two concentrations of ancistrotectoine (after a preincubation time of 15 min) Graph was represented of the mean S.E.M. of % maximum contraction.

CONDITION	EC_{50} of 5-HT(M)
5-HT	4.04×10^{-7}
Control	5.78×10^{-7}
Methysergide 1×10^{-7} M	1.97×10^{-7}
+ Ancistrotectorine 1×10^{-7} M	3.67×10^{-6}
+ Diltiazem 1×10^{-7} M	2.59×10^{-5}
+ Verapamil 1×10^{-7} M	

Table. 1 EC_{50} of 5-HT in various conditions.

CONDITION	EC_{50} of NE(M)
NE	
Control	9.00×10^{-10}
+ Ancistrotectorine 1.00×10^{-7} M	2.37×10^{-8}
+ Diltiazem 1×10^{-7} M	1.29×10^{-8}
+ Verapamil 1×10^{-7} M	3.27×10^{-8}

Table. 2 EC_{50} of NE in various conditions.

CONDITION	EC_{50} of $CaCl_2$ (M)
Control	8.01×10^{-4}
+ Ancistrotectorine 1×10^{-7} M	5.04×10^{-3}
+ Diltiazem 1×10^{-7} M	2.10×10^{-3}
+ Verapamil 1×10^{-7} M	2.20×10^{-2}

Table. 3 EC_{50} of $CaCl_2$ in various conditions.

CONDITION	EC_{50} of KCl(M)
KCl	
Control	2.28×10^{-3}
+ Ancistrotectorine 1×10^{-7} M	3.37×10^{-2}
+ Diltiazem 1×10^{-7} M	5.94×10^{-2}
+ Verapamil 1×10^{-7} M	1.78×10^{-1}

Table. 4 EC_{50} of KCl in various conditions.

