RESULTS

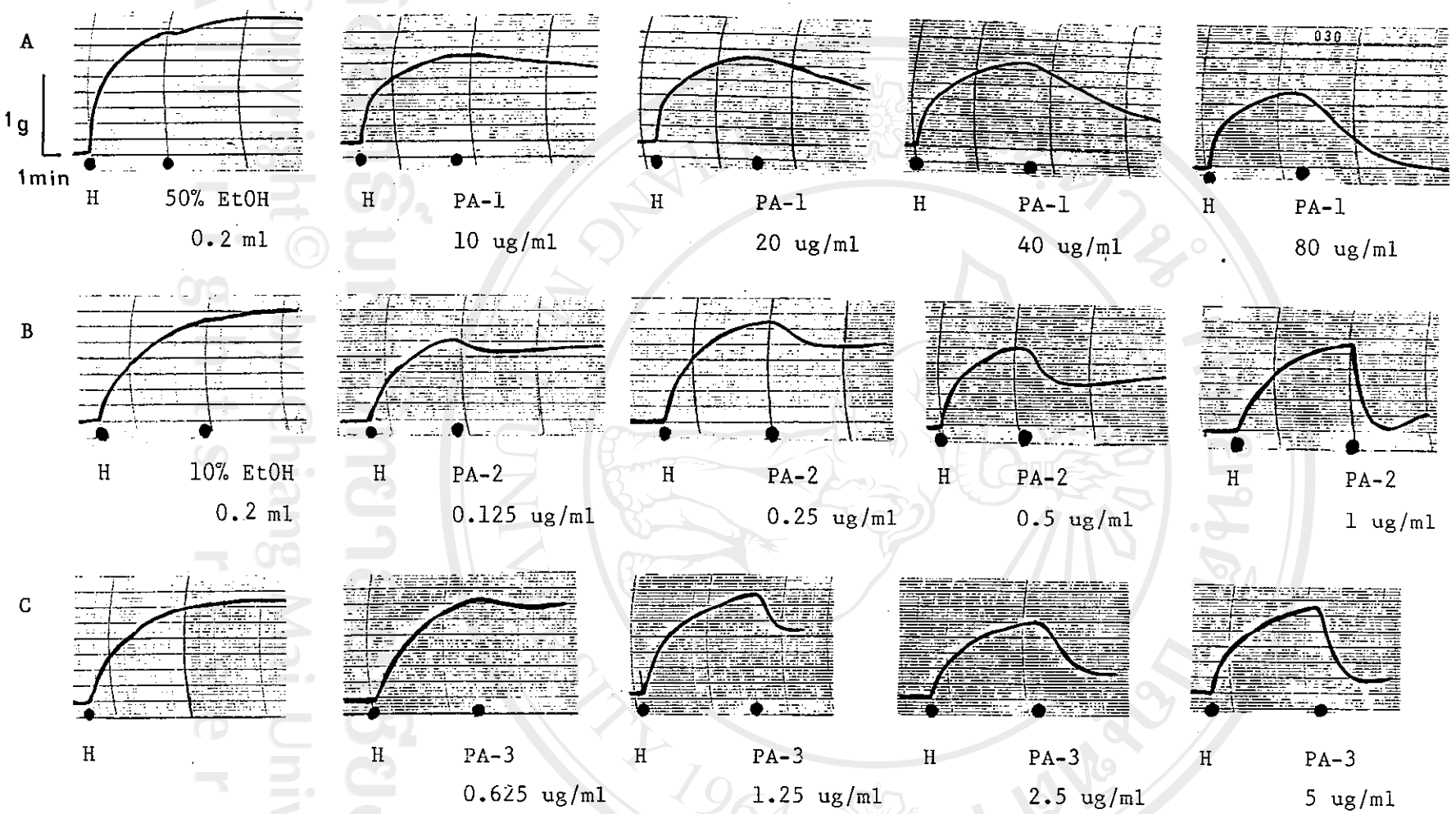
A. Experiments in vitro

1. Effect of phenylalkane derivatives and reference drugs on isolated guinea-pig tracheal chain preparation.

1.1 Screening for potential bronchodilator activity of phenylalkane derivatives.

Bronchodilator activity of five phenylalkane derivatives which are both butane and butene congeners (PA-1, PA-2, PA-3, PA-4 and PA-5) were evaluated on the histamine-induced contraction of the guinea-pig tracheal chain. At appropriate concentration, each of the phenylalkane derivatives exhibited a significant antagonistic effect on a maximal contraction of tracheal muscle caused by 0.3 ug/ml of histamine as shown in Fig. 7 and Table 4.

The dose-response relationship between various doses of phenylalkane derivatives and the percent relaxation of the histamine-induced contraction (by employing the least Square method) was expressed as linear regression equations which were found to be $Y = - 613.64 + 89.40 X$ (PA-1), $Y = - 466.16 + 93.24 X$ (PA-2), $Y = - 482.68 + 86.38 X$ (PA-3), $Y = - 541.47 + 75.95 X$ (PA-4) and $Y = - 472.06 + 70.12 X$ (PA-5) and are graphically illustrated in Fig 8. Because of the marked positive correlation coefficient values ($r = 0.9888$, 0.9888, 0.9702, 0.9605 and 0.9917 for PA-1, PA-2, PA-3, PA-4 and PA-5, respectively) of



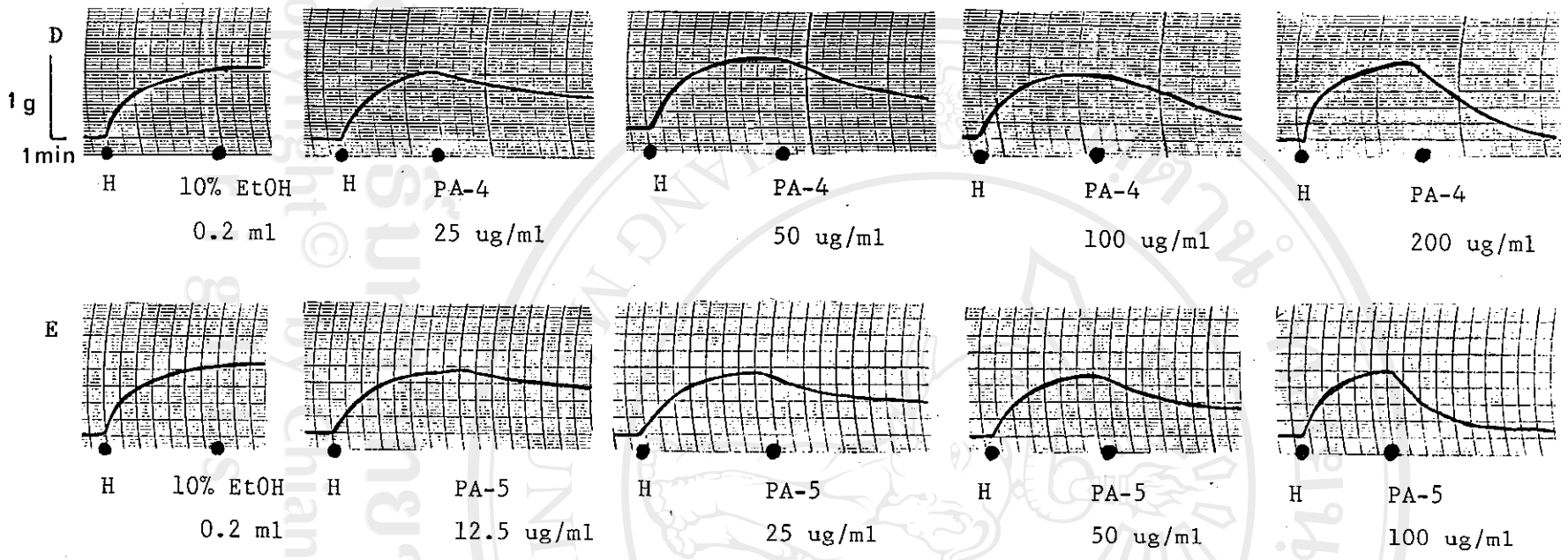


Fig. 7 Effect of phenylalkane derivatives, PA-1 (panel A), PA-2 (panel B) PA-3 (Panel C) PA-4 (panel D) and PA-5 (panel E), on histamine-induced contraction of the isolated guinea-pig tracheal chain

Table 4 Effect of phenylalkane derivatives (PAs) on histamine-induced contraction of isolated guinea-pig tracheal chain.

PAs (ug/ml)	% relaxation
PA-1	
10.00	19.89 ± 2.86
20.00	28.87 ± 1.85
40.00	63.24 ± 1.78
80.00	98.14 ± 1.30
PA-2	
0.125	15.04 ± 1.57
0.25	32.72 + 3.15
0.50	61.27 + 4.54
1.00	97.33 + 2.67
PA-3	
0.625	19.49 ± 2.71
1.25	42.69 ± 3.22
2.50	68.83 ± 3.53
5.00	97.14 ± 1.32
PA-4	
25.00	21.78 ± 3.44
50.00	48.04 ± 4.39
100.00	60.64 ± 3.17
200.00	88.49 ± 1.54
PA-5	
12.50	29.17 ± 4.69
25.00	43.42 ± 2.61
50.00	63.58 ± 2.96
100.00	92.81 ± 1.09

Value expressed as mean ± S.E.M., (n = 6)

Dose of histamine = 0.3 ug/ml

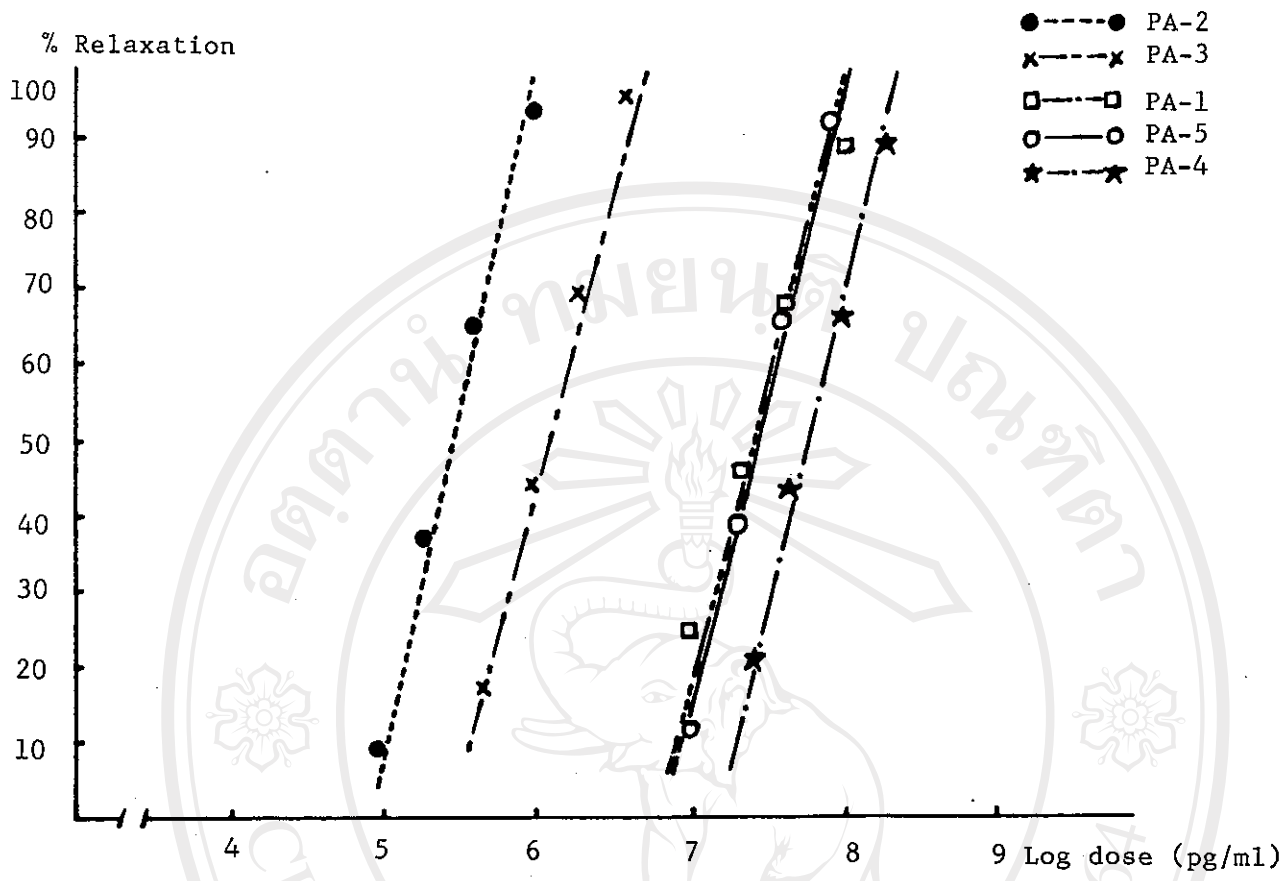


Fig. 8 Comparison of the dose-response relationship of phenylalkane derivatives on the histamine-induced contraction of isolated guinea-pig tracheal chain.

The dose-response relationship was expressed as a linear regression equation Y = a +bX, and the correlation coefficinet (r) :

PA-2	Y = - 466.16 + 93.24 X	r = 0.9888	EC_{50} = 0.35 ug/ml
PA-3	Y = - 482.68 + 86.38 X	r = 0.9702	EC_{50} = 1.47 ug/ml
PA-1	Y = - 613.64 + 89.40 X	r = 0.9888	EC_{50} = 26.05 ug/ml
PA-5	Y = - 472.06 + 70.12 X	r = 0.9917	EC_{50} = 27.88 ug/ml
PA-4	Y = - 541.47 + 75.95 X	r = 0.9605	EC_{50} = 61.32 ug/ml

the dose-response relationships of the phenylalkane derivatives, it indicated that the antagonistic effect of those phenylalkane derivatives on the histamine-induced tracheal contraction is dose-related ($P < 0.05$).

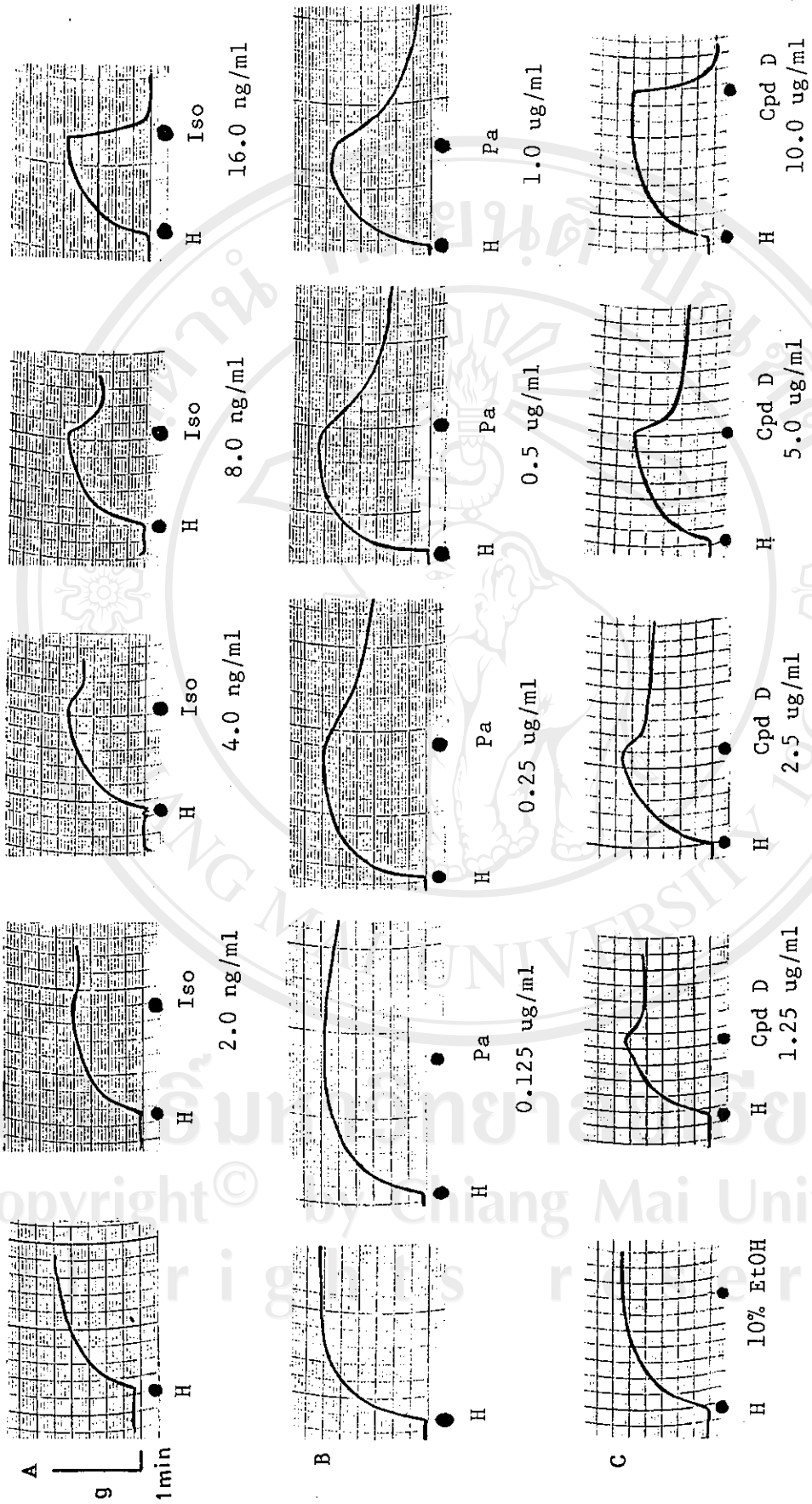
The antagonistic effect of the phenylalkane derivatives gradually increased as higher concentrations were used. The doses of the phenylalkane derivatives which caused maximum relaxation of the histamine-induced tracheal contraction were 80, 1, 5, 200 and 100 ug/ml for PA-1, PA-2, PA-3, PA-4 and PA-5, respectively. By employing an Analysis of Variance, it was found that the linear regression lines of PA-1, PA-2, PA-3, PA-4 and PA-5 were parallel to each other ($P < 0.05$).

Bronchodilator activity of these phenylalkane derivatives was compared at doses which caused a 50% inhibition of histamine-induced trachea contraction (EC_{50}), It was found that PA-2 was the most potent (EC_{50} = 0.34 ug/ml) followed by PA-3 (EC_{50} = 1.47 ug/ml), PA-1 (EC_{50} = 26.05 ug/ml), PA-5 (EC_{50} = 27.88 ug/ml) and PA-4 (EC_{50} = 61.32 ug/ml), respectively.

Although PA-2 exhibited the highest potency among the five phenylalkane derivative, it is not soluble in distilled water or NSS. The solvent used for PA-2 in the isolated experiment was 10% ethanol, which can potentiate the agonist-induced bronchoconstriction when injected intravenously to the animals in the in vivo experiment. PA-3, the second potent congener, which is soluble in NSS, was therefore selected to be the representative of the phenylalkane derivatives for the detail study.

