CHAPTER III

RESULTS

In Vivo Preparation.

1. Effects of intravenous piperine on blood pressure and repiration

The effects of intravenously administered piperine in the rat are recorded in Fig. 2 and table 1. Piperine 1 mg/kg introduced by a vein temporarily reduced blood pressure by 46 mmHg, producing bradycardia and apnea. The duration of the low pressure was very short, and soon after the tension rose by 60 mmHg above the initial value. The heart rate declined from 386 beats/min to 118 beats/min and apnea lasted for 8 to 15 seconds. The sudden drop in blood pressure was followed by a rise in blood pressure. The sudden drop in blood pressure combined with bradycardia and apnea indicate with great probability that the symptoms are due to the Bezold-Jarisch reflex. The afferent branch of the Bezold-Jarisch reflex being furnished by the vagus nerve, it was attempted to decide this question by examining how the above symptoms would be affected by bilateral vagotomy. Fig 3 shows that with the vagus nerve cut on both sides, 1 mg/kg of intravenously administered piperine no longer elicited a fall in blood pressure, bradycardia and apnea; instead pressor effect was obtained. Further experiments in rats treated with atropine (0.2 mg/kg) or hexamethonium (5 mg/kg), Piperine failed to cause hypotension and bradycardia, but it still elicited apnea. (as shown in Fig. 4 and Fig. 5)

Experiments were also performed to study the effects of hexamethonium (5 mg/kg) on the pressor response of intravenous piperine.

The results are shown in Fig. 9,13 and Table 4,5,6. It is seen that prior administration of hexamethonium produced a statistically significant ($p < 0.01$) on reducing blood pressure (about 57 %). Also, This pressor response could be attenuated by propranolol (0.7 mg/kg) and phentolamine (1 mg/kg) approximately 50 % and 26 % respectively (see Fig. 10,11,13 and Table 4,5,6). In addition, the combination antagonists effect of propranolol and phentolamine could lessen the pressor response by 65 % (see Fig. 12,13 and Table 4,5,6).

2. Effect on circulation of piperine administered into carotid artery

In order to avoid receptors in the lung and heart eliciting reflex which occurred immediately after intravenous injection, piperine was injected into the carotid artery. Fig. 6 shows that after intracarotidal piperine injection, blood pressure rose by 56 mm.Hg. while depression, bradycardia and apnea concomitant to intravenous administration failed to set in.

Heymans et al. (1933) postulated that lobeline injected into the internal carotid artery raised the blood pressure and doubling the volume of respiration. Gokhan (1953) stated that when applied in this manner, lobeline does not pass the blood brain barrier. With the sinus denerved, depresses repiration and blood pressure rather than increasing them. Not only denervation of the carotid sinus, but also its infiltration with local anesthetics inhibits nerve impulses to originate in chemoreceptors. It was therefore examined how infiltrating the sinus caroticus region with 2 % cocaine, influences the action of piperine given into the carotid artery. As the effect of piperine cannot be abolished by local anesthetics (see Fig 7), it must be assumed that piperine has a direct stimulating effect on the vasomotor center. Thus, further experiments were performed to study the effects of ganglionic

blocking drug on the pressor response of intracarotidal piperine. Fig.8 shows that the pressor effect can be greatly reduced by hexamethonium (5 mg/kg).

3. Effects of intravenous piperine in pithed rat,

As shown in Fig. 14, after intravenous injection of 1 mg/kg piperine blood pressure rose by 60 mmHg. without hypotension and bradycardia. Phentolamine (1 mg/kg) and propranolol (0.7 mg/kg) could attenuate the pressor response 52 % and 34 % respectively (see Fig. 15, 16,19,20,21). Again the results in Fig. 17,19,20 and 21, prior administration of combined propranolol and phentolamine, show a statistically significant ($P < 0.005$) on reducing blood pressure. This antagonized effect did not differ from the antagonizing effect of phentolamine alone.

Experiments were also performed to study the effect of reserpine pretreatment on the pressor response in pithed rats. The results are shown in Fig. 18 in which 1 mg/kg piperine was given into femoral vein of pithed, reserpine-pretreated rats. It is seen that reserpine pretreatment almost completely abolished the pressor effect of piperine in pithed rat (about 91 %)

In Vitro Preparation:

1. Effects of piperine on isolated rat atria.

: Positive chronotropic and inotropic effect.