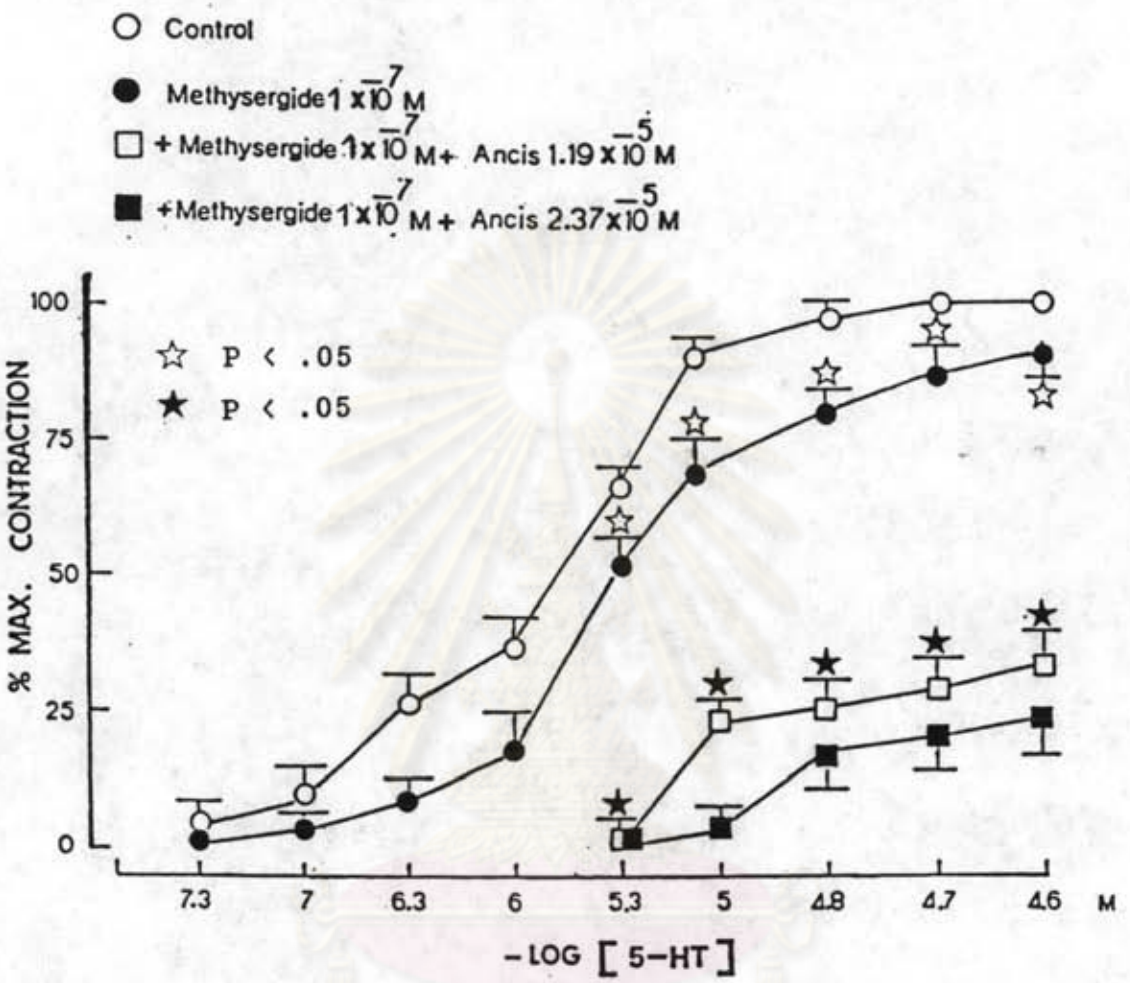

Figure. 15 The effect of ancistrotectorine on contractions induced by the cumulative addition of 5-HT in the presence of methysergide

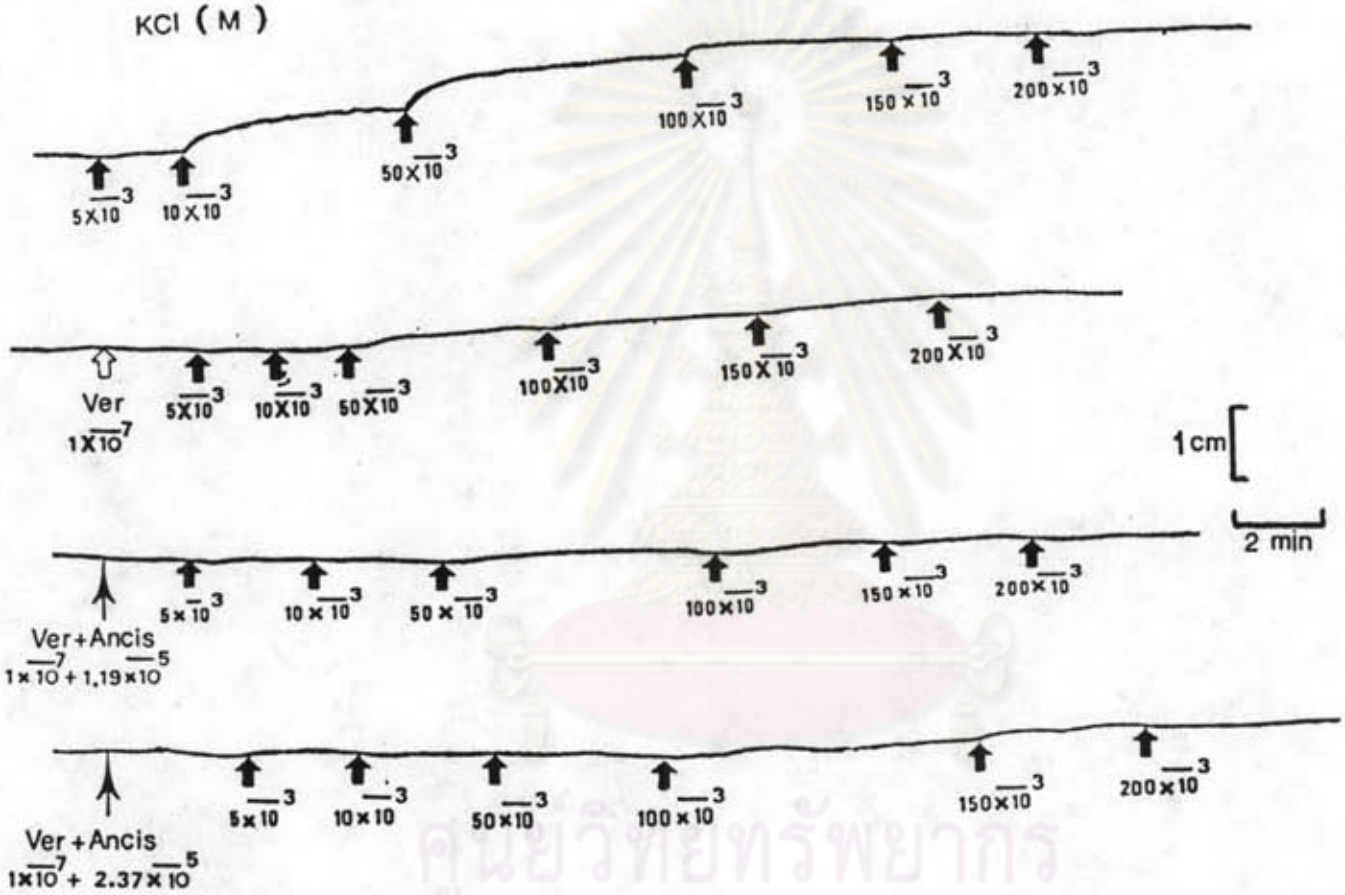

Figure. 16 The effect of ancistrotectorine on contractions induced by the cumulative addition of KCl in the presense of verapamil.