1.2 Comparison of the dose-response relationship of PA-3 with reference drugs.

A maximum contraction of guinea-pig tracheal muscle preparation occurred within 5-7 min after challenge with histamine. The dose-response relationships of the PA-3 and reference drugs (aminophylline, isoproterenol, papaverine and verapamil) as well as compound D, a proven bronchodilator (Kiatyingunsulee et al, 1979; Soparat, 1984) were examined. Addition of PA-3 at the concentration of 0.625, 1.25, 2.5 and 5 ug/ml to contracted tracheal muscle, induced by histamine, resulted in a dose dependent relaxation of tracheal muscle. Fig. 9 A, 9 B, 9 C, 9 D and 9 E illustrate the antagonistic effect of isoproterenol, papaverine, compound D, verapamil and aminophylline, respectively, on the maximal contraction of tracheal muscle induced by histamine, and the results obtained are shown in Table 5. By the least Square method, the linear regression lines of the dose-response relationship of reference drugs were determined and are graphically illustrated according to their regression equations in Fig. 10.



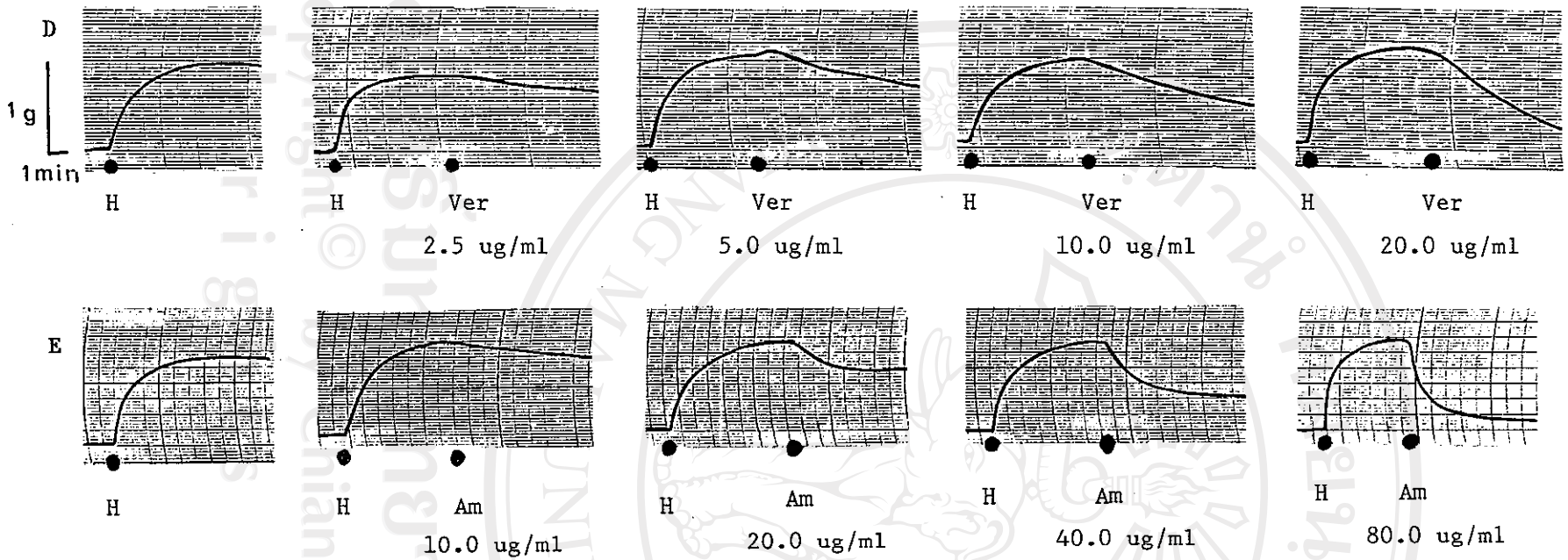


Fig. 9 Effect of isoproterenol (Iso) (panel A), papaverine (Pa) (panel B), compound D (panel D) verapamil (ver) (panel D), and aminophylline (Am) (panel E) on histamine-induced contraction of isolated guinea-pig tracheal chain.

Table 5 Effect of isoproterenol, papaverine, compound D, verapamil and aminophylline on histamine-induced contraction of isolated guinea-pig tracheal chain.

Drugs	% relaxation
Isoproterenal (ng/ml)	
2	9.38 ± 1.95
4	27.05 ± 3.29
8	52.17 ± 3.34
16	100.00 ± 0.00
Papaverine (ug/ml)	
0.125	36.99 ± 3.21
0.25	54.32 ± 2.90
0.50	77.41 ± 2.95
1.00	95.26 ± 1.20
Compound D (ug/ml)	
1.25	20.38 ± 3.57
2.50	34.23 ± 2.09
5.00	51.22 ± 2.94
10.00	93.45 ± 2.77
Verapamil (ug/ml)	
2.50	22.03 ± 2.46
5.00	36.35 ± 3.78
10.00	73.14 ± 1.30
20.00	96.26 ± 1.20
Aminophylline (ug/ml)	
10.00	22.06 ± 0.92
20.00	39.36 ± 1.25
40.00	58.98 ± 0.35
80.00	94.36 ± 0.36

Value expressed as mean ± S.E.M., (n = 6)

Dose of histamine = 0.3 ug/ml

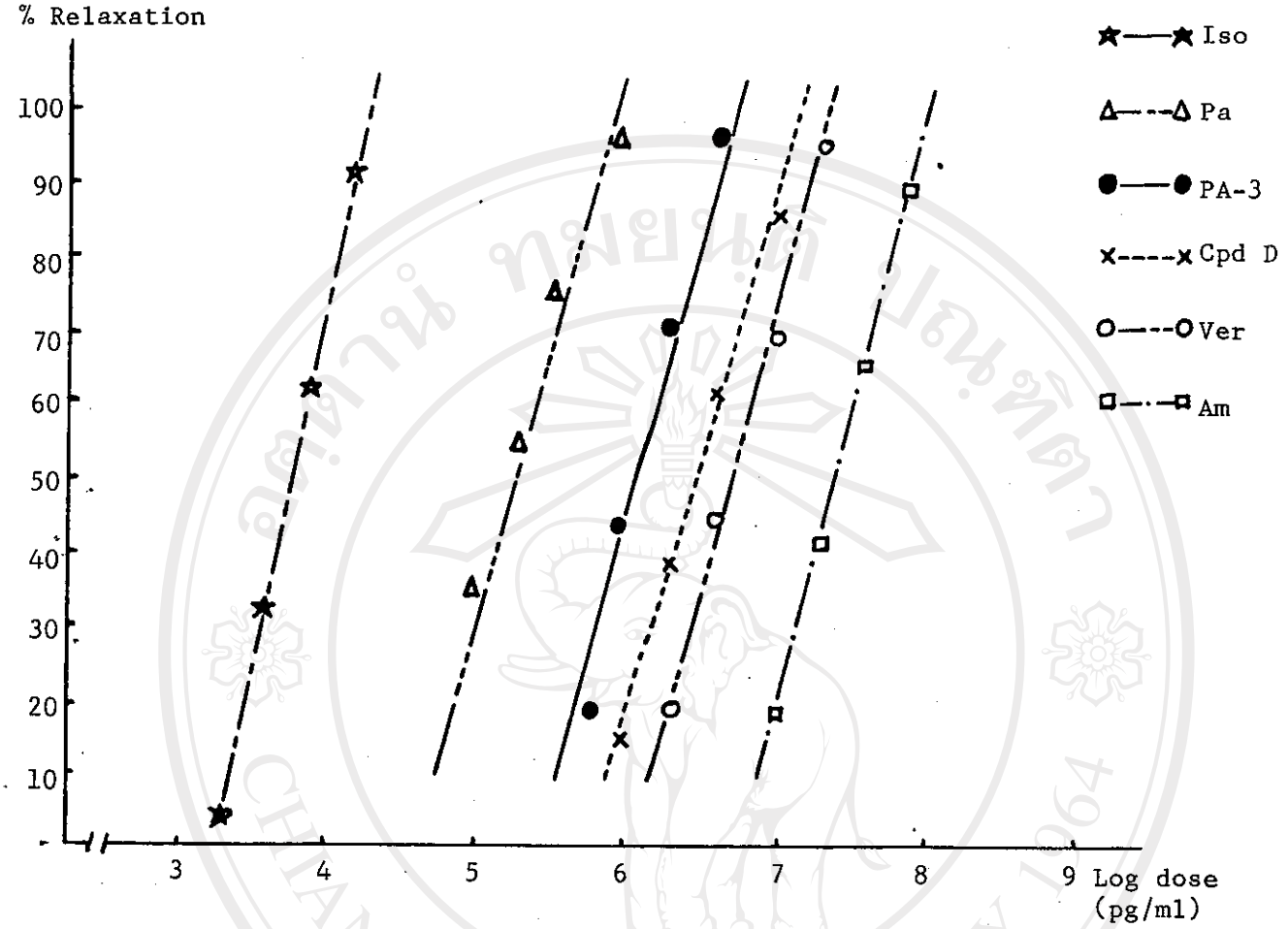


Fig 10 Comparison of the dose-response relationship of PA-3 and reference drugs on the histamine-induced contraction of isolated guinea-pig tracheal chain.

The dose-response relationship was expressed as linear regression equation of Y = a + bX, and the correlation coefficient (r) :

Isoproterenol	Y = -323.07 + 98.66 X	r = 0.9981	EC_{50} = 6.04 X 10^{-3} ug/ml
Papaverine	Y = -306.83 + 67.16 X	r = 0.9910	EC_{50} = 0.21 ug/ml
PA-3	Y = -482.68 + 86.38 X	r = 0.9702	EC_{50} = 1.47 ug/ml
Compound D	Y = -463.97 + 78.46 X	r = 0.9986	EC_{50} = 3.55 ug/ml
Verapamil	Y = -533.44 + 86.19 X	r = 0.9625	EC_{50} = 5.88 ug/ml
Aminophylline	Y = -531.76 + 78.57 X	r = 0.9934	EC_{50} = 25.37 ug/ml

The order of antagonistic potency of those tested drugs on histamine-induced tracheal contraction, by comparison of the EC_{50} values, from high to low is as follows: isoproterenol, papaverine, PA-3, compound D, verapamil and aminophylline, of which the EC_{50} values were found to be 6.04×10^{-3} ug/ml, 0.21 ug/ml, 1.47 ug/ml, 3.55 ug/ml, 5.88 ug/ml and 25.37 ug/ml, respectively.

According to their correlation coefficient values, r = 0.9981, 0.9910, 0.9986, 0.9625 and 0.9934 for isoproterenol, papaverine, compound D, verapamil and aminophylline, respectively, it is suggested that the antagonistic effect of those tested drugs is dose-related ($P < 0.05$).

By employing an Analysis of Variance, it was found that the linear regression line of the dose-response relationship of PA-3 was parallel to those of papaverine, compound D, verapamil and aminophylline but not to that of isoproterenol ($P < 0.05$).

1.3 Comparison of the antagonistic effect of PA-3 and reference drugs on histamine-induced tracheal contraction in the presence of beta-adrenergic antagonist.

In order to examine the effect of the PA-3 and reference drugs on a β - adrenergic receptor in the guinea-pig trachea, a dose of each tested drugs i.e. PA-3 (5 ug/ml), isoproterenol (16 ug/ml), papaverine (1 ug/ml), aminophylline (80 ug/ml) and verapamil (20 ug/ml), which exerted a maximum relaxation of histamine-induced contraction, was added

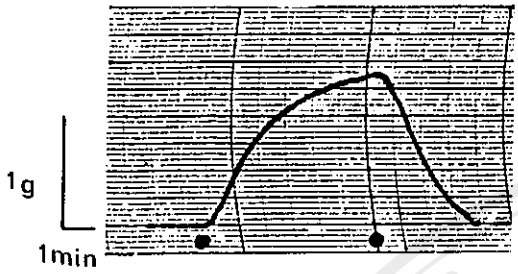
into the tissue bath at the plateau state of the histamine-induced tracheal contraction after pretreatment with 0.25 ug/ml of propranolol (a β -adrenergic antagonist). The dose of propranolol used could effectively block the effect of isoproterenol (16 ug/ml) as shown in Fig 11. In the presence of propranolol, PA-3, papaverine, aminophylline and verapamil still possessed the antagonistic effect on the histamine-induced tracheal contraction (Fig. 11) with the same intensity as in the experiment without propranolol.

2. Effect of PA-3 and reference drugs on isolated rat tracheal strip preparation.

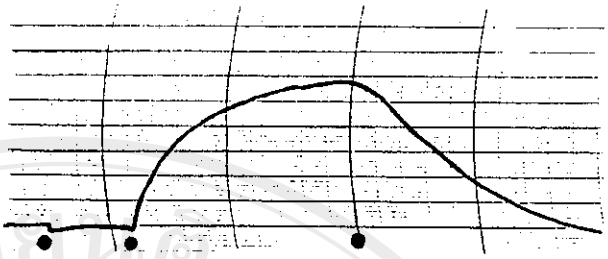
2.1 Effect of PA-3 and reference drugs.

The antagonistic effect of PA-3 was also tested on methacholine-induced rat tracheal muscle contraction. The dose of methacholine (0.4 ug/ml) which produced the submaximal contraction was determined previously and used to induce tracheal contraction. PA-3 and reference drugs exhibited a significant antagonistic effect on the methacholine-induced tracheal contraction (Fig. 12). The percent relaxation of methacholine-induced contraction of PA-3 and reference drugs gradually increased as higher concentrations were used, as shown in Table 6. The doses which caused maximum relaxation of the methacholine-induced contraction of PA-3, atropine, papaverine, verapamil, compound D and aminophylline were found to be 5 mg/ml, 6 ng/ml, 32 ug/ml, 80 ug/ml, 500 ug/ml and 2 mg/ml, respectively.

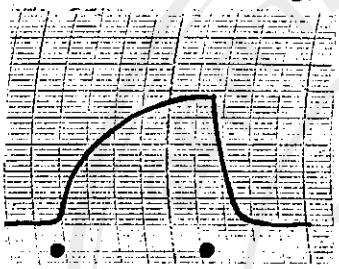
Fig. 11 Antagonistic activity of PA-3 (panel A), isoproterenol (Iso) (panel B), verapamil (Ver) (panel C), papaverine (Pa) (panel D) and aminophylline (Am) (panel E) on histamine-induced tracheal contraction in the presence of propranolol (β -adrenergic antagonist).