The effect of piperine on right atrial rate and left atrial isometric tension is recorded in Fig. 22 and Fig. 23. Five doses of piperine, 3,6,12,24 and 48 ug/ml, were tested in these experiments. At all other concentrations tested, piperine produced both positive chronotropic and inotropic effects, reaching a peak at 3-5 min exposure

a subsequent declining of intensity of effect can be seen cleary for all concentrations during 30 min of continuous exposure. The positive chronotropic and inotropic showed dose dependent. Piperine at 24 and 48 ug/ml produced a large initial increase in rate and contractile force which were followed by depression. In this study a dose of 12 ug/ml was used to investigate for the mechanism of piperine. The positive chronotropic and inotropic effects at this dose were about 18 % and 11 % increase from normal value respectively.

2. Effects of a beta-blocking agent (propranolol) on positive chronotropic and inotropic actions of piperine.

As shown in Fig. 24 and Fig. 25 propranolol 0.15 ug/ml caused no significant reduction on both right atrial rate and left atrial force of contraction. Prior administration of propranolol (5 min before piperine) could significantly ($p < 0.05$) reduce the positive chronotropy of piperine at every periods along 30 min. The average percentage of this reduction was about 50 % from the effect of piperine alone (see Fig. 24). In Fig. 25, propranolol (0.15 ug/ml) gradually reduced the positive inotropic effect of piperine but they were not statistically significance.

Experiments were also performed to study the antagonized effect of propranolol at dose 0.03 and 0.07 ug/ml on the positive inotropy of piperine. Also, the results observed in Fig. 26 were shown that propranolol at dose 0.03 and 0.07 ug/ml could not significantly reduce the positive inotropic effect of piperine.

3. Effects of piperine on isolated reserpinized rat atria.

The rats were pretreated with reserpine 5 mg/kg i.p. 2 days before experiment. The average body weight was decreased about 47.12 $\pm$

1.35 gm. (n = 35). Normal right atrial rate collected from 35 rats was about 261.5 $\pm$ 20.5 beats/min which was similar to non-treated rats (266.4 $\pm$ 24.6, n = 82). As shown in Fig 27, the positive chronotropic effect of piperine in reserpinized rats was less than those observed in nonreserpinized rats. The percentage of this reduction was about 41 % at 1 min, 60 % at 3 min and 100 % at 5 min after addition of piperine and all of the points were highly significant ($P < 0.01$). Again in the left atria, reserpine pretreatment almost completely abolished the positive inotropic effect of piperine (about 90 %). The peak maximum effect was 103 $\pm$ 0.84 % at 3 min after addition of piperine (see Fig.28).

4. Effects of 5-HT antagonist (Methysergide, Cyproheptadine) on positive chronotropic and inotropic actions of piperine.

As shown in Fig. 29 and Fig. 30, Prior administration of methysergide 0.47 ug/ml (5 min before addition of piperine) could not reduce both the positive chronotropic and inotropic effects of piperine at every periods along 30 min.

Similar to methysergide, cyproheptadine could not antagonized both the positive chronotropy and inotropy of piperine (see Fig. 31,32).

5. Effects of α - blocking agent (Phentolamine) on positive chronotropic and inotropic actions of piperine.

In the presence of phentolamine (.32 ug/ml; added 5 min before) the effects of piperine were not altered in any significant way (Fig. 33,34).

6. Effects of neuronal uptake inhibitor (Desipramine, Cocaine) on positive chronotropic and inotropic actions of piperine.

Prior administration of desipramine (.27 ug/ml; 15 min before

piperine) caused a dramatic reduction (about 60 %) on right atrial rate (see Fig. 35) and also 90 % on the left atrial isometric tension (see Fig. 36). The peak maximum effect in the left atria was 101.03 $\pm$ 3.18 at 1 min after addition of piperine.

Similarly, in the presence of cocaine (9.1 ug/ml, added 15 min before) the positive chronotropy and inotropy of piperine were reduced by approximately 60 % and 90 % respectively (Fig. 37,38).