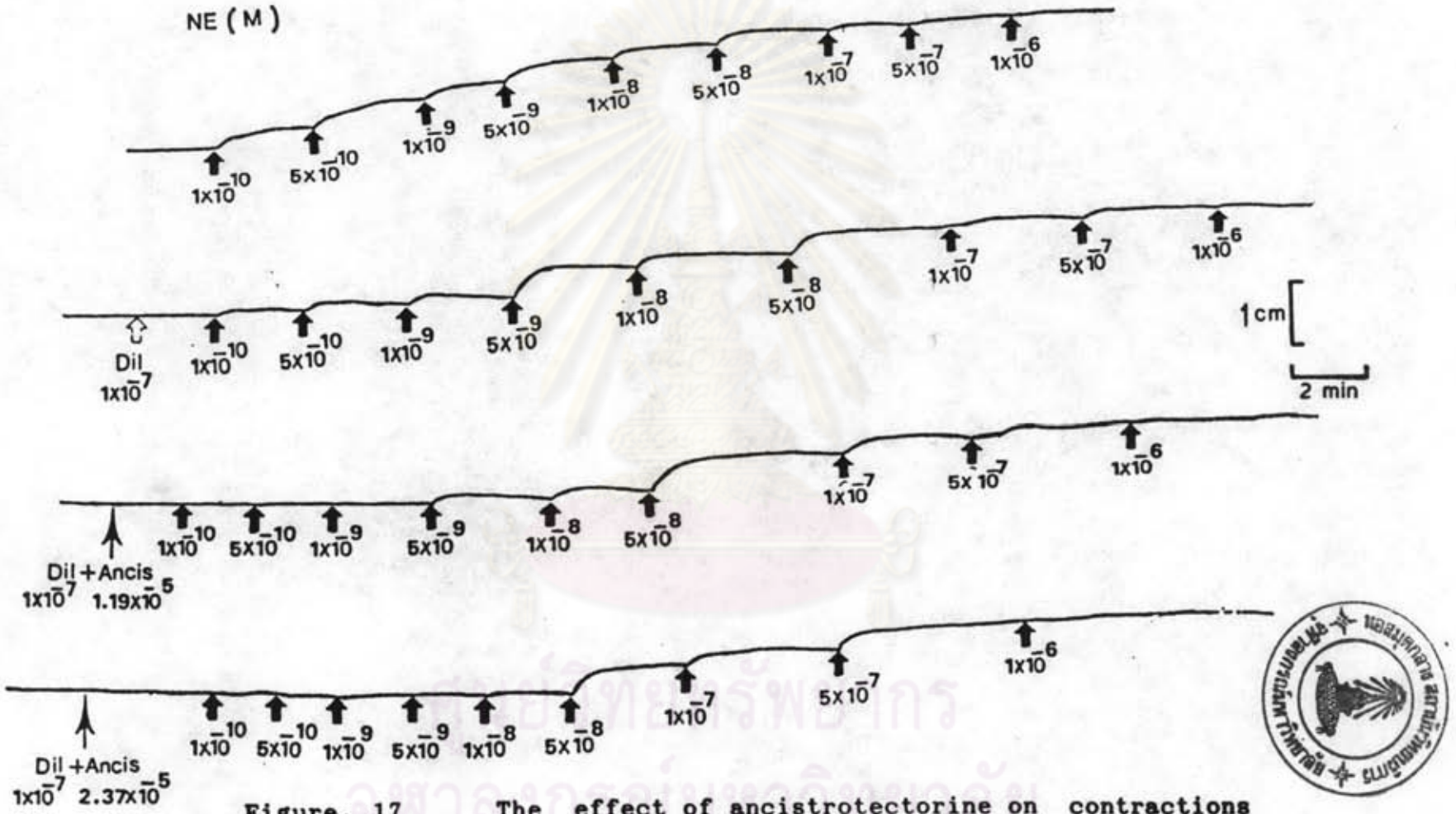

Figure. 17 The effect of ancistrotectorine on contractions induced by the cumulative addition of NE in the presense of diltiazem.