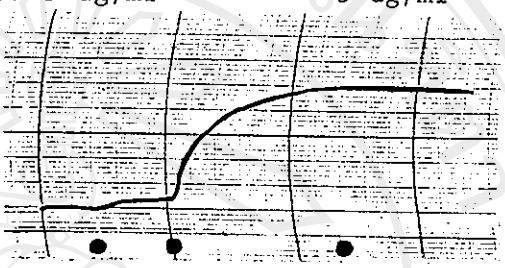
H PA-3
5 ug/ml



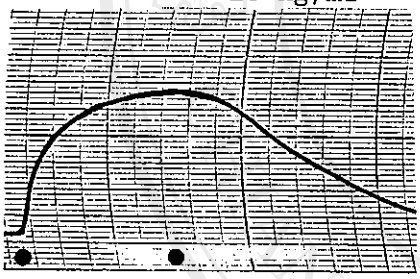
Prop H PA-3
0.25 ug/ml 5 ug/ml



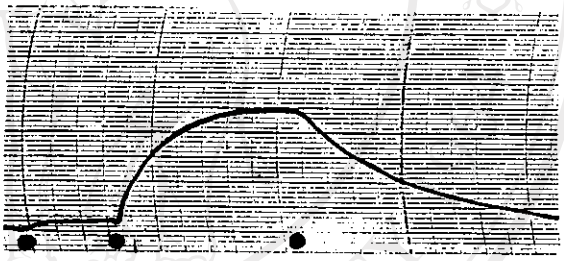
H Iso
16 ng/ml



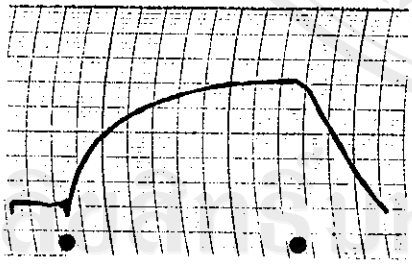
Prop H ISO
0.25 ug/ml 16 ng/ml



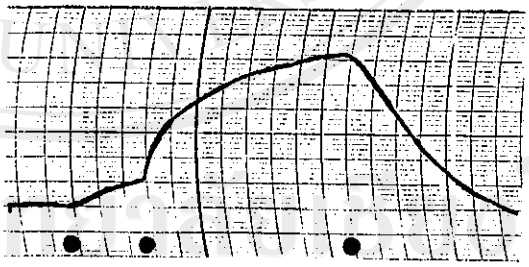
H Ver
20 ug/ml



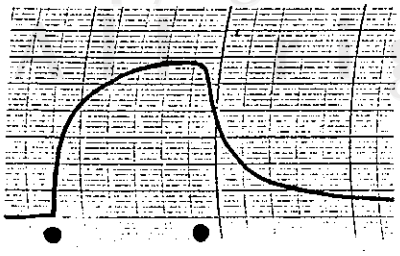
Prop H Ver
0.25 ug/ml 20 ug/ml



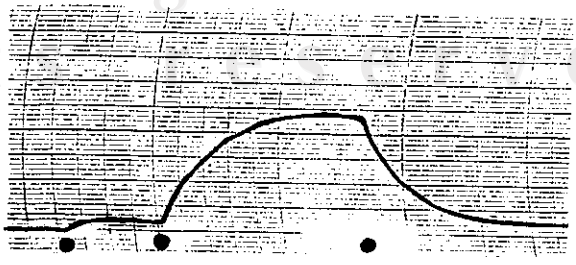
H Pa
1 ug/ml



Prop H Pa
0.25 ug/ml 1 ug/ml

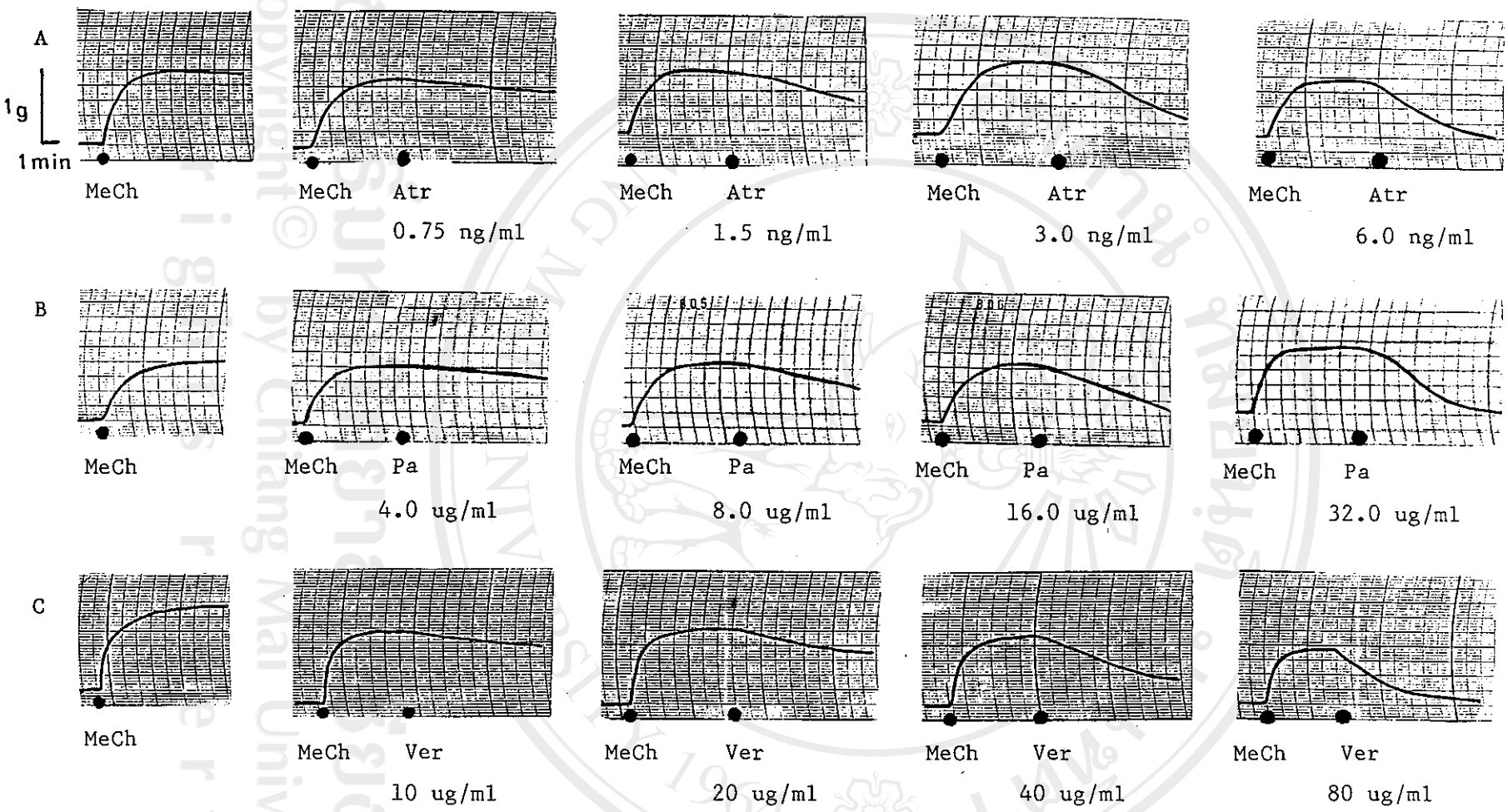


H Am
80 ug/ml



Prop H Am
0.25 ug/ml 80 ug/ml

Fig. 12 Effect of atropine (Atr) (panel A), papaverine (Pa) (panel B), verapamil (Ver) (panel C), aminophylline (Am) (panel D) compound D (Cpd D) (panel E) and PA-3 (panel F) on methacholine-induced contraction of isolated rat tracheal strip.



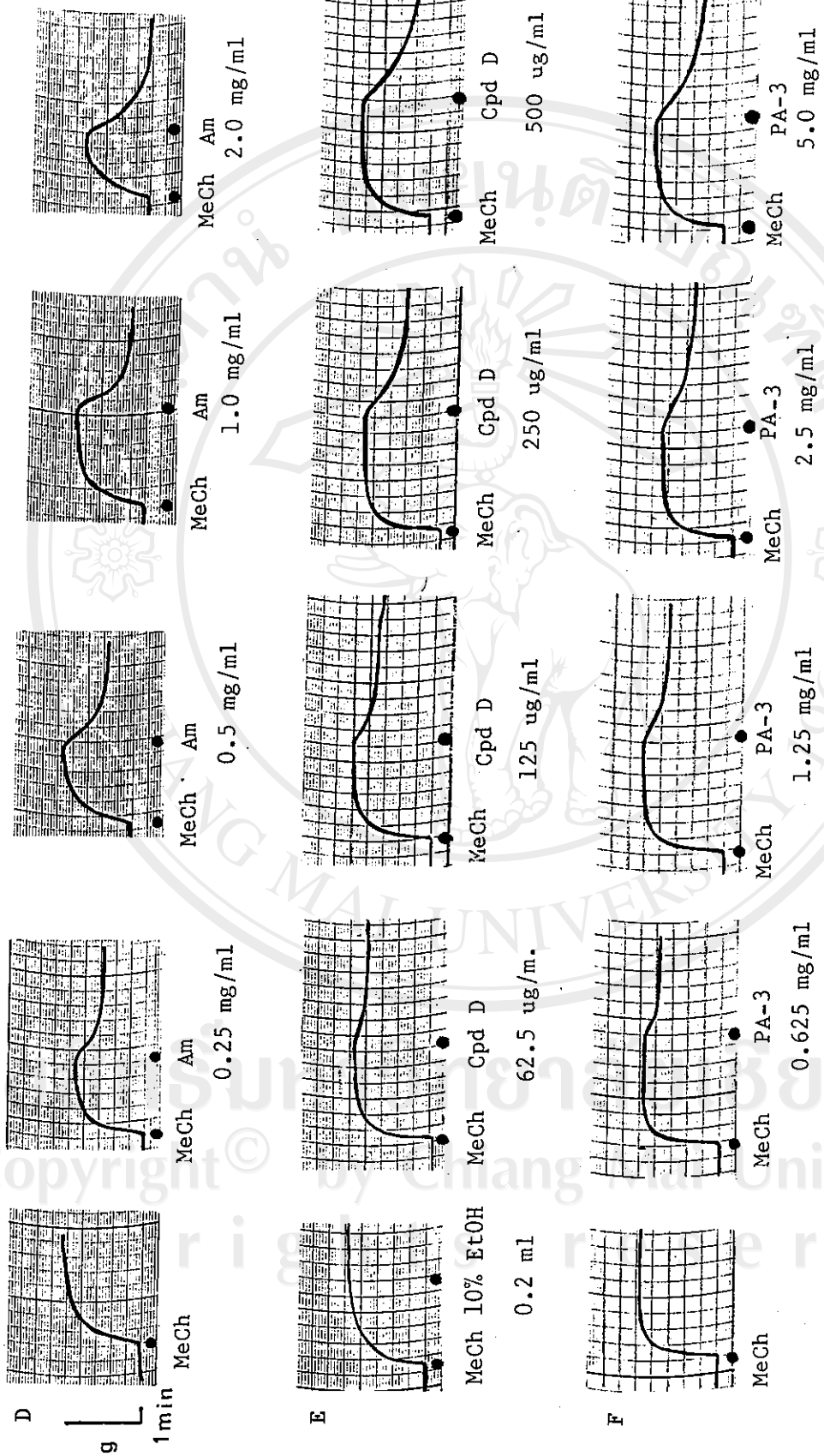


Table 6 Effect of PA-3 and reference drugs (atropine, papaverine, verapamil and aminophylline) as well as compound D on methacholine-induced contraction of isolated rat tracheal strip.

PA-3 and Drugs		% relaxation
Atropine	(ng/ml)	
0.75		18.87 ± 2.62
1.50		32.83 ± 1.46
3.00		76.71 ± 4.57
6.00		89.82 ± 4.32
Papaverine	(ug/ml)	
4.00		23.56 ± 0.75
8.00		39.24 ± 1.57
16.00		68.42 ± 2.40
32.00		85.91 ± 2.91
Verapamil	(ug/ml)	
10.00		18.88 ± 1.91
20.00		36.97 ± 2.92
40.00		56.96 ± 3.02
80.00		90.54 ± 2.63
Compound D	(ug/ml)	
62.50		28.03 ± 0.89
125.00		43.79 ± 2.65
250.00		63.53 ± 5.02
500.00		92.56 ± 2.94
Aminophylline	(mg/ml)	
0.25		28.71 ± 1.25
0.50		48.02 ± 3.96
1.00		60.08 ± 1.77
2.00		89.47 ± 1.85

PA-3 and Drugs	% relaxation
PA-3 (mg/ml)	
0.625	20.41 ± 1.68
1.250	32.35 ± 1.58
2.50	53.91 ± 1.46
5.00	88.78 ± 1.87

Value expressed as mean ± S.E.M., (n = 6)

Dose of methacholine = 0.4 ug/ml

2.2 Comparison of the dose-response relationship of PA-3 with reference drugs.

The dose-response relationship of PA-3 and reference drugs on the methacholine-induced tracheal contraction was evaluated similarly to that on the histamine-induced contraction of guinea-pig trachea. The linear regression lines of the dose-response relationship of PA-3 and reference drugs were determined and graphically illustrated in Fig. 13.

By comparison of the EC_{50} values of the tested drugs on methacholine-induced rat tracheal contraction, atropine was found to be the most potent bronchodilator followed by papaverine, verapamil, compound D, aminophylline and PA-3, of which EC_{50} values were 1.8×10^{-3} ug/ml, 9.85 ug/ml, 27.81 ug/ml 141.03 ug/ml, 572.13 ug/ml and 1,836.22 ug/ml, respectively.

The positive correlation coefficient values of tested drugs ($r = 0.9218, 0.9529, 0.9889, 0.9893, 0.9844$ and 0.9977 for atropine, papaverine, verapamil, compound D, aminophylline and PA-3, respectively), suggest their dose-related manner of the antagonistic effect on the methacholine-induced contraction ($P < 0.01$).

A comparison of the linear regression lines of those tested drugs was done by employing an Analysis of Variance, it was found that the linear regression line of the dose-response relationship of PA-3 was parallel to those of aminophylline, verapamil, papaverine and compound D but not to that of atropine ($P < 0.05$).

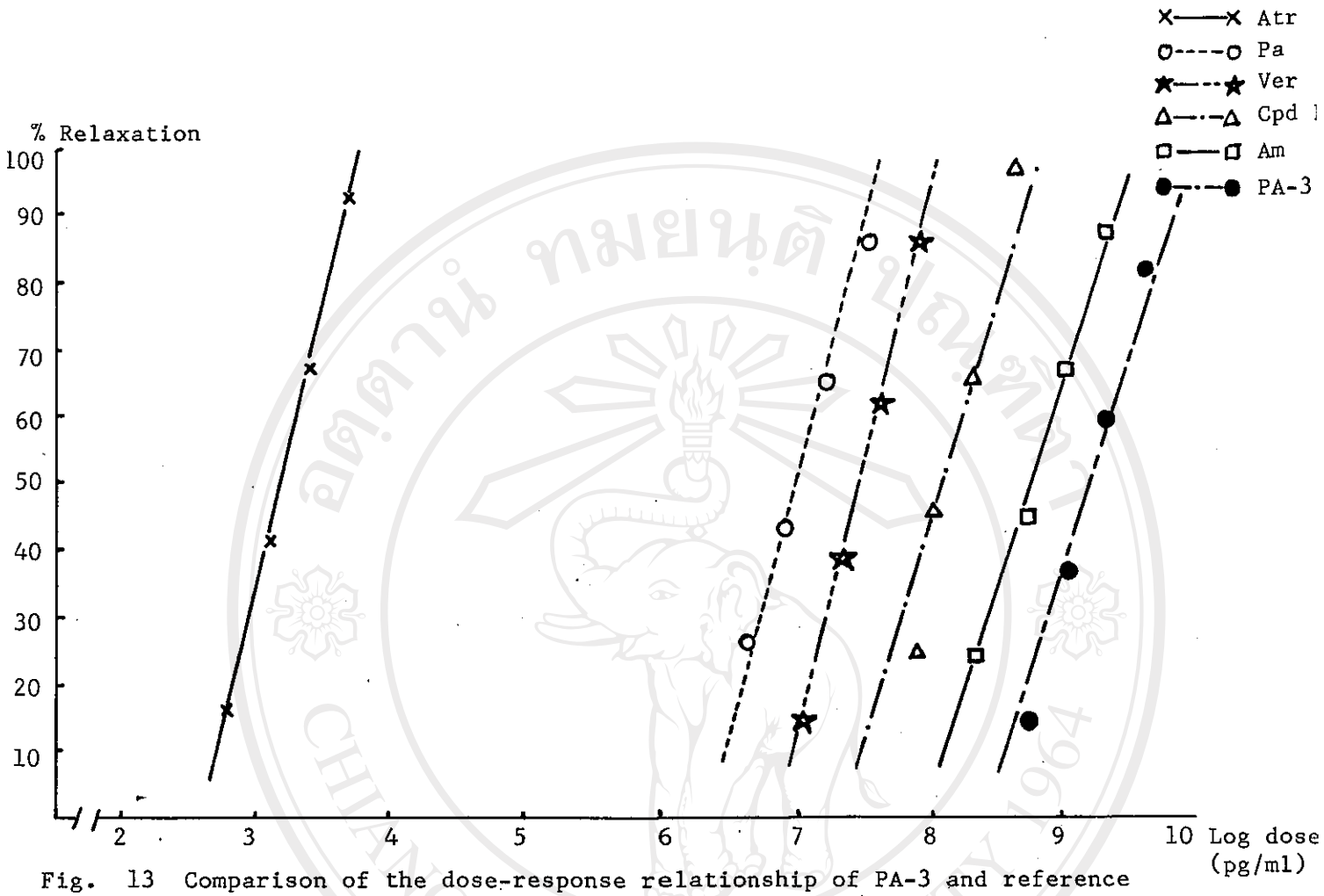


Fig. 13 Comparison of the dose-response relationship of PA-3 and reference drugs on the methacholine-induced tracheal contraction of isolated rat tracheal strip.

The dose-response relationship was expressed as a linear regression equation Y = a +bX, and the correlation coefficient (r):

Atropine	Y = -229.13 + 85.28 X	r = 0.9218	EC_{50} = 1.8 X 10^{-3} ug/ml
Papaverine	Y = -452.35 + 71.83 X	r = 0.9529	EC_{50} = 9.85 ug/ml
Verapamil	Y = -538.46 + 79.05 X	r = 0.9889	EC_{50} = 27.81 ug/ml
Compound D	Y = -527.46 + 70.86 X	r = 0.9893	EC_{50} = 141.03 ug/ml
Aminophylline	Y = -572.22 + 71.05 X	r = 0.9844	EC_{50} = 572.13 ug/ml
PA-3	Y = -650.26 + 75.59 X	r = 0.9977	EC_{50} = 1,836.22 ug/ml

Isoproterenol exhibited a less sensitive action on the isolated rat tracheal strip preparation, its effectiveness was therefore not evaluated in this study.

B. Experiment in vivo

1. Effect of PA-3 and reference drugs on histamine-induced bronchoconstriction in pentobarbital anesthetized guinea-pig.

The dose of histamine which produced a maximum increase in intratracheal pressure which represents bronchoconstriction was first determined and found to be 4 ug/kg body weight. The increase in intratracheal pressure, caused by a supramaximal dose of histamine (8 ug/kg body weight) given intravenously prior to the administration of the tested drug, was used as a control value for determination of the antagonistic effect of tested drugs as shown in Fig. 14 A. Fig 14 B shows the antagonistic effect of chlorpheniramine, an H_1 receptor antagonist, on histamine-induced bronchoconstriction. Tested drugs were given 2 min before the administration of the second dose of histamine, which was given 20 minutes after the first one. It was found that a statistically significant ($P<0.01$) inhibition of the histamine-induced bronchoconstriction was seen with all dosage levels (10, 20, 40 and 80 mg/kg body weight) of PA-3. The antagonistic effect of PA-3 was linearly related to the dose as shown in Fig. 15 and Table 7. The dose-response relationship of the antagonistic effect in terms of both intratracheal pressure response (PIPR) and response area (RA) of PA-3 was expressed

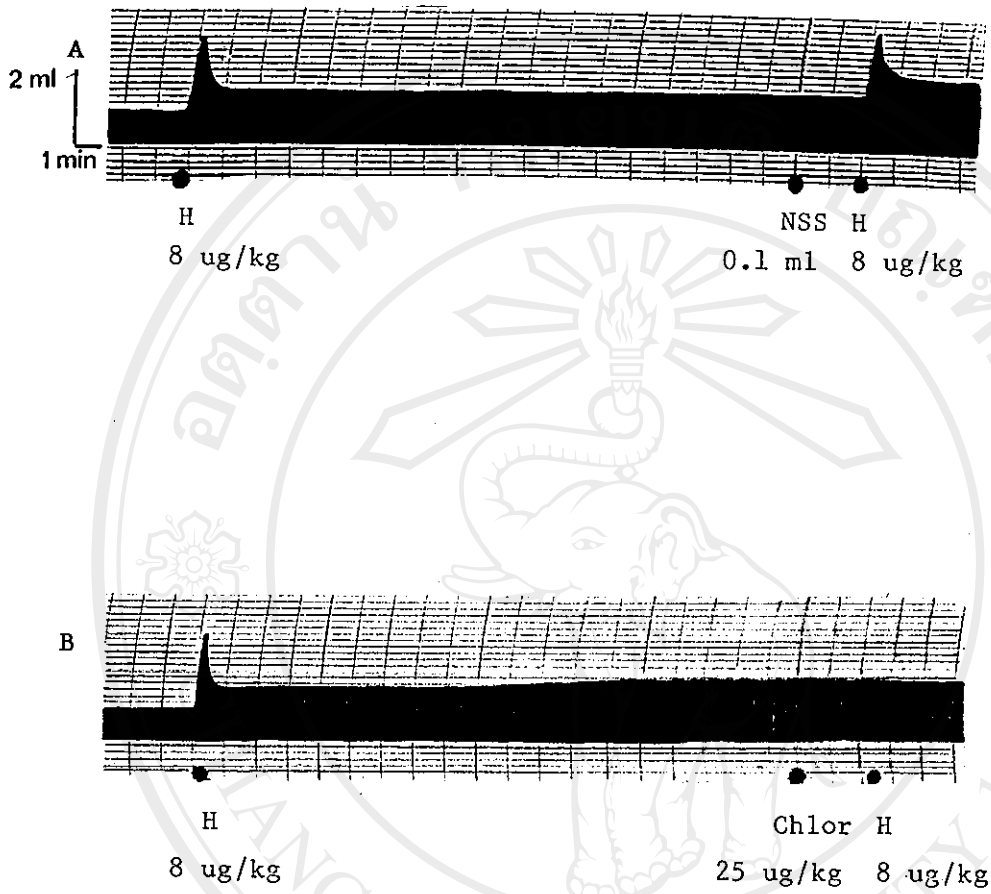


Fig 14 A. Increase in intratracheal pressure in response to the first and the second dose of histamine in pentobarbital anesthetized guinea-pigs.

Fig. 14 B. Blocking effect of chlorpheniramine (Chlor) on histamine-induced bronchoconstriction in pentobarbital anesthetized guinea-pigs.