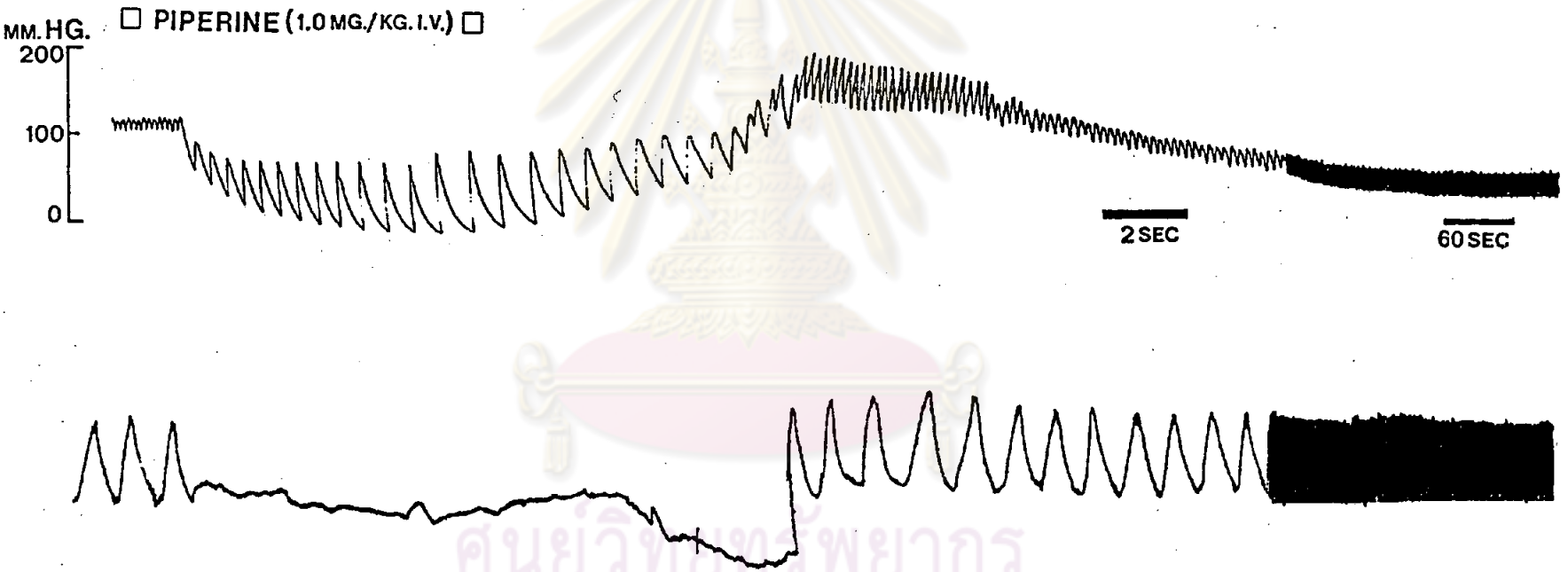

Figure 2. Effect of 1 mg/kg of intravenous piperine on blood pressure and respiration. Readings from top to bottom : blood pressure, respiration.

Piperine 1 mg/kg (n = 20)	Systemic blood pressure (mm.Hg)			Heart rate (beats/min)	Duration of apnea (sec)
	Systolic blood pressure	Diastolic blood pressure	Mean blood pressure		
Before piperine injection	110.44±1.76	74.44±1.92	86.11±2.21	386.67±2.11	-
After piperine injection	85.56±1.81	17.78±2.31	40.37±2.19	118.78±1.51	11.89±2.13
% decrease	22.49±1.38	76.09±2.01	53.13±2.31	69.13±2.28	

Table 1. Effect of 1 mg/kg of intravenous piperine on blood pressure, heart rate and respiration. (mean±S.E.M.)