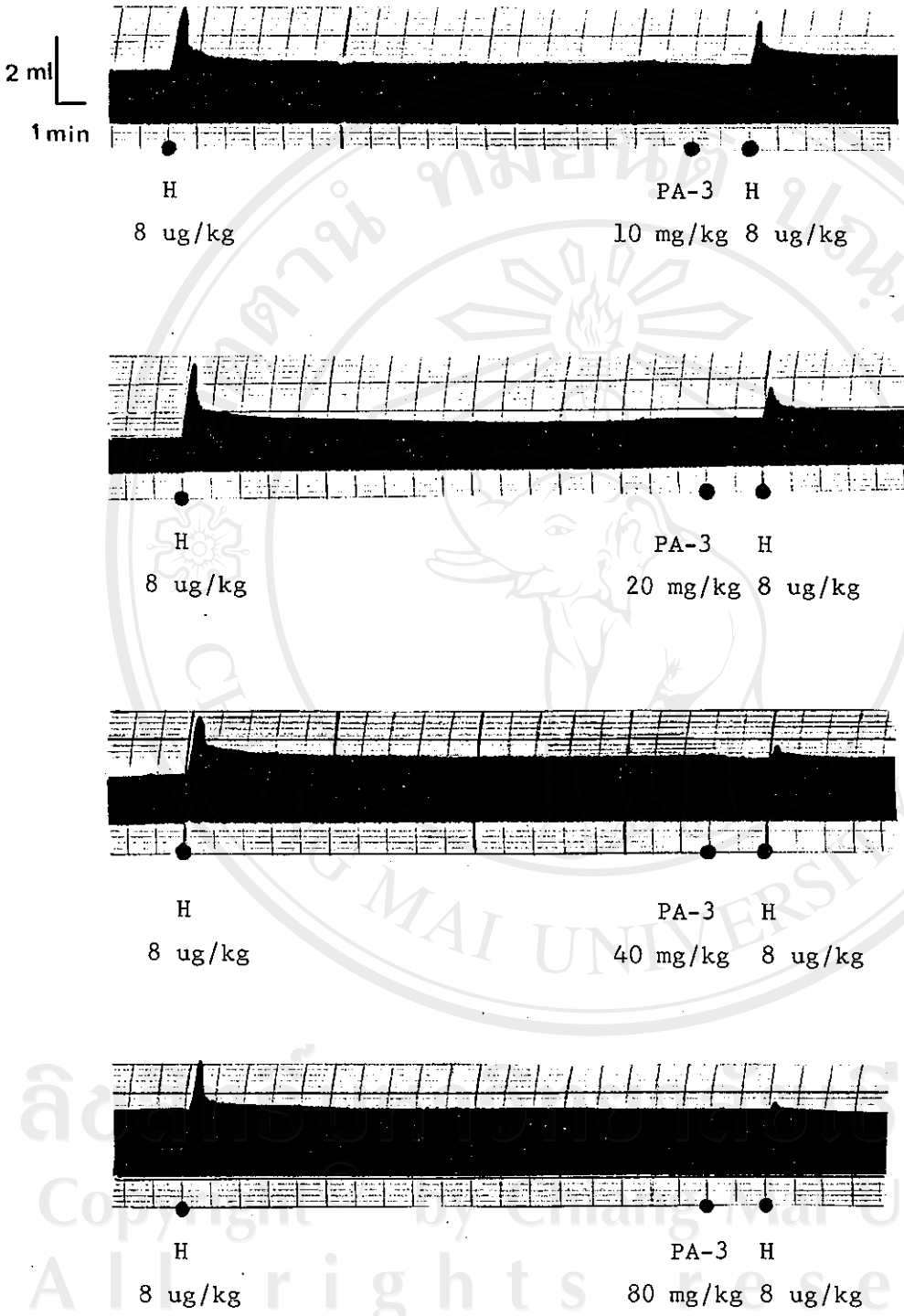


Fig 15 Effect of PA-3 on histamine-induced bronchoconstriction in pentobarbital anesthetized guinea-pigs.

Table 7 Effect of PA-3 on histamine-induced bronchoconstriction in pentobarbital anesthetized guinea-pigs.

Dose of PA-3 (mg/kg)	% inhibition of bronchoconstriction	
	PIPR	RA
10.00	22.05 ± 2.69	39.48 ± 6.67
20.00	39.38 ± 4.13	59.15 ± 6.43
40.00	61.91 ± 2.75	70.57 ± 4.28
80.00	96.43 ± 3.57	97.73 ± 2.27

Value expressed as mean ± S.E.M. (n = 4)

Dose of histamine = 8 ug/kg

as a linear regression equations, Y = -553.20 + 81.61 X for PIPR: Y = -394.11 + 61.84 X for RA, and is graphically illustrated in Fig. 20 and Fig. 21. The EC_{50} values calculated from the linear regression equations of PIPR and RA were found to be 24.62 and 15.19 mg/kg body weight, respectively.

According to the correlation coefficient values (r = 0.9917, 0.9777 for PIPR and RA, respectively), it suggests that the antagonistic effect of PA-3 on histamine-induced increase in intratracheal pressure is dose-related ($P < 0.05$).

The bronchodilator effect of reference drugs on histamine-induced bronchoconstriction was evaluated similary to that of PA-3. The drugs, i.e. isoproterenol, aminophylline, verapamil and papaverine, exihibited significant antagonistic effect on the histamine-induced bronchoconstriction as shown in Table 8 and Fig 16, 17, 18 and 19, respectively.

The linear regression lines of the dose-response relationship of all reference drugs were determined in term of both PIPR and RA. The order of the antagonistic potency of these tested drugs, in term of PIPR, from high to low is as follows; isoproterenol, verapamil, papaverine, PA-3 and aminophylline, of which the EC_{50} values were found to be 1.1×10^{-3}, 3.30, 5.52, 24.62 and 26.11 mg/kg body weight, respectively. In term of RA, isoproterenol still exhibited the most potent bronchodilator effect followed by verapamil, papaverine, aminophylline and PA-3, of which EC_{50} values were 1.3×10^{-3}, 3.22, 5.86, 14.95 and 15.19 mg/kg body weight,

Table 8 Effect of reference drugs (isoproterenol, verapamil, papaverine and aminophylline) on histamine-induced bronchoconstriction in pentobarbital anesthetized guinea-pigs.

Drug	% Inhibition of bronchoconstriction	
	PIPR	RA
Isoproterenol (ug/kg)		
0.75	34.17 ± 2.10	28.44 ± 8.50
1.5	68.04 ± 4.05	63.33 ± 3.26
3.0	80.91 ± 3.65	75.17 ± 6.81
6.0	96.55 ± 1.99	94.27 ± 3.93
Verapamil (mg/kg)		
1.25	19.93 ± 3.35	21.66 ± 6.15
2.5	27.36 ± 3.03	38.26 ± 8.48
5.0	66.37 ± 5.82	53.98 ± 5.87
10.0	97.22 ± 2.78	99.45 ± 0.56
Papaverine (mg/kg)		
2.5	16.61 ± 3.81	18.03 ± 3.21
5.0	51.11 ± 7.54	47.45 ± 6.67
10.0	70.63 ± 2.95	63.05 ± 5.24
20.0	100.00 ± 0.00	100.00 ± 0.00
Aminophylline (mg/kg)		
10	21.22 ± 4.89	39.88 ± 4.05
20	33.47 ± 2.43	57.23 ± 8.20
40	57.32 ± 6.11	76.05 ± 1.85
80	100.00 ± 0.00	100.00 ± 0.00

Value expressed as mean ± S.E.M. (n = 4)

Dose of histamine = 8 ug/kg

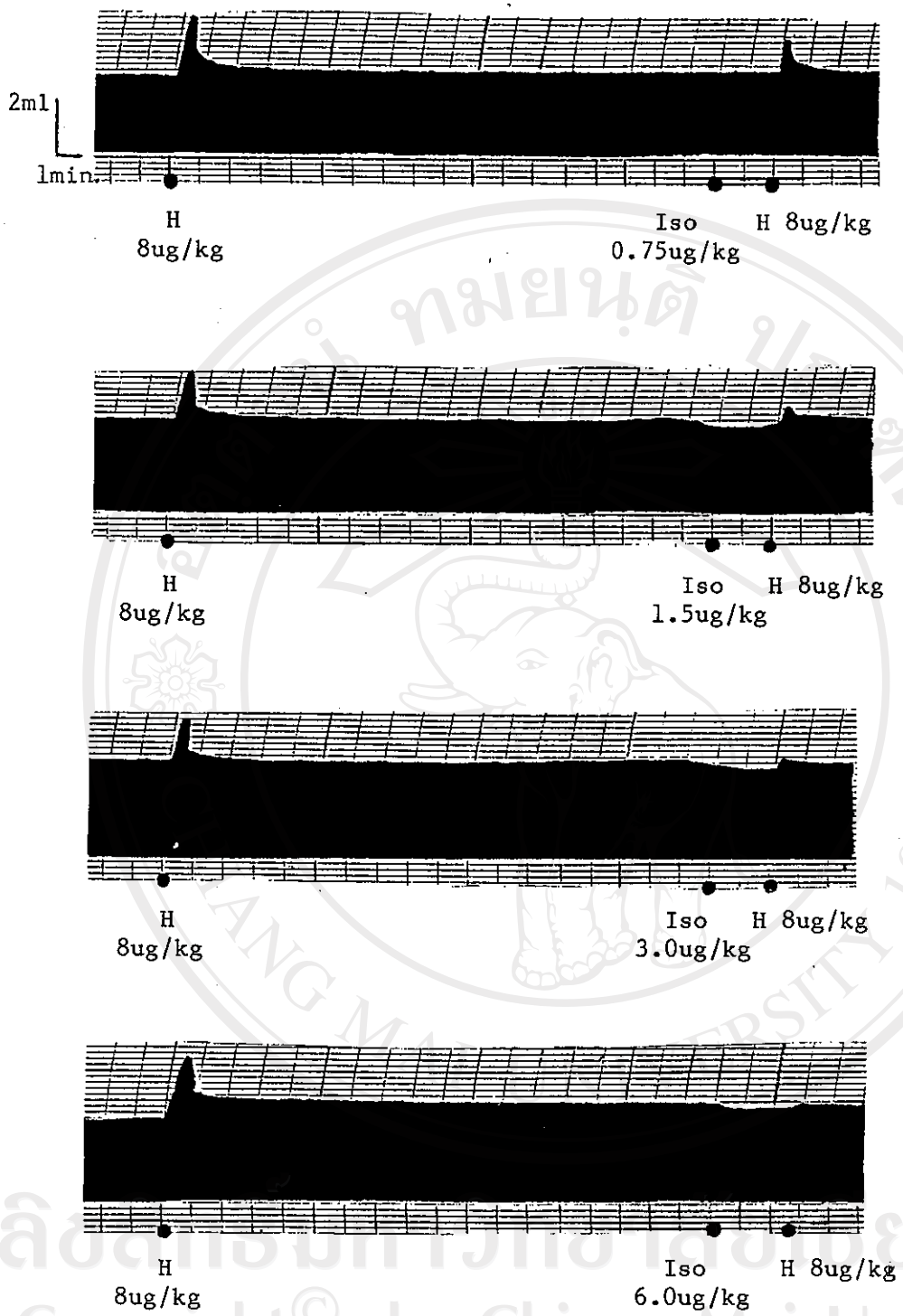


Fig. 16 Effect of isoproternol (Iso) on histamine-induced bronchoconstriction in pentobarbital anesthetized guinea-pigs.

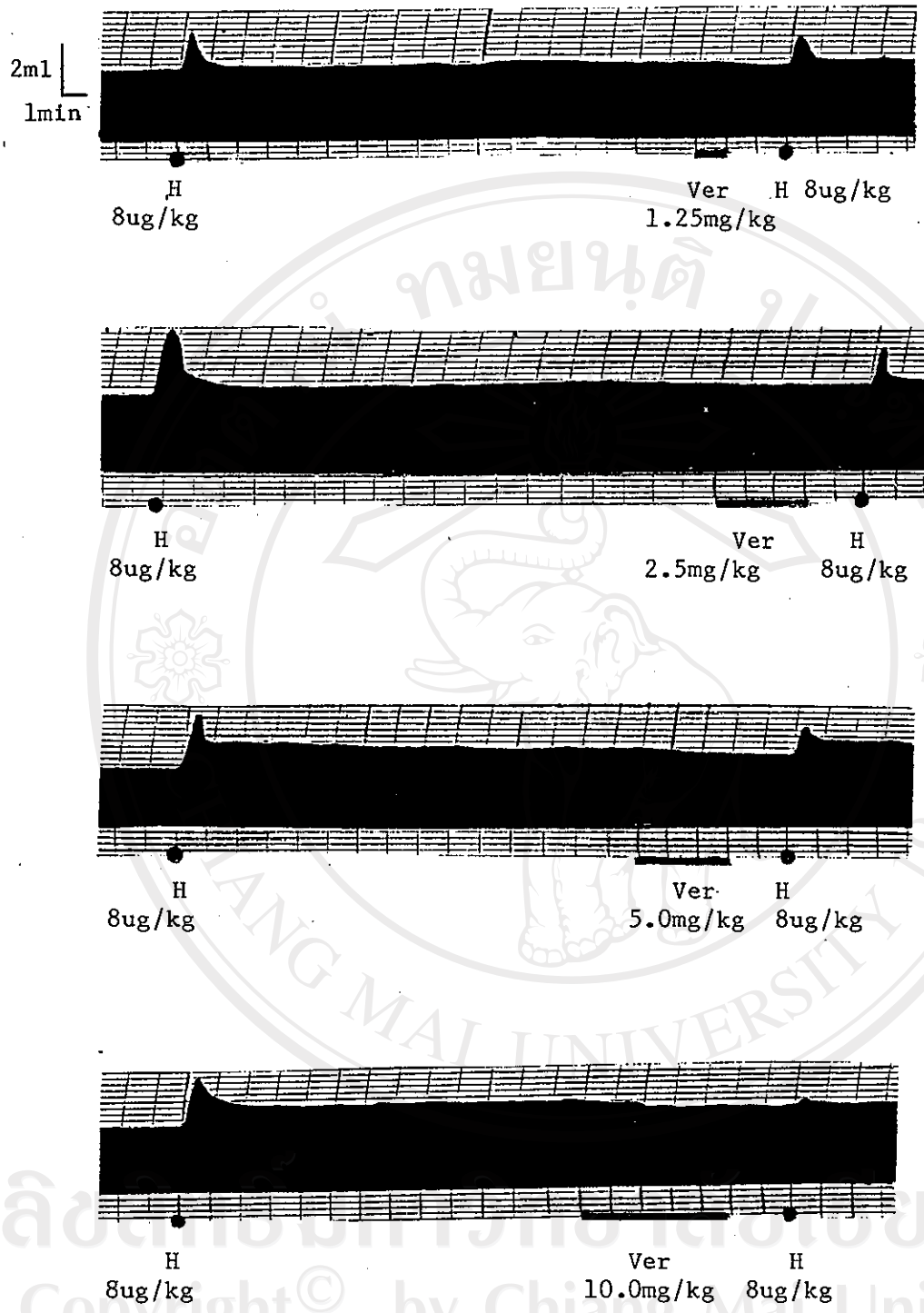


Fig. 17 Effect of verapamil (Ver) on histamine-induced bronchoconstriction in pentobarbital anesthetized guinea-pigs.

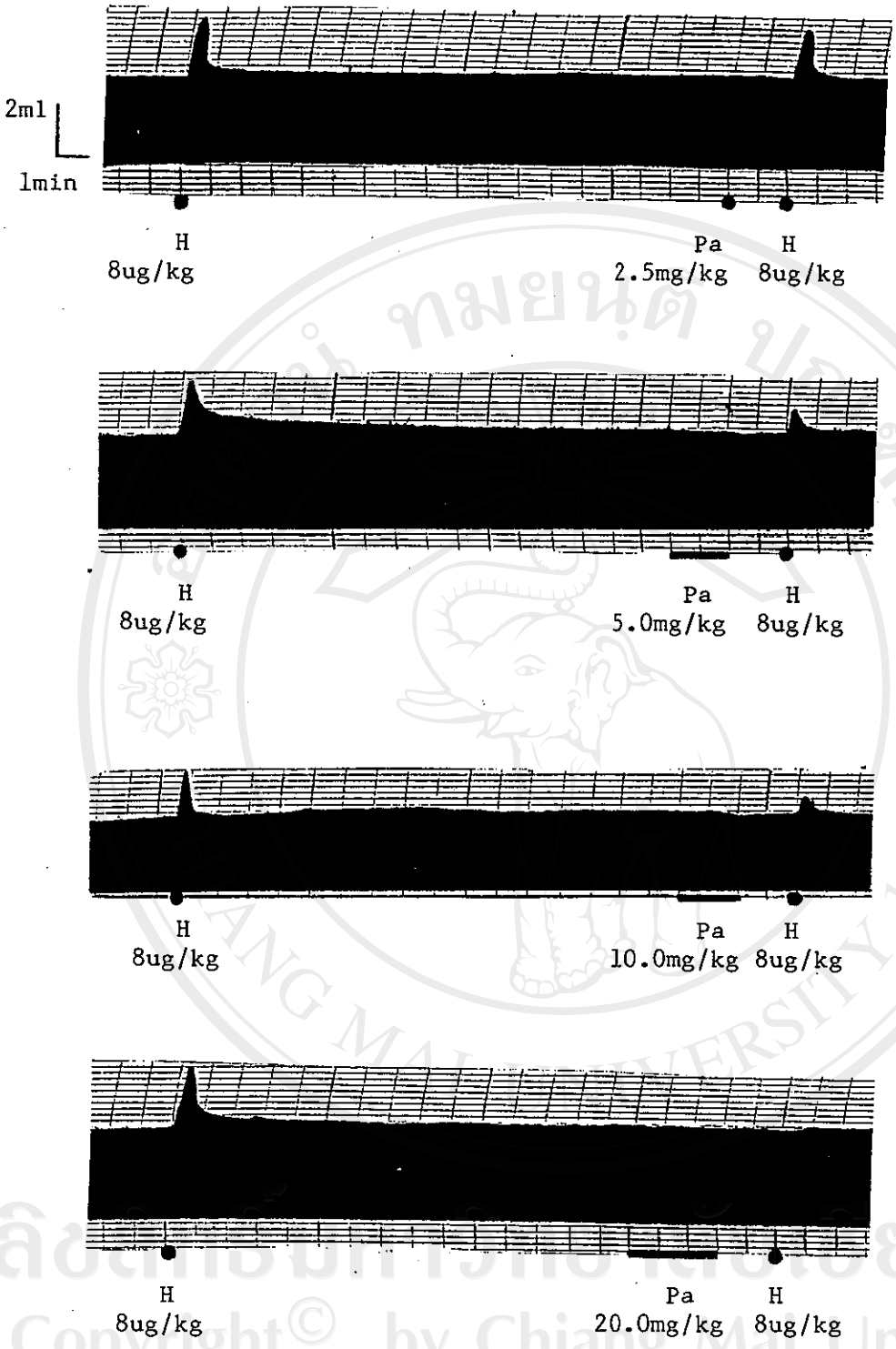


Fig. 18 Effect of papaverine (Pa) on histamine-induced bronchoconstriction in pentobarbital anesthetized guinea-pigs.

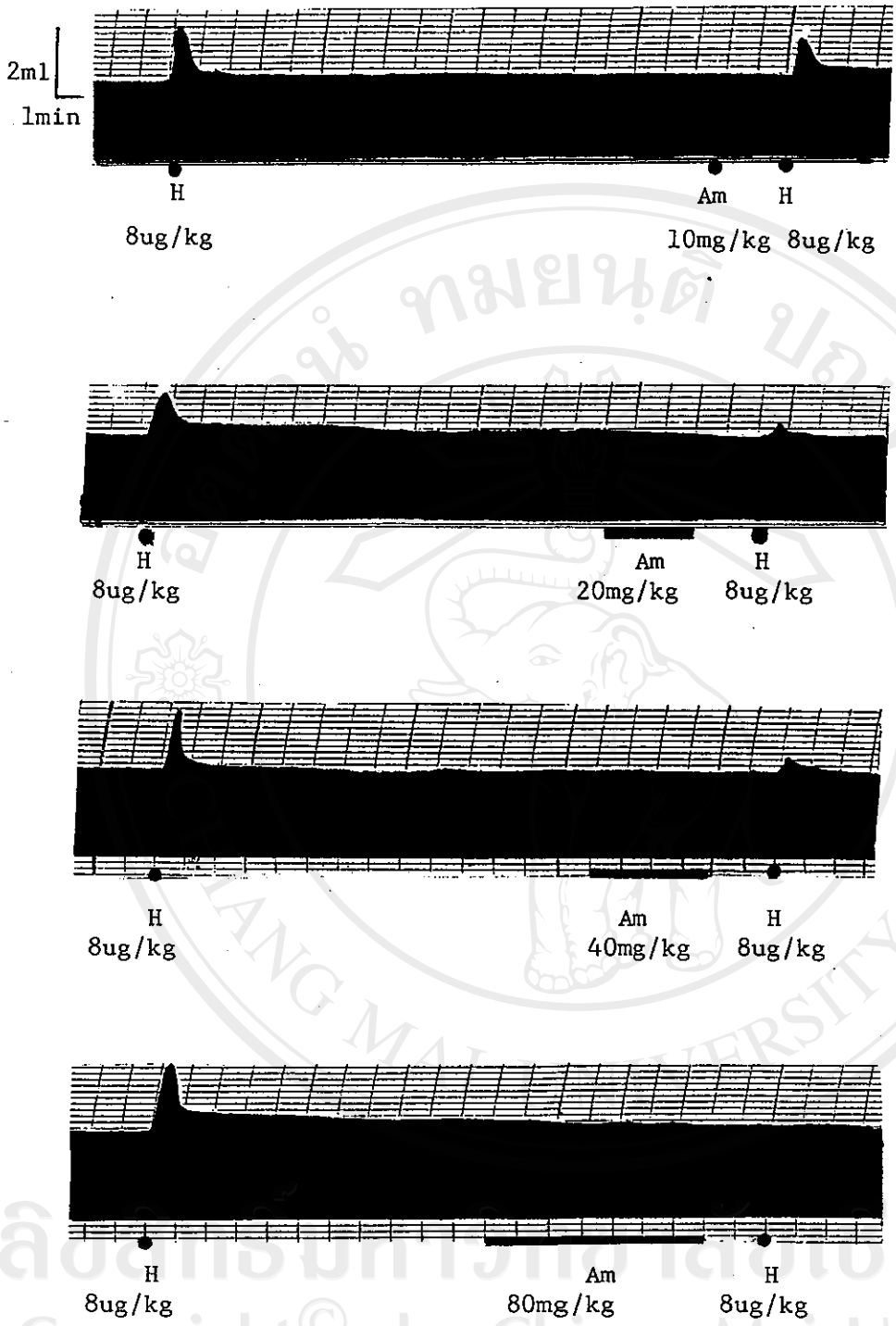


Fig. 19 Effect of aminophylline (Am) on histamine-induced bronchoconstriction in pentobarbital anesthetized guinea-pigs.

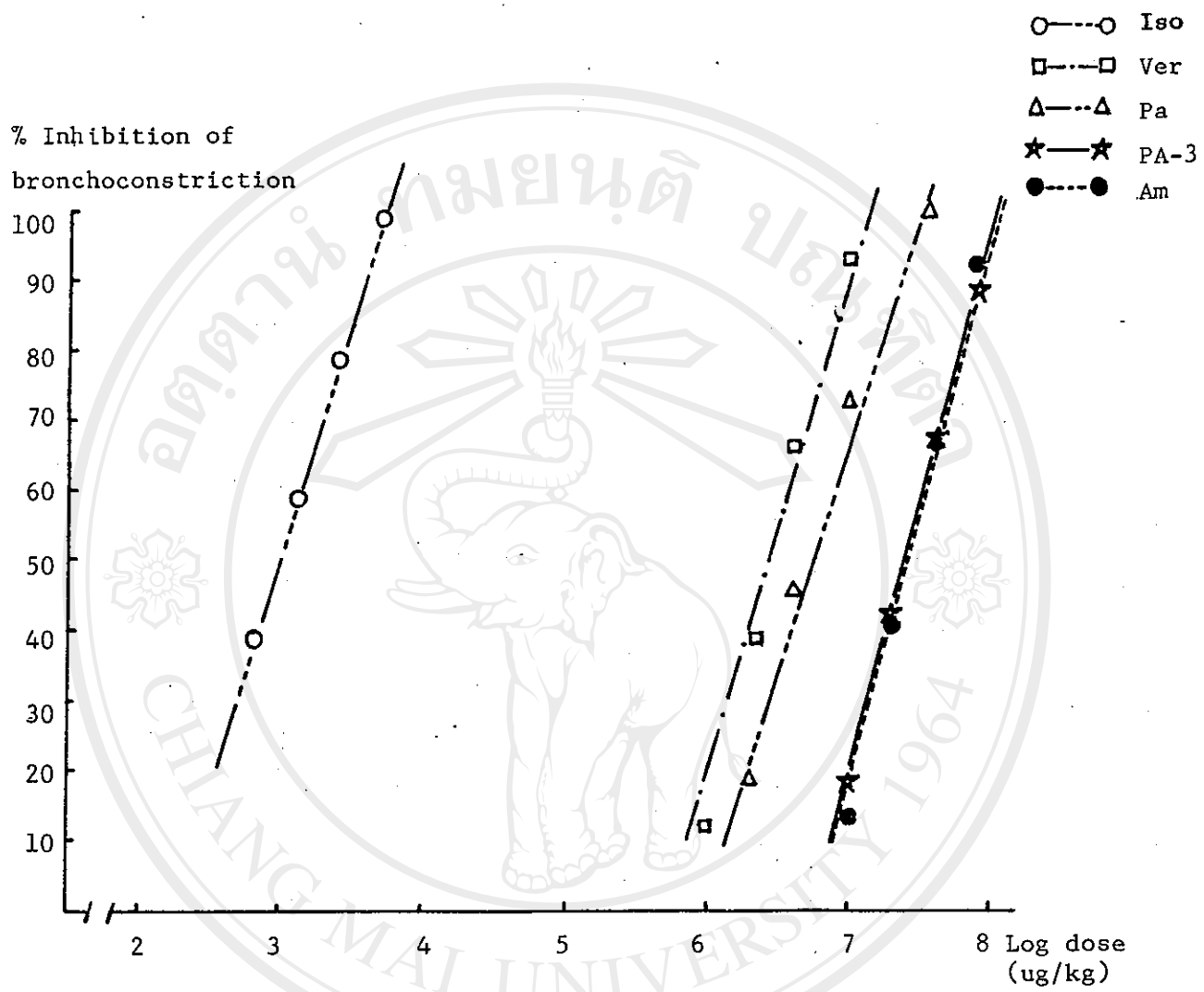


Fig. 20 Comparison of the dose-response relationship of PA-3 and reference drugs on the histamine-induced bronchoconstriction in pentobarbital anesthetized guinea-pig in term of PIPR.
The dose-response relationship was expressed as a linear regression equation of Y = a + bX, and the correlation coeffeicient (r) :

Isoproterenol	Y = -151.13 + 66.45X	r = 0.8829	EC_{50} = 1.1×10^{-3} mg/kg
Verapamil	Y = -536.57 + 89.99X	r = 0.9787	EC_{50} = 3.30 mg/kg
Papaverine	Y = -554.04 + 89.59X	r = 0.9463	EC_{50} = 5.52 mg/kg
PA-3	Y = -553.20 + 81.61X	r = 0.9917	EC_{50} = 24.62 mg/kg
Aminophylline	Y = -591.11 + 86.44X	r = 0.9995	EC_{50} = 26.11 mg/kg

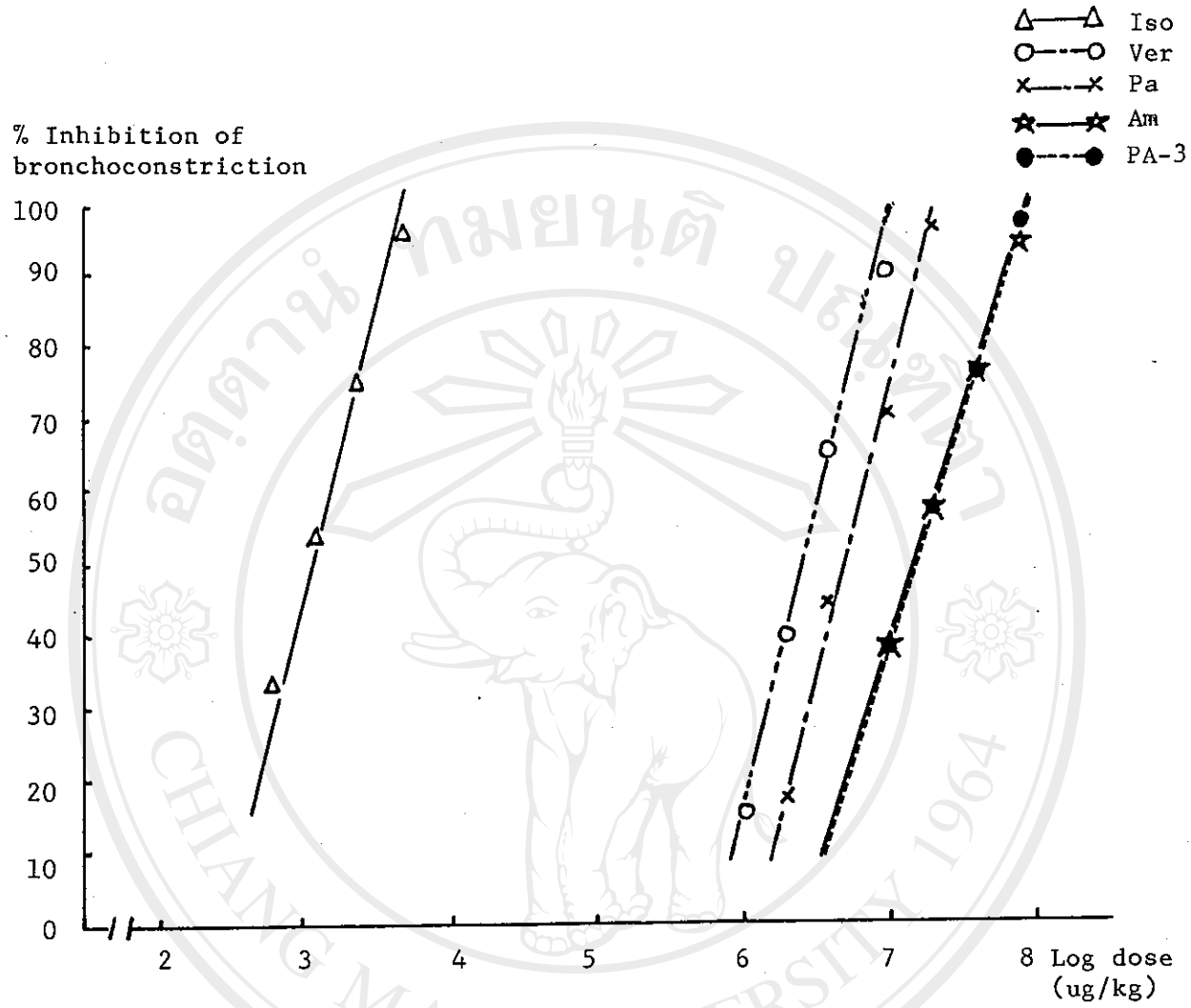


Fig. 21 Comparison of the dose-response relationship of PA-3 and reference drugs on the histamine-induced bronchoconstriction in pentobarbital anesthetized guinea-pig in term of response area (RA). The dose-response relationship was expressed as a linear regression equation of Y = a + bX, and the correlation coefficient (r) :

Isoproterenol	Y = -166.05 + 69.55X	r = 0.8961	EC_{50} = 1.3×10^{-3} mg/kg
Verapamil	Y = -488.49 + 82.74X	r = 0.9966	EC_{50} = 3.22 mg/kg
Papaverine	Y = -537.90 + 86.87X	r = 0.9720	EC_{50} = 5.86 mg/kg
Aminophylline	Y = -424.75 + 66.17X	r = 0.9778	EC_{50} = 14.95 mg/kg
PA-3	Y = -394.11 + 61.84X	r = 0.9777	EC_{50} = 15.19 mg/kg

respectively. The linear regression lines of the dose-response relationship of all reference drugs and PA-3 are graphically illustrated in Fig. 20 and 21.

The positive correlation coefficient values (r = 0.8829, 0.9787, 0.9463, 0.9995 and 0.9917 for isoproterenol, verapamil, papaverine and aminophylline, respectively) indicated that the antagonistic effect of these reference drugs on histamine-induced bronchoconstriction in term of PIPR is dose-dependent ($P < 0.05$).

In terms of RA, all reference drugs also showed dose-related antagonistic effects on histamine-induced bronchoconstriction ($P < 0.05$) with the correlation coefficient values (r) of 0.8961, 0.9966, 0.9720 and 0.9778 for isoproternol, verapamil, papaverine and aminophylline, respectively.

The parallelism between dose-response curves of PA-3 and reference drugs were analyzed, and it was found that the dose-response curve of PA-3, in term of PIPR, was parallel to those of aminophylline, verapamil and papaverine but not to that of isoproterenol ($P < 0.05$).

2. Effect of PA-3 and reference drugs on the cumulative doses of methacholine (MeCh)-induced bronchoconstriction in pentobarbital anesthetized rats.

The first cumulative doses of methacholine-challenged set (1.5, 3 and 4.5 ug) were given intravenously to test the sensitivity of the

animals. The second set (3, 6 and 9 ug) was administered similary to the first set in order to determine the antagonistic effect of the tested drugs which were pretreated 2 min before.

Fig. 22 A illustrates the increase in intratracheal pressure (representing bronchoconstriction) in the response to the first and the second set of cumulative methacholine-doses. Peak height intratracheal pressure response (PIPR) was determined from the difference between the peak response value and the basal resting value of each single dose. The respective response area (RA) was measured using transparent millimeter paper for each single dose of methacholine in 2 min. Fig. 22 B shows the blocking effect of atropine (an antimuscarinic drug) on the second set of cumulative methacholine-induced bronchoconstriction.

The increase in PIPR and RA by both sets of methacholine-induced bronchoconstriction was found to be dose-related ($r = 0.9915$ for PIPR and $r = 0.9975$ for RA) as shown in Table 9 and Fig. 23.

The antagonistic effect of tested drugs was determined by comparing the PIPR and the RA in the response to the second set of methacholine-doses between drug-treated group and control group (received NSS).

To assure that various groups of animals used in each experiment exibited the similar sensitivity in the response to cumulative methacholine-doses, both PIPR and RA of each group were compared.

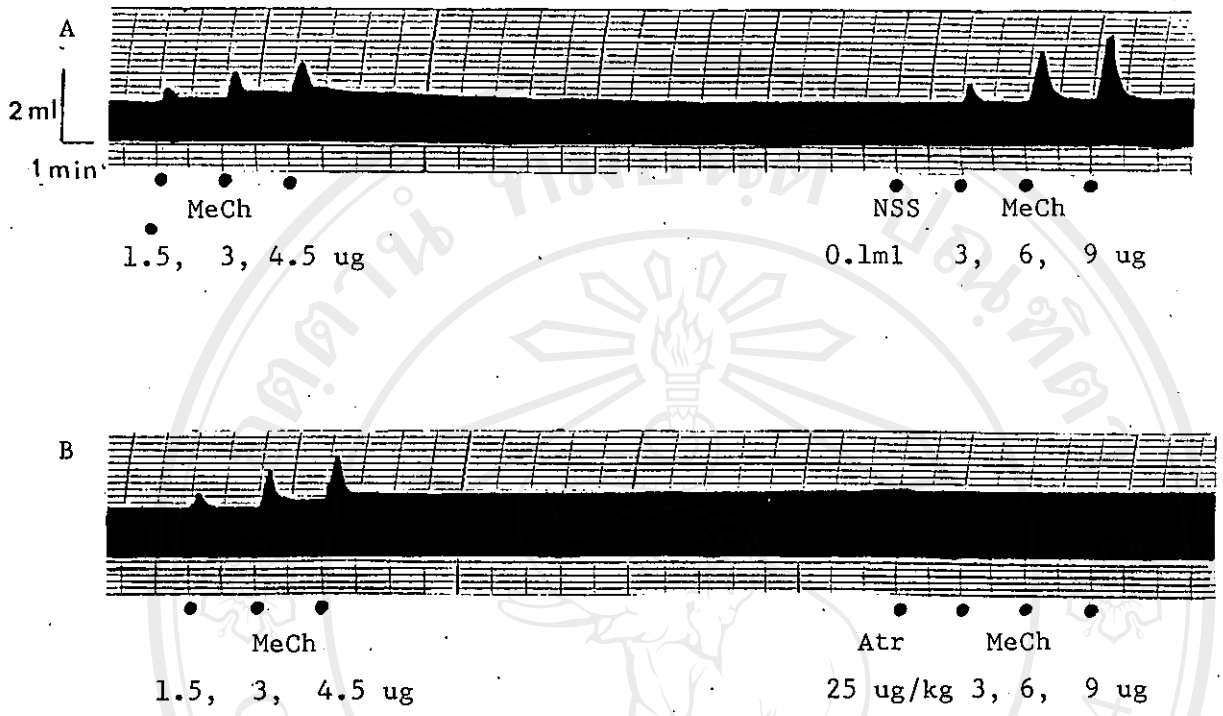


Fig. 22 A. Increase in intratracheal pressure in response to the first (1.5, 3, 4.5 ug) and the second cumulative doses (3, 6, 9 ug) of methacholine in pentobarbital anesthetized rats.

Fig. 22 B. Blocking effect of atropine (Atr) on the second cumulative doses of methacholine-induced bronchoconstriction in pentobarbital anesthetized rats.

Table 9 Effect of the cumulative doses of methacholine-induced bronchoconstriction in pentobarbital anesthetized rats.