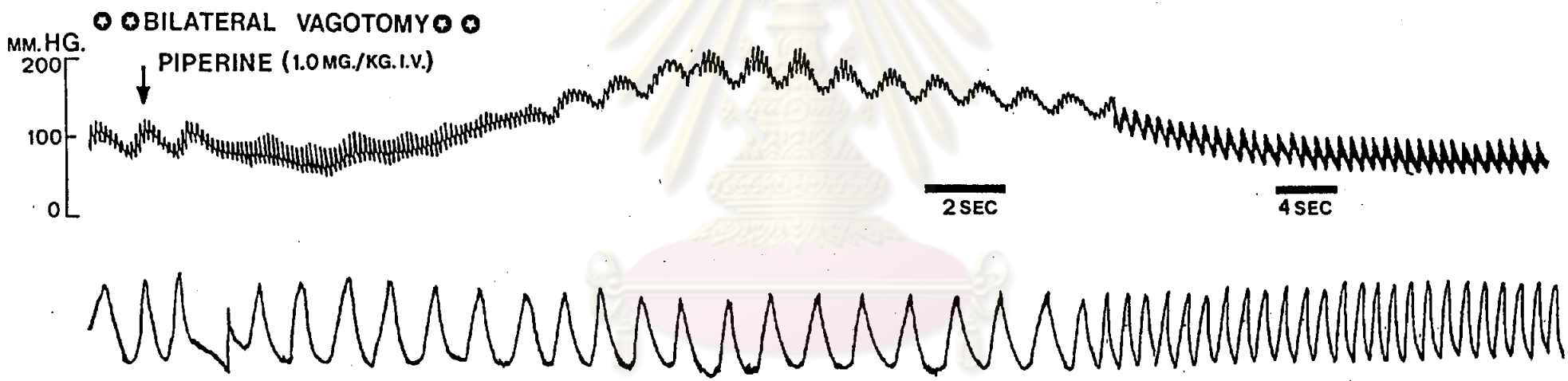

Figure 3. Effect of intravenous piperine on blood pressure and respiration in the vagotomized rat. Reading from top to bottom : blood pressure, respiration.

Piperine 1 mg/kg (n = 12)	Systemic blood pressure			Heart rate (beats/min)
	Systolic blood pressure	Diastolic blood pressure	Mean blood pressure	
Before piperine injection	116.38±2.91	90.34±2.12	98.18±2.46	358.74±2.45
After piperine injection	186.13±2.36	160.24±3.15	168.13±92	360.81±2.52
% change	60.11±2.89	76.37±3.28	70.25±2.86	0.54±2.39

Table 2. Effect of intravenous piperine on blood pressure and heart rate in the vagotomized rat. (mean±S.E.M.).

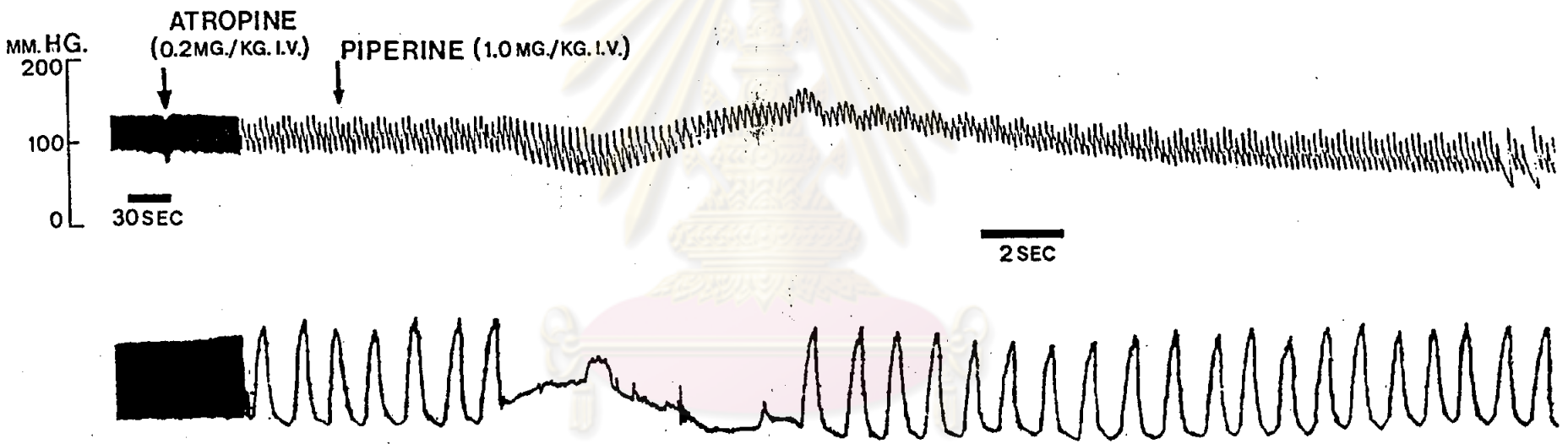

Figure 4. Atropine inhibits the depressor- and bradycardia - producing effects of intravenous piperine, without affecting apnea (0.2 mg/kg of atropine had been administered previously.) Readings from top to bottom : blood pressure, respiration.