Cumulative doses of MeCh	PIPR (mm)	RA (mm^2)
First set		
1.5 ug	2.6 ± 0.68	6.0 ± 0.84
3.0	5.0 ± 0.32	19.6 ± 1.25
4.5	6.5 ± 0.39	30.2 ± 2.67
Second set		
3.0 ug	2.6 ± 0.55	7.0 ± 1.45
6.0	7.2 ± 0.86	20.2 ± 2.11
9.0	9.5 ± 0.55	29.2 ± 1.98

Values expressed as mean ± S.E.M.

n = 4

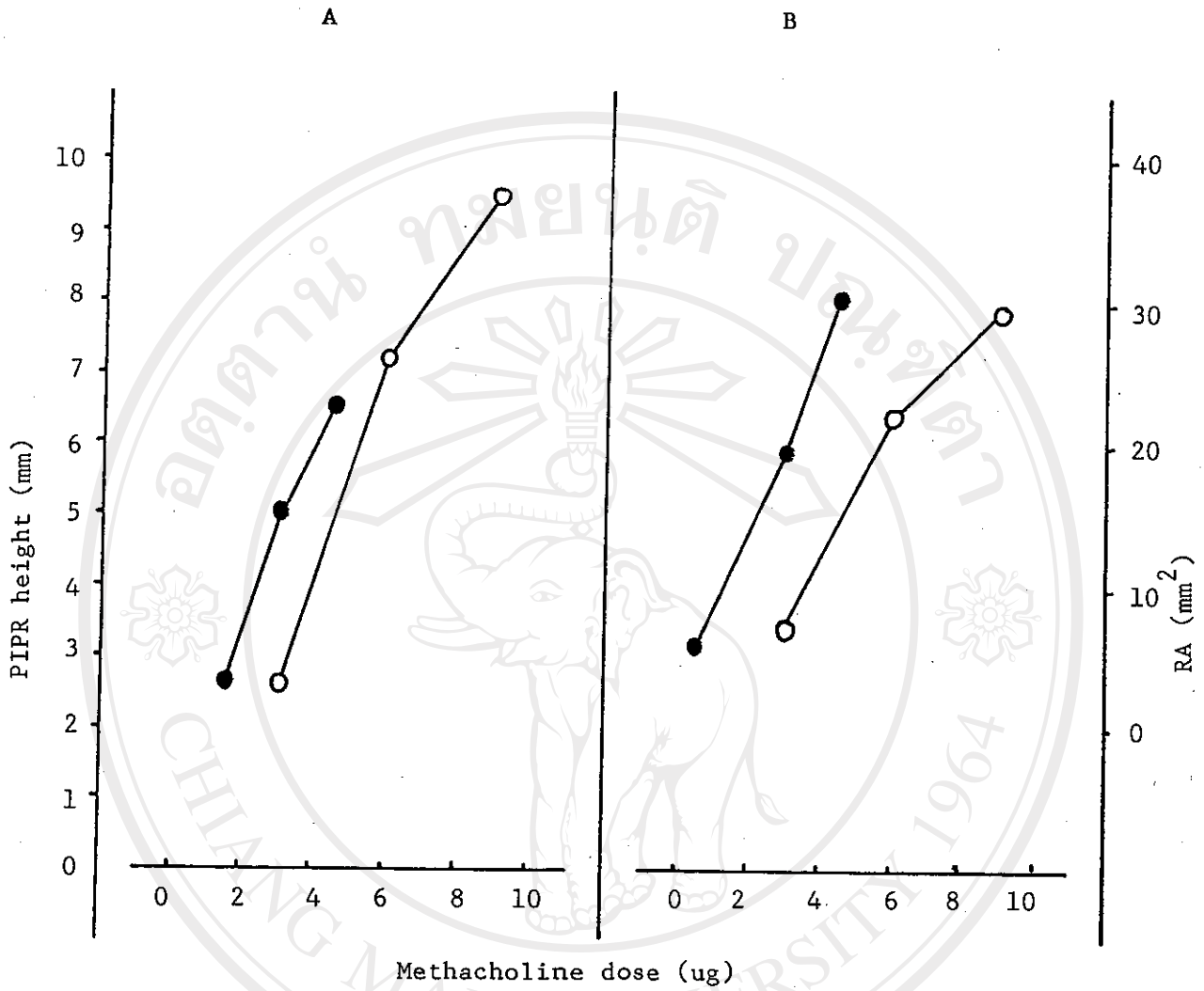


Fig. 23 Dose-related bronchoconstriction caused by methacholine (intravenously) in pentobarbital anesthetized rats.

A : Peak height of intratracheal pressure response (PIPR)

B : Response area (RA)

●—● First set of MeCh doses

○—○ Second set of MeCh doses

Table 10 is an example of the responses of 5 groups of animals used for testing of the antagonistic effect of PA-3 on cumulative methacholine-induced bronchoconstriction. It was found that all groups of animals exhibited similar response to the first set of methacholine-doses in term of PIPR. Anyhow, in term of RA, some groups of animals showed different responses to the doses of 3 and 4.5 ug, as shown in Table 10. In other experiments using reference drugs (isoproterenol, verapamil, papaverine and aminophylline) each group of animals exhibited the responses to the first set of methacholine-doses similarly to the groups of animals in experiment with PA-3.

PA-3 at the doses of 10, 20, 40 and 80 mg/kg body weight caused a statistically significant inhibition of the dose-related methacholine-induced increase in intratracheal pressure when given intravenously 2 min before the second set of methacholine challenges (Fig. 24 and Table 11). PA-3 at the dose of 80 mg/kg body weight could nearly completely antagonize methacholine-induced bronchoconstriction. The inhibitory effect, in term of PIPR, on each dose of the cumulative methacholine-doses was found to depend on the doses of PA-3 used, as shown in Fig. 25 and Table 11.

Pretreatment of the animals with various doses of reference drugs 2 min before the administration of the second set of mechacholine-doses could also effectively inhibit methacholine-induced bronchoconstriction. Table 12 and Fig. 26 show the antagonistic effect of isoproterenol

Table 10 Effect of the first set of cumulative methacholine-induced bronchoconstriction in various groups of anesthetized rats used for testing the activity of PA-3.

Methacholine was given intravenously at 2 min intervals. the PIPR and RA caused by the first methacholine challenge set in each tested group were compared with those of control group (1.5 versus 1.5 ug; 3 versus 3 ug; 4.5 versus 4.5 ug), using paired t-test.

NSS and PA-3 were given intravenously 2 min prior to the administration of the second set of methacholine-doses (20 min-pause from the first set).

Control	group	:	NSS	0.2 ml
Group	A	:	PA-3	10 mg/kg
Group	B	:	PA-3	20 mg/kg
Group	C	:	PA-3	40 mg/kg
Group	D	:	PA-3	80 mg/kg

MeCh	Control		Group A		Group B		Group C		Group D	
(ug)	PIPR	RA	PIPR	RA	PIPR	RA	PIPR	RA	PIPR	RA
1.5	2.6 ±0.68	6.0 ±0.84	2.25 ±0.25	5.75 ±1.44	3.75 ±0.85	7.00 ±1.29	3.00 ±0.58	6.00 ±0.58	2.25 ±0.25	5.00 ±0.58
3.0	5.0 ±0.32	19.6 ±1.25	6.5 ±0.50	21.00 ±3.19	7.00 ±0.71	23.25** ±2.50	8.50 ±0.29	17.75* ±1.18	6.75 ±1.11	19.75 ±3.20
4.5	6.5 ±0.39	30.2 ±2.67	9.75 ±0.85	45.5* ±6.08	9.75 ±0.85	35.0* ±2.86	8.75 ±0.48	32.00 ±4.14	8.75 ±1.11	34.5* ±6.54

Values expressed as mean ± S.E.M. (n = 4)

* $P < 0.05$

** $P < 0.01$

Table 11 Effect of PA-3 on the second set of methacholine-induced bronchoconstriction in pentobarbital anesthetized rats.

MeCh (ug)	Control		PA-3 (mg/kg)							
	NSS		10		20		40		80	
	PIPR	RA	PIPR	RA	PIPR	RA	PIPR	RA	PIPR	RA
3.0	2.6 ±0.55	7.0 ±1.45	1.88* ±0.43	3.25* ±1.31	1.25* ±0.28	3.00* ±0.58	0.63* ±0.24	0.88* ±0.43	0.25* ±0.25	0.25* ±0.25
6.0	7.2 ±0.86	20.2 ±2.11	3.75* ±1.44	13.00* ±3.94	3.38* ±0.69	10.25* ±1.65	3.25* ±0.25	9.25* ±3.64	1.5* ±0.29	7.25* ±2.17
9.0	9.5 ±0.55	29.2 ±1.98	5.75* ±1.11	21.00* ±4.18	5.50* ±1.55	19.50* ±3.79	3.75* ±0.25	16.00* ±1.68	2.75* ±0.48	12.75* ±1.75

Mean values ± S.E.M. from 4 rats in each group are given.

Statistical significance (t-test; * $P < 0.05$; ** $P < 0.01$) refers to difference from the control group.

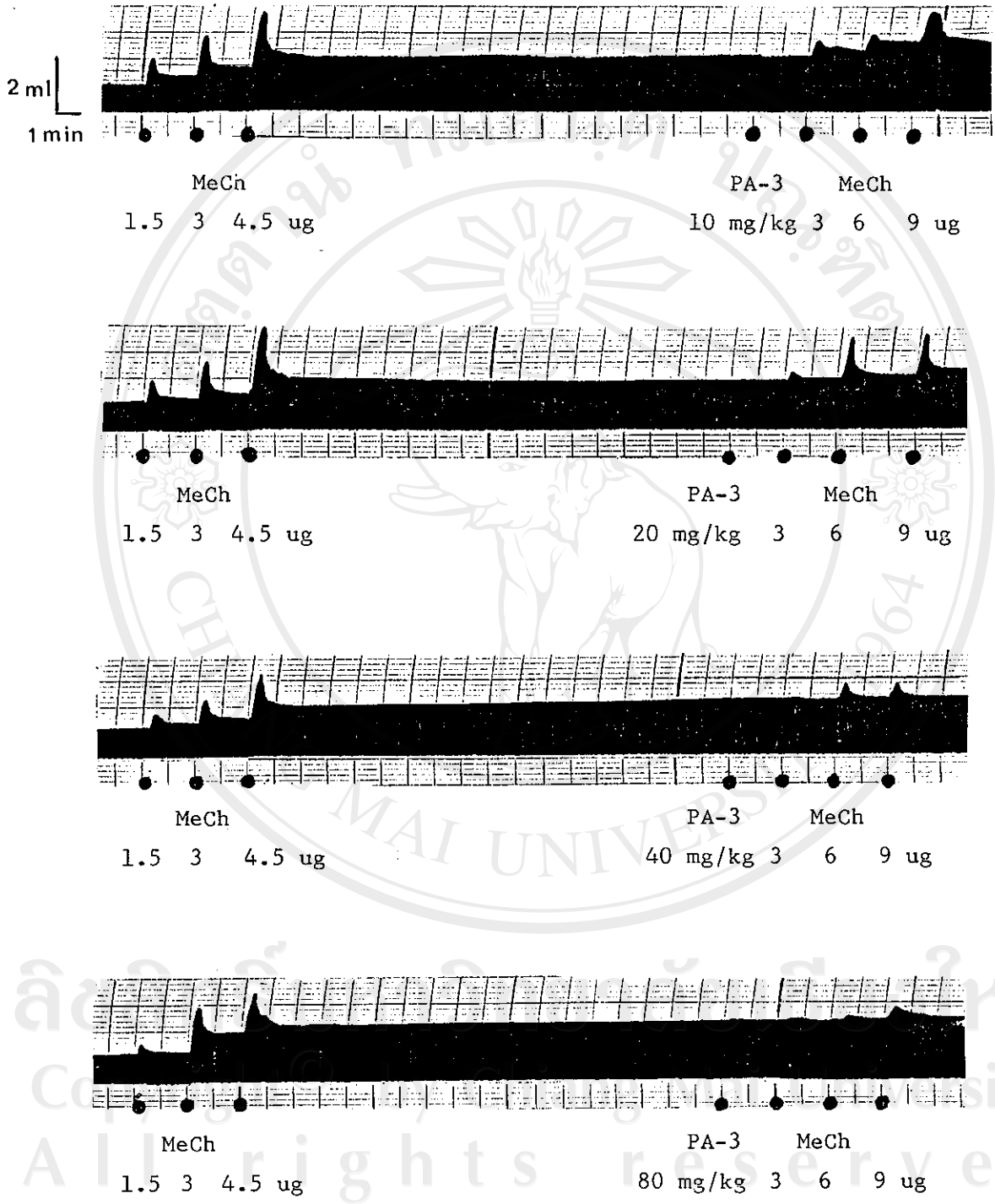


Fig. 24 Effect of PA-3 on dose-related methacholine-induced bronchoconstriction in pentobarbital anesthetized rats.

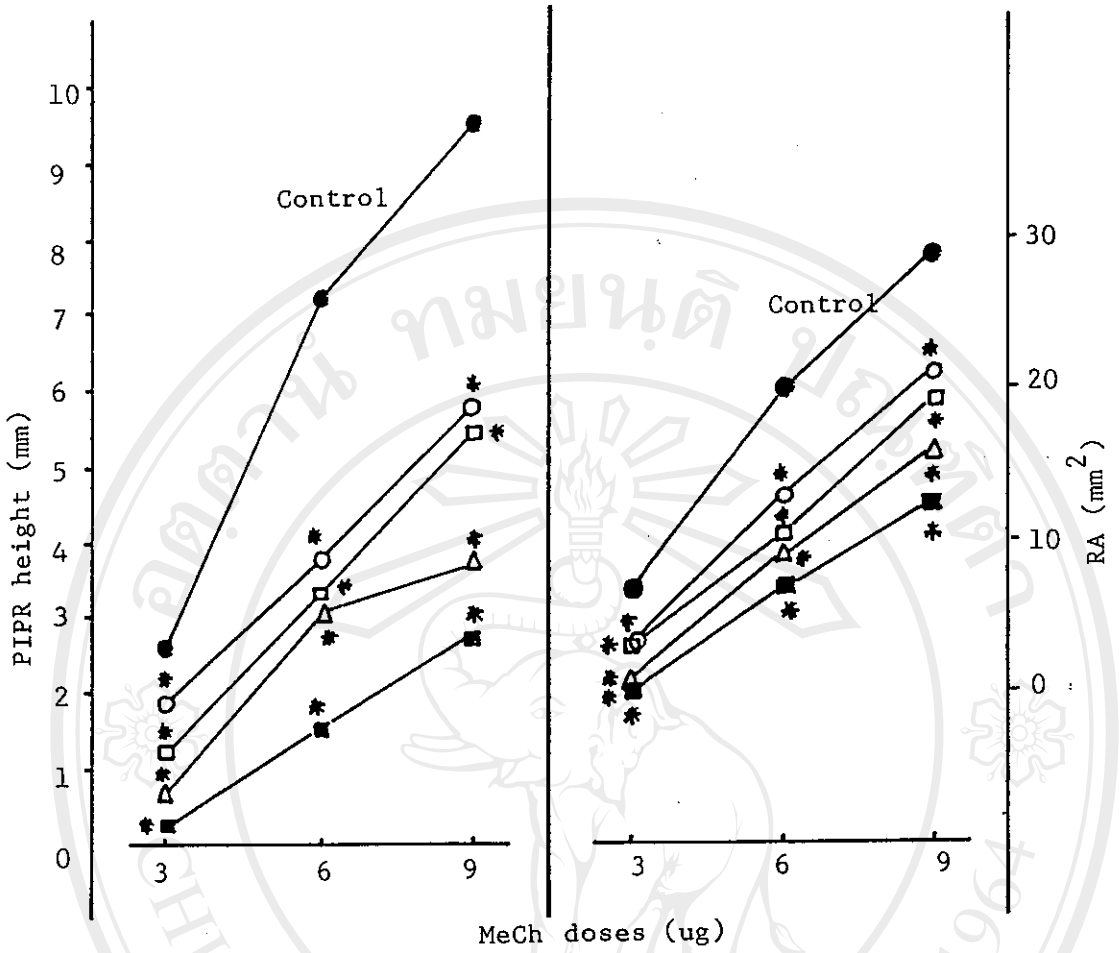


Fig. 25 Effect of PA-3 on methacholine-induced bronchoconstriction in pentobarbital anesthetized rats, assessed in term of peak height of intratracheal pressure response (PIPR) and response area (RA).

Control

PA-3 10 mg/kg body weight

PA-3 20 mg/kg body weight

PA-3 40 mg/kg body weight

PA-3 80 mg/kg body weight

* P < 0.05

** P < 0.01

Table 12 Effect of isoproterenol on the second set of methacholine-induced bronchoconstriction in pentobarbital anesthetized rats.

	Control		Isoproterenol (ug/kg)							
MeCh	NSS		12.5		25		50		100	
(ug)	PIPR	RA	PIPR	RA	PIPR	RA	PIPR	RA	PIPR	RA
3.0	2.6 ±0.55	7.0 ±1.45	1.00** ±0.00	1.75* ±0.48	0.63* ±0.24	1.75* ±0.85	0.75* ±0.25	1.75** ±0.75	0.50* ±0.29	0.50** ±0.29
6.0	7.2 ±0.86	20.20 ±2.11	5.00* ±0.71	18.00 ±2.34	3.88* ±0.88	15.00* ±1.68	3.75* ±0.63	10.50** ±3.20	2.63* ±0.24	6.0** ±1.35
9.0	9.5 ±0.55	29.20 ±1.98	9.50 ±0.29	30.75 ±3.12	7.50* ±0.65	41.75 ±5.68	7.25* ±0.48	23.25* ±4.49	7.00* ±1.22	29.0 ±8.26

Mean values ± S.E.M. from 4 rats in each group are given. Statistical significance (t-test; * $P < 0.05$; ** $P < 0.01$) refers to difference from the control group.

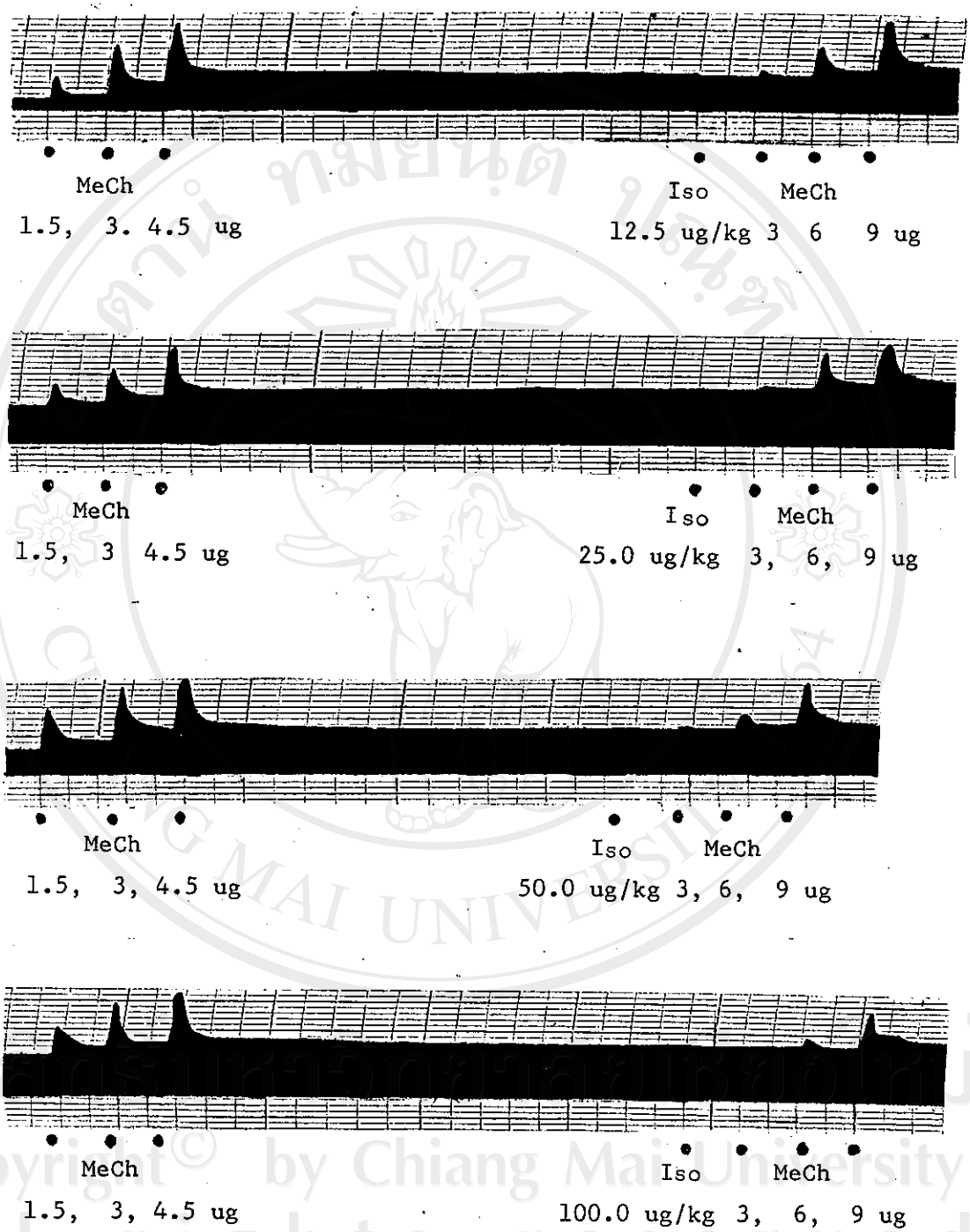


Fig. 26 Effect of isoproterenol (Iso) on the dose-related methacholine-induced bronchoconstriction in pentobarbital anesthetized rats.

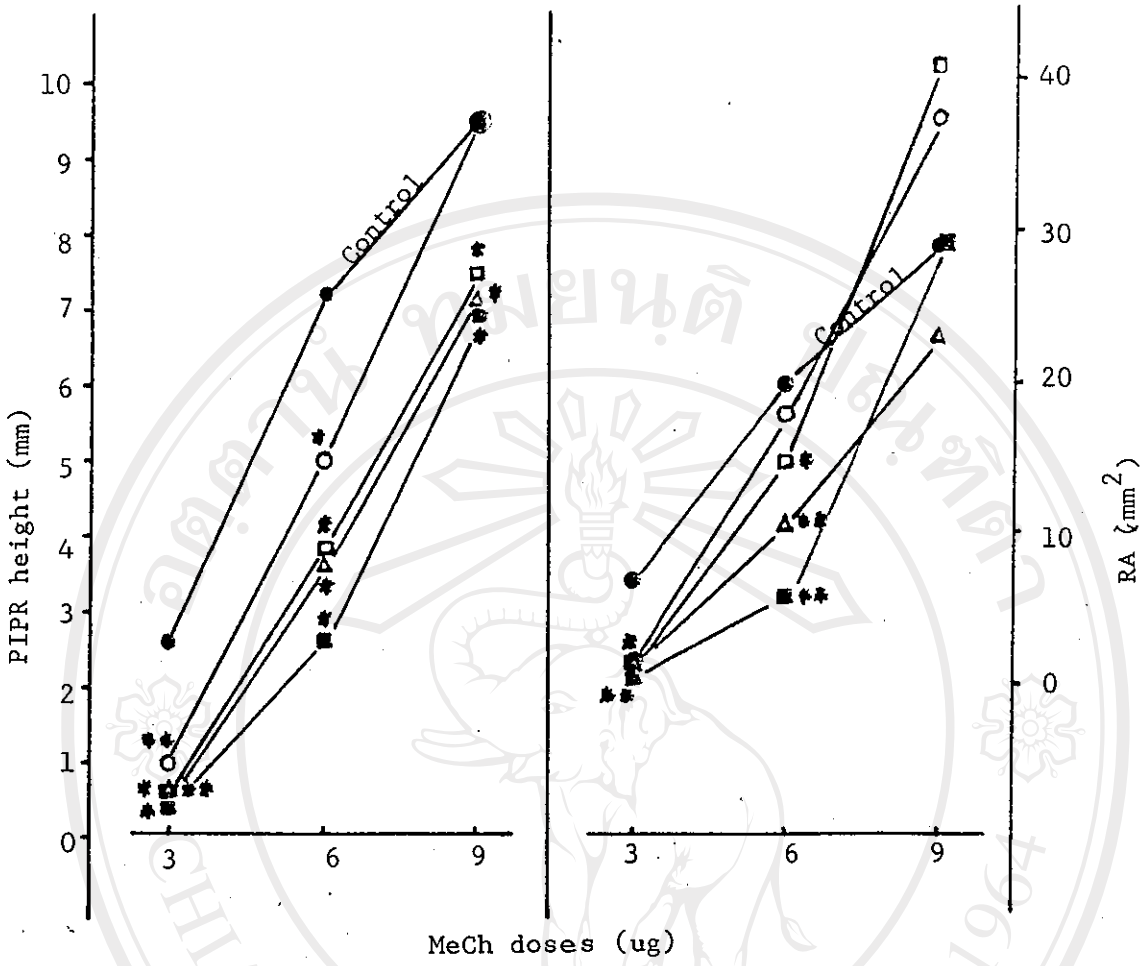


Fig. 27 Effect of isoproterenol (Iso) on methacholine-induced bronchoconstriction in pentobarbital anesthetized rats, assessed in terms of peak height of intratracheal pressure response (PIPR) and response area (RA).

Control
Iso 72.5 ug/kg body weight
Iso 25.0 ug/kg body weight
Iso 50.0 ug/kg body weight
Iso 100.0 ug/kg body weight

* $P < 0.05$

** $P < 0.01$

on cumulative methacholine-induced bronchoconstriction. At the dose of 100 ug/kg body weight, isoproterenol completely prevented bronchoconstriction induced by the first dose (3 ug) of the second set of methacholine-doses. The inhibitory effect of isoproterenol on methacholine-induced bronchoconstriction was also found to depend on its doses used, as shown in Fig. 27. Similary, other reference drugs, i.e. verapamil, papaverine and aminophylline, could effectively prevent the bronchoconstriction induced by cumulative methacholine-doses as shown in Table 13, Fig. 28 and Fig. 29; Table 14, Fig. 30 and Fig. 31, as well as in Table 15, Fig. 32 and Fig. 33, respectively. Their inhibitory effects were also found to depend on their doses used.

3. Hippocratic screening test

This experiment was performed to observe the changes in general behaviours in conscious rats. The responses of the animals to intraperitoneal injection of various doses of PA-1 were recorded according to the standardized working sheet. The signs and symptoms caused by PA-1 at doses of 125, 250, 500 and 1000 mg/kg body weight are summarized in Table 14.

Sixty minutes after PA-1 administration the following signs and symptoms were observed:

Table 13 Effect of papaverine on the second set of methacholine-induced bronchoconstriction in pentobarbital anesthetized rats.

	Control		Papaverine (ml/kg)							
MeCh	NSS		2.5		5.0		10.0		20.0	
(ug)	PIPR	RA	PIPR	RA	PIPR	RA	PIPR	RA	PIPR	RA
3.0	2.6 ±0.55	7.0 ±1.45	2.50 ±0.29	12.5 ±2.96	2.25** ±0.25	5.00 ±1.00	1.13* ±0.31	2.13* ±0.59	1.63* ±0.13	3.75* ±0.48
6.0	7.2 ±0.86	20.2 ±2.11	7.0 ±1.19	23.5 ±4.09	6.75** ±0.71	11.50** ±2.94	6.50* ±1.31	19.50 ±3.77	4.00* ±0.71	11.25** ±2.87
9.0	9.5 ±0.55	29.2 ±1.98	9.0 ±0.41	33.25 ±4.17	8.00* ±0.65	21.25** ±2.36	7.50* ±1.35	25.75 ±5.22	6.25* ±0.48	16.00* ±2.80

Mean values ± S.E.M. from 4 rats in each group are given.
Statistical significance (t-test: * $P < 0.05$;
** $P < 0.01$) refers to difference from the control group.

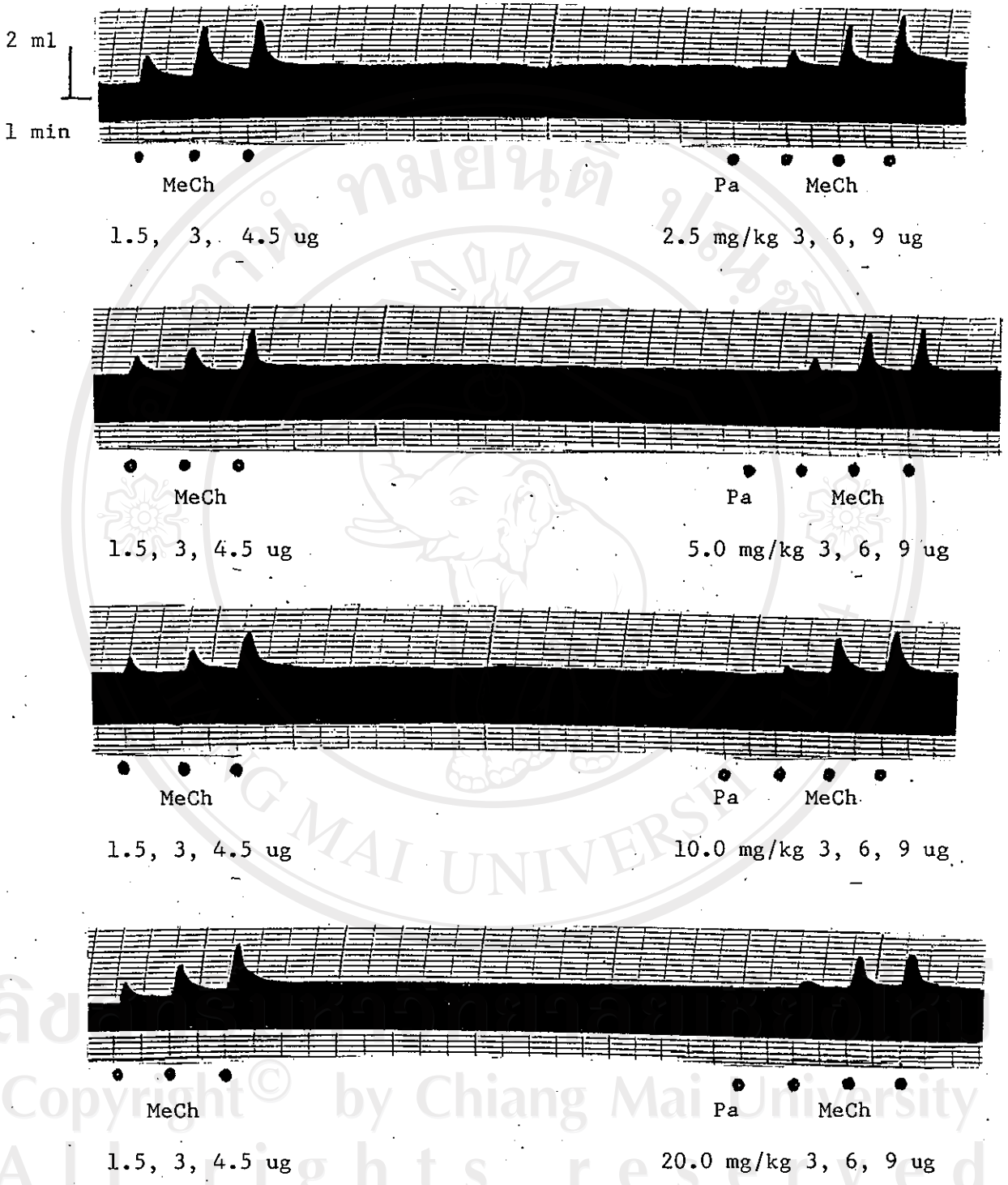


Fig. 28 Effect of papaverine (Pa) on the dose-related methacholine-induced bronchoconstriction in pentobarbital anesthetized rats.

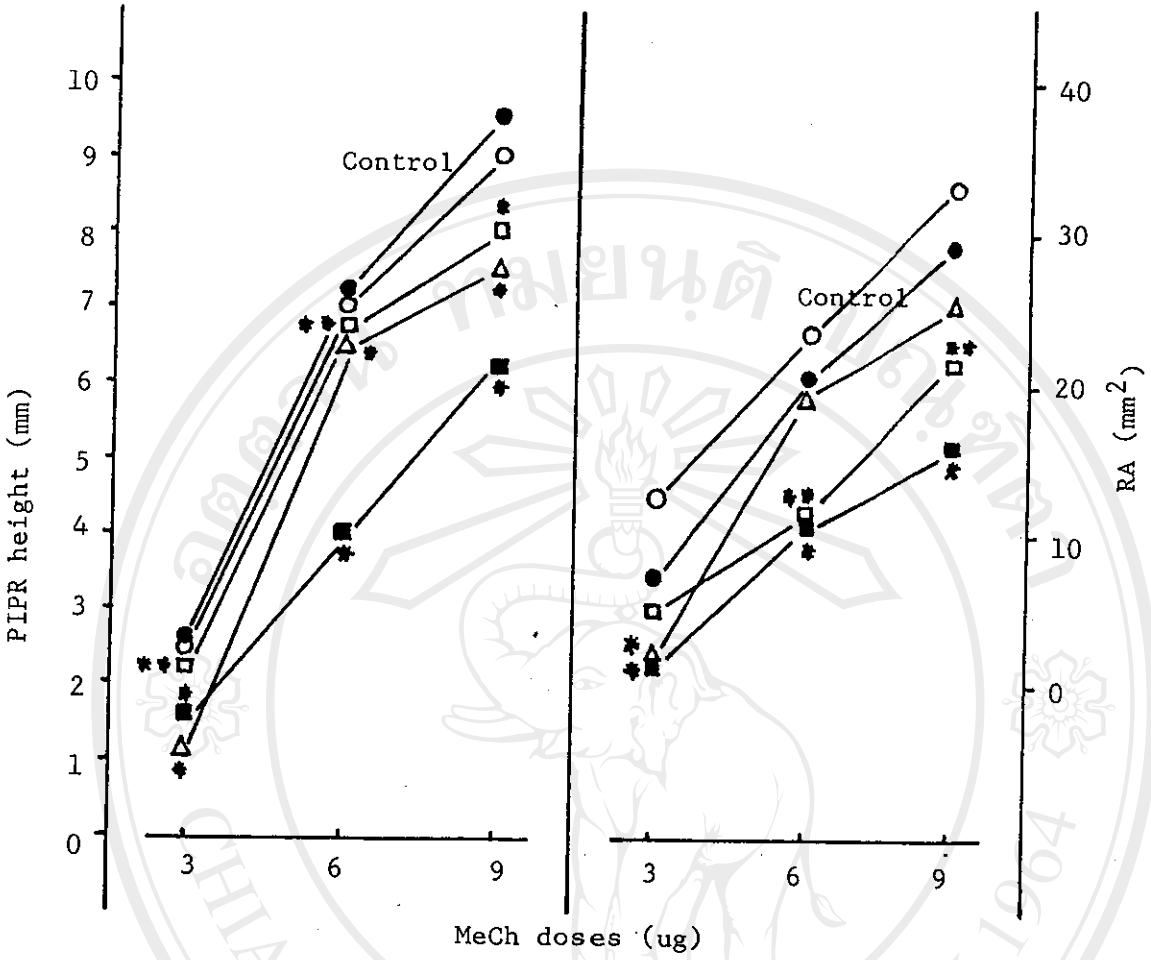


Fig. 29 Effect of papaverine (Pa) on methacholine-induced bronchoconstriction in pentobarbital anesthetized rats, assessed in terms of peak height of intratracheal pressure response (PIPR) and response area (RA).

Control
Pa 2.5 mg/kg body weight
Pa 5.0 mg/kg body weight
Pa 10.0 mg/kg body weight
Pa 20.0 mg/kg body weight

* $P < 0.05$

** $P < 0.01$

Table 14 Effect of verapamil on the second set of the methacholine-induced bronchoconstriction in pentobarbital anesthetized rats.

	Control		Verapamil (mg/kg)							
MeCh	NSS		1.25		2.5		5.0		10.0	
(ug)	PIPR	RA	PIPR	RA	PIPR	RA	PIPR	RA	PIPR	RA
3.0	2.60 ±0.55	7.00 ±1.45	2.75 ±0.48	8.75 ±2.39	2.50 ±0.63	6.50 ±0.87	2.25* ±0.29	6.50 ±1.89	2.13* ±0.31	4.50* ±0.29
6.0	7.20 ±0.86	20.00 ±2.11	7.00 ±0.41	17.75* ±4.84	4.63* ±1.25	12.75* ±2.39	4.38* ±0.90	12.25* ±2.43	4.00* ±0.41	12.75* ±0.63
9.0	9.50 ±0.55	29.20 ±1.98	9.75 ±0.75	30.25 ±4.03	7.25** ±1.31	19.50* ±3.28	6.00* ±0.41	24.75** ±2.93	5.75* ±0.63	26.25** ±3.07

Mean values ± S.E.M. from 4 rats in each group are given.
Statistical significance (t-test; * $P < 0.05$, ** $P < 0.01$) refers to difference from the control group.

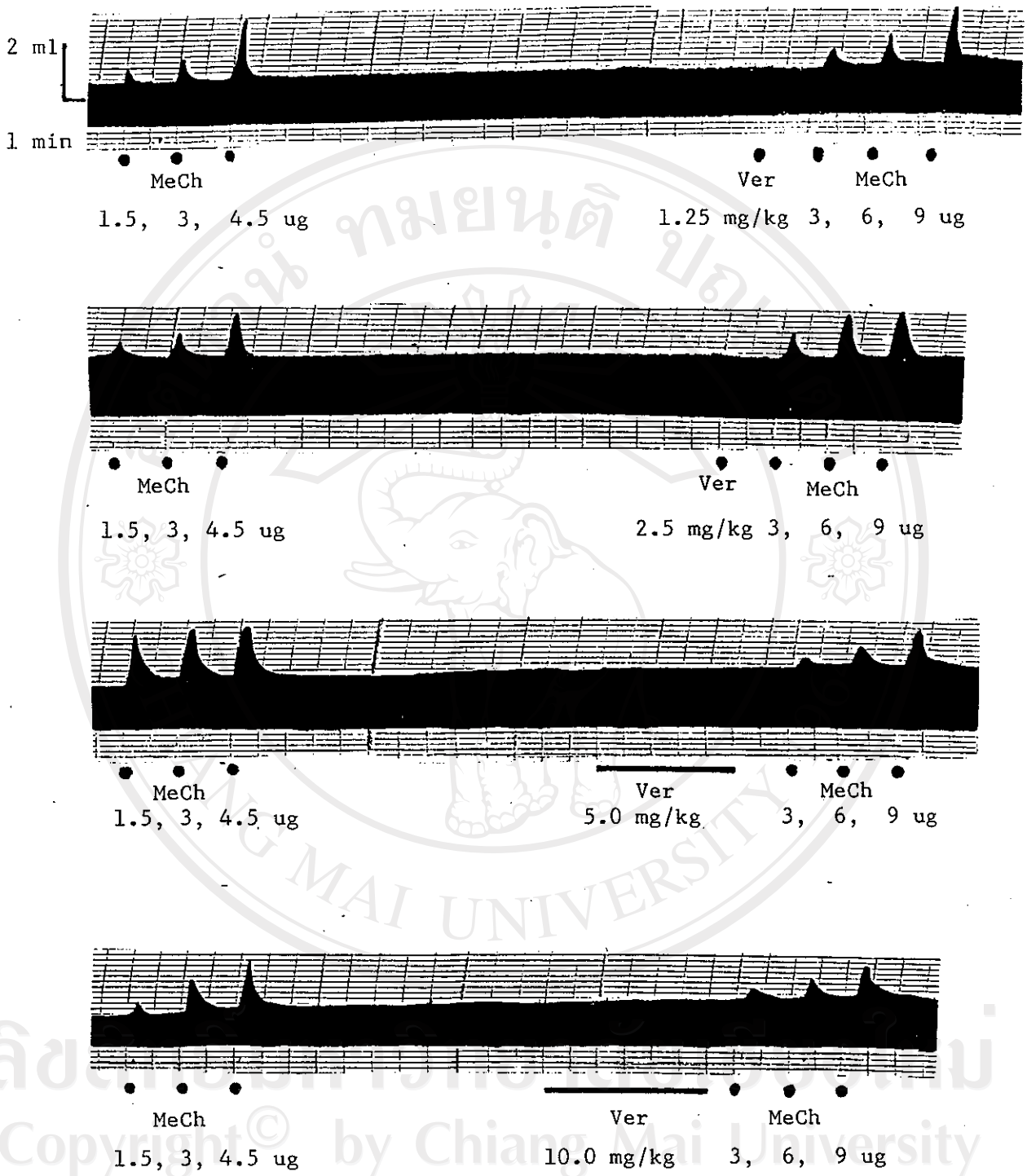


Fig. 30 Effect of verapamil (Ver) on the dose-related methacholine-induced bronchoconstriction in pentobarbital anesthetized rats.

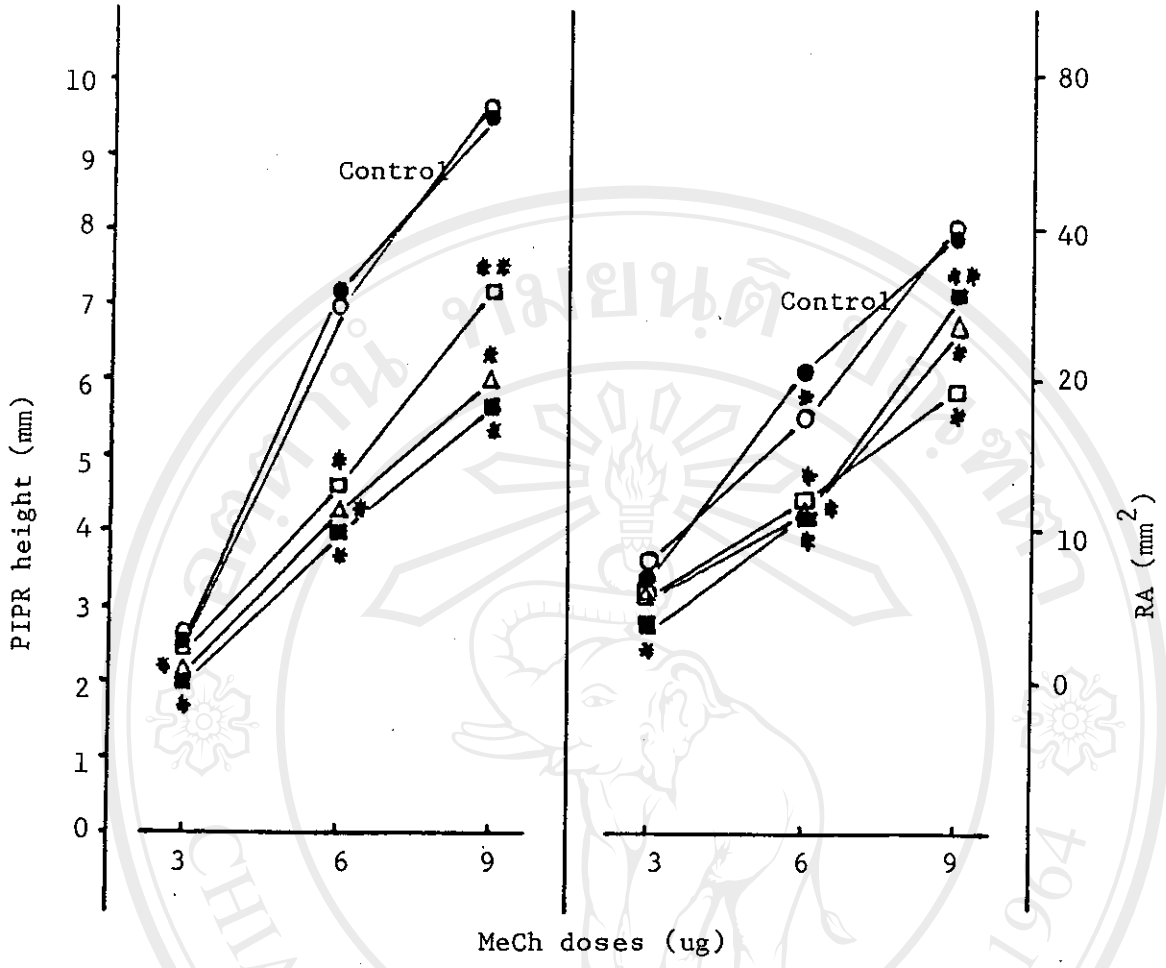


Fig. 31 Effect of verapamil (Ver) on methacholine-induced bronchoconstriction in pentobarbital anesthetized rats, assessed in terms of peak height of intratracheal pressure response (PIPR) and response area (RA).

Control

Ver 1.25 mg/kg body weight

Ver 2.50 mg/kg body weight

Ver 5.00 mg/kg body weight

Ver 10.00 mg/kg body weight

* $P < 0.05$

** $P < 0.01$

Table 15 Effect of aminophylline on the second set of methacholine-induced bronchoconstriction in pentobarbital anesthetized rats.

	Control		Aminophylline (mg/kg)							
MeCh	NSS		10		20		40		80	
(ug)	PIPR	RA	PIPR	RA	PIPR	RA	PIPR	RA	PIPR	RA
3.0	2.60 ±0.55	7.00 ±1.45	1.75* ±0.25	3.25* ±0.25	0.88* ±0.31	1.75* ±0.85	0.25* ±0.14	0.25* ±0.14	0.00* ±0.00	0.00* ±0.00
6.0	7.20 ±0.86	20.2 ±2.11	6.50* ±0.50	15.75* ±0.85	5.25* ±1.71	9.50* ±0.25	1.75* ±1.93	5.75* ±0.48	0.75* ±0.48	2.00* ±1.22
9.0	9.50 ±0.55	29.2 ±1.98	7.00** ±0.35	17.75* ±1.89	8.00* ±0.00	21.00* ±1.73	4.00* ±0.41	11.75* ±2.50	2.00* ±0.00	3.88** ±0.66

Mean values ± S.E.M. from 4 rats in each group ar given.

Statistical significance (t-test; * $P < 0.05$; ** $P < 0.01$) refers to difference from the control group

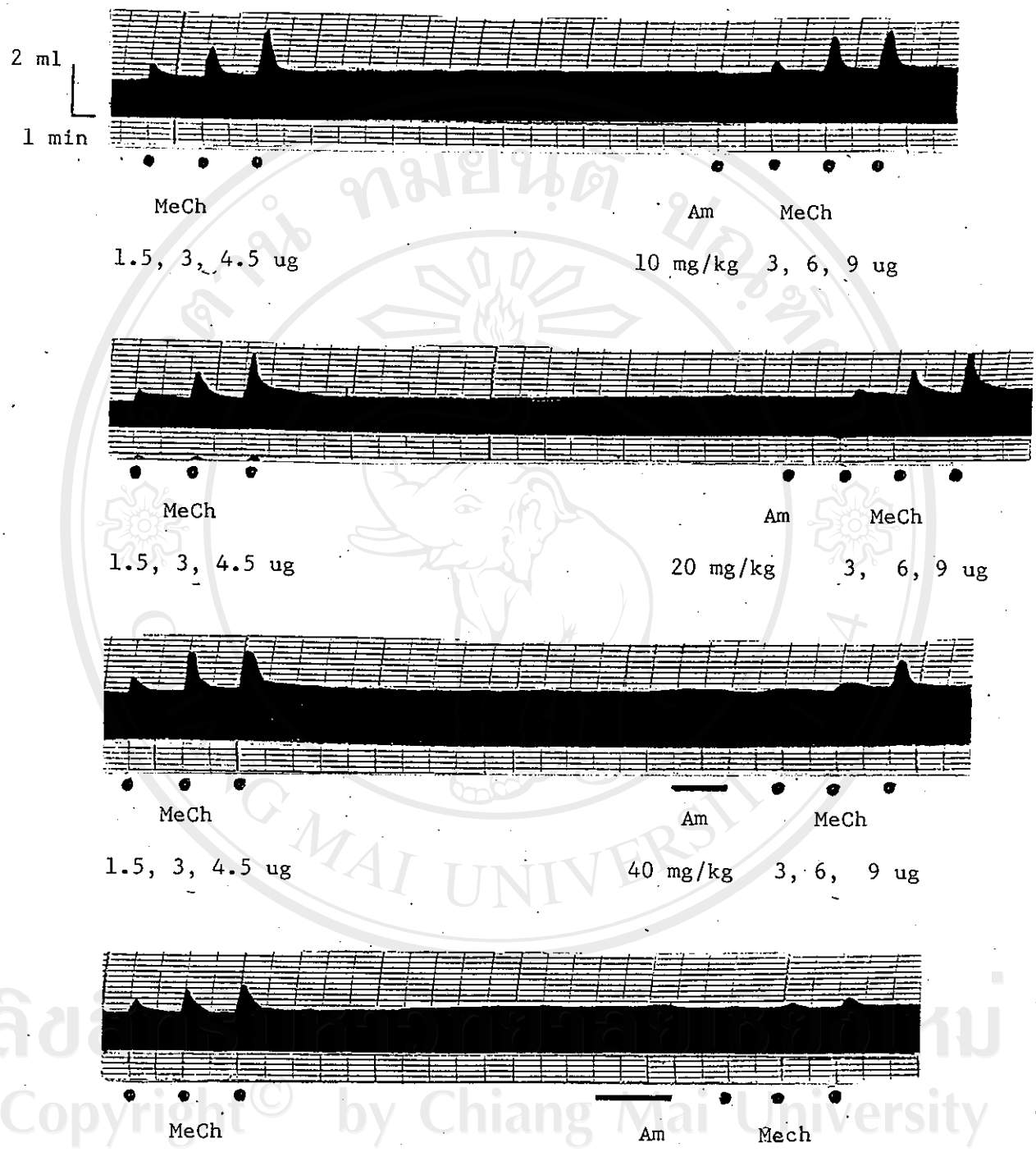


Fig. 32 Effect of aminophylline (Am) on the dose-related methacholine-induced bronchoconstriction in pentobarbital anesthetized rats.

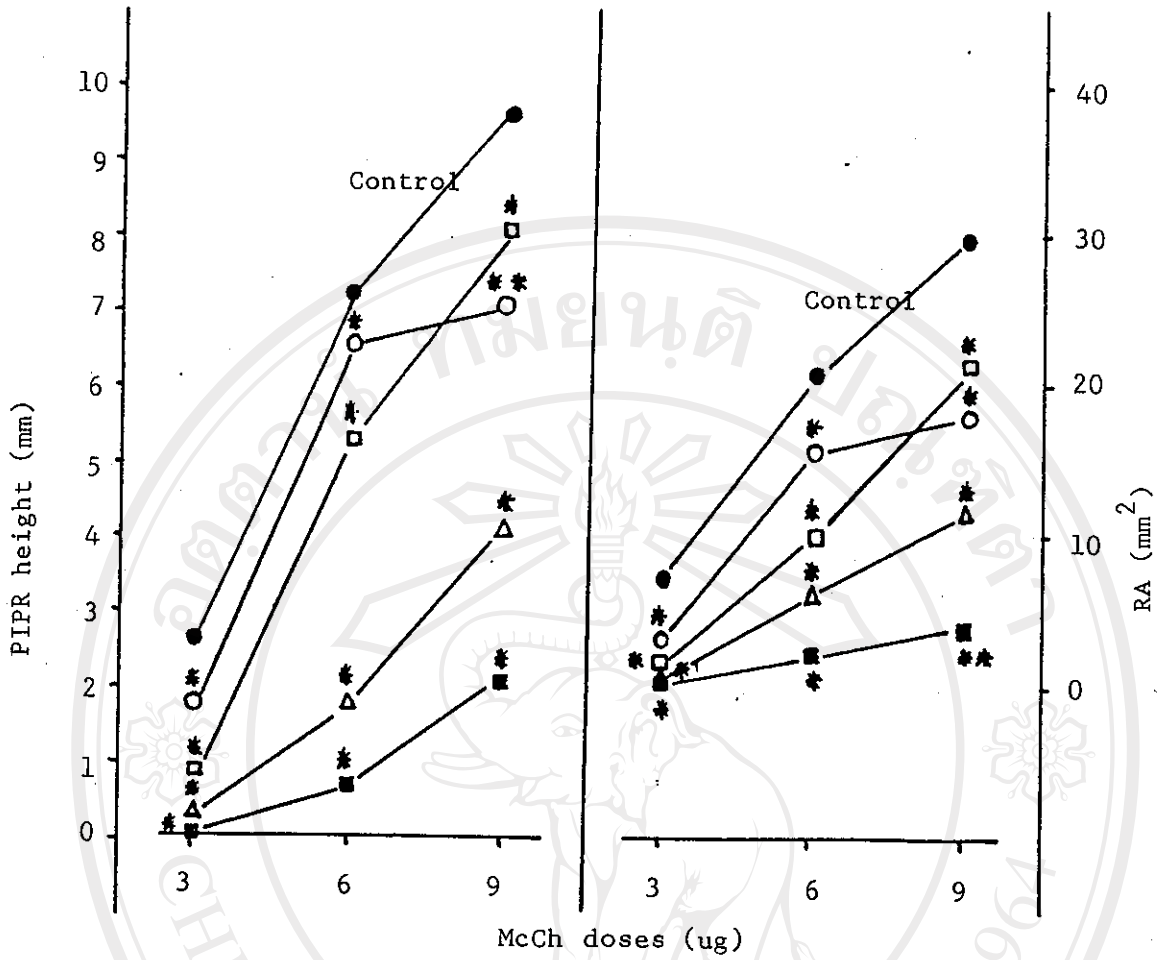


Fig. 33 Effect of aminophylline (Am) on methacholine-induced bronchoconstriction in pentobarbital anesthetized rats, assessed in terms of peak height of intratracheal pressure response (PIPR) and response area (RA).

●——● Control
○——○ Am 10 mg/kg body weight
□——□ Am 20 mg/kg body weight
△——△ Am 40 mg/kg body weight
■——■ Am 80 mg/kg body weight

* P < 0.05

** P < 0.01

a. Decrease of respiratory rate.

The decrease of respiratory rate was seen with all doses of PA-1. A gradual increase in respiratory depression occurred when the dose of PA-1 was increased. Respiratory arrest was seen within 90 minutes following the dose of 1000 mg/kg body weight, and apparently was the cause of death.

b. Decrease of motor activity.

This effect gradually increased with the increased dose of PA-1. The threshold dose of PA-1 which caused the decrease of motor activity was found to be 250 mg/kg body weight. High doses (500 and 1000 mg/kg body weight) caused marked decrease of motor activity (no movement at all).

c. Loss of righting reflex.

At low doses (125 and 250 mg/kg body weight) PA-1 had no effect on the righting reflex. A moderate loss of righting reflex (can be placed on either side equally well) was seen with the dose of 500 mg/kg body weight. Complete loss of righting reflex (cannot be aroused from back position by the hind leg toe pinch) was seen at the dose of 1000 mg/kg body weight.

d. Loss of screen grip.

Loss of screen grip was seen at doses of 500 and 1000 mg/kg

body weight.

e. Enophthalmus.

Mild enophthalmus (equivocal response) was seen with all doses of PA-1.

f. Ataxia.

Ataxia was seen only when high doses of 500 and 1000 mg/kg were given.

All surviving animals were killed and autopsied after 7 days of experiment. Normal size and color of internal organs i.e. liver, lungs, spleen and gastro-intestinal tract were observed.

PA-3 was studied previously by Pichipalakorn (1983) and it was found that intraperitoneal injections of PA-3 at high doses (2, 3 mg/kg body weight) caused the CNS depression which was evidenced by the decrease of motor activity, loss of righting reflex, decrease of respiratory rate and loss of screen grip. Marked CNS depression leading to respiratory arrest was seen with doses of 2 and 3 gm/kg body weight. Enophthalmus, which was the symptom derived from eyes, was observed with all doses of PA-3 tested. It was also noted that blanching of the ear and oral mucosa occurred with all doses of PA-3.

PA-2, PA-4 and PA-5 were not studied in this part of the study because the test requires quite large amount of compounds.

Effect of solvent used

0.5% methyl cellulose, the solvent for PA-1, did not cause any observable signs and symptoms in the tested animals when compared to control animals.

Table 16 Effect of PA-1 given intraperitoneally in conscious rats, recorded 1 hr after drug administration.

Signs and Symptoms	PA-1 (mg/kg)			
	125	250	500	1000
Decrease of motor activity	-	+	+	+
Loss of righting reflex	-	-	+	+
Decrease of respiratory rate	+	+	+	+
Loss of screen grip	-	-	+	+
Enophthalmus	+	+	+	+
Ataxia	-	-	+	+

\- = not detected

\+ = detected