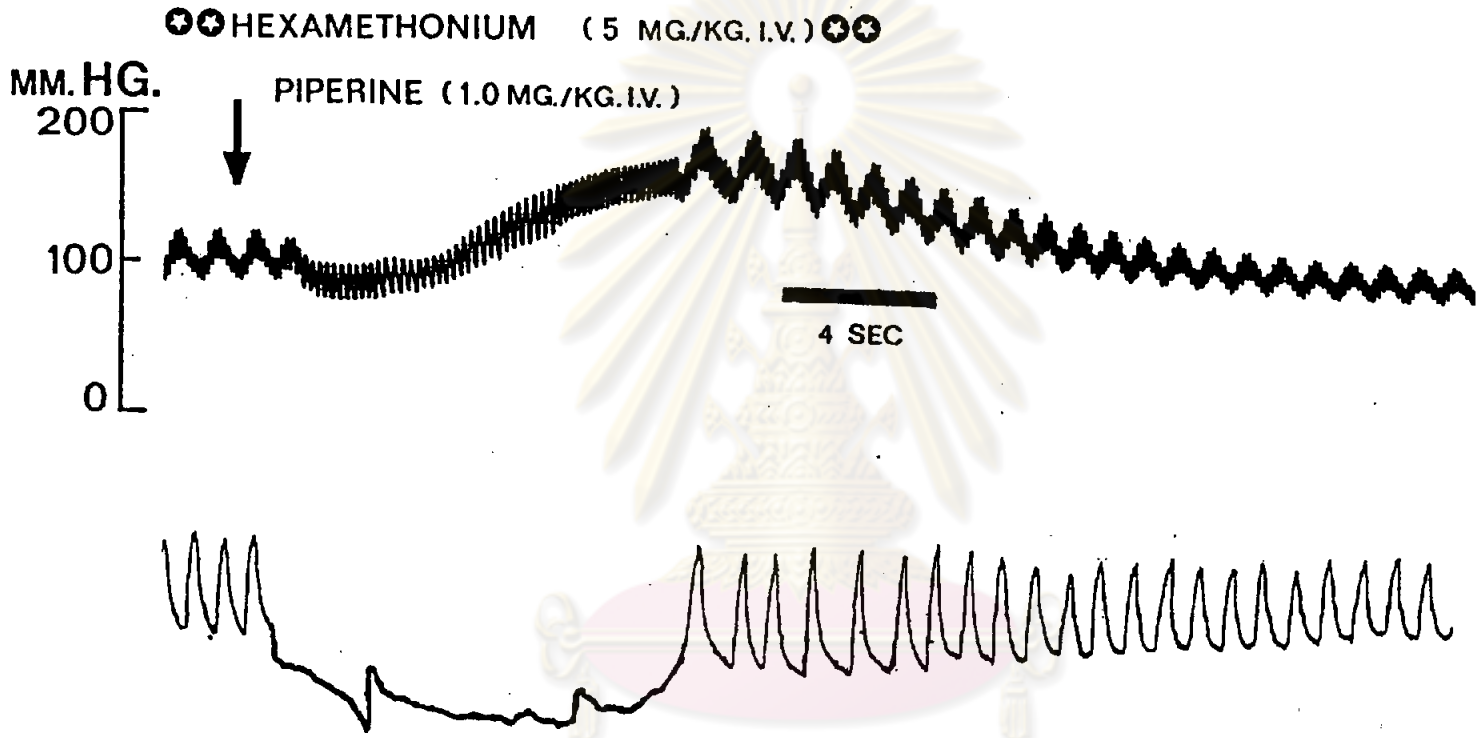

Figure 5. Hexamethonium inhibits the depressor- and bradycardia- producing effects of intravenous piperine, without affecting apnea (5 mg/kg of hexamethonium had been administered previously.) Readings from top to bottom : blood pressure, respiration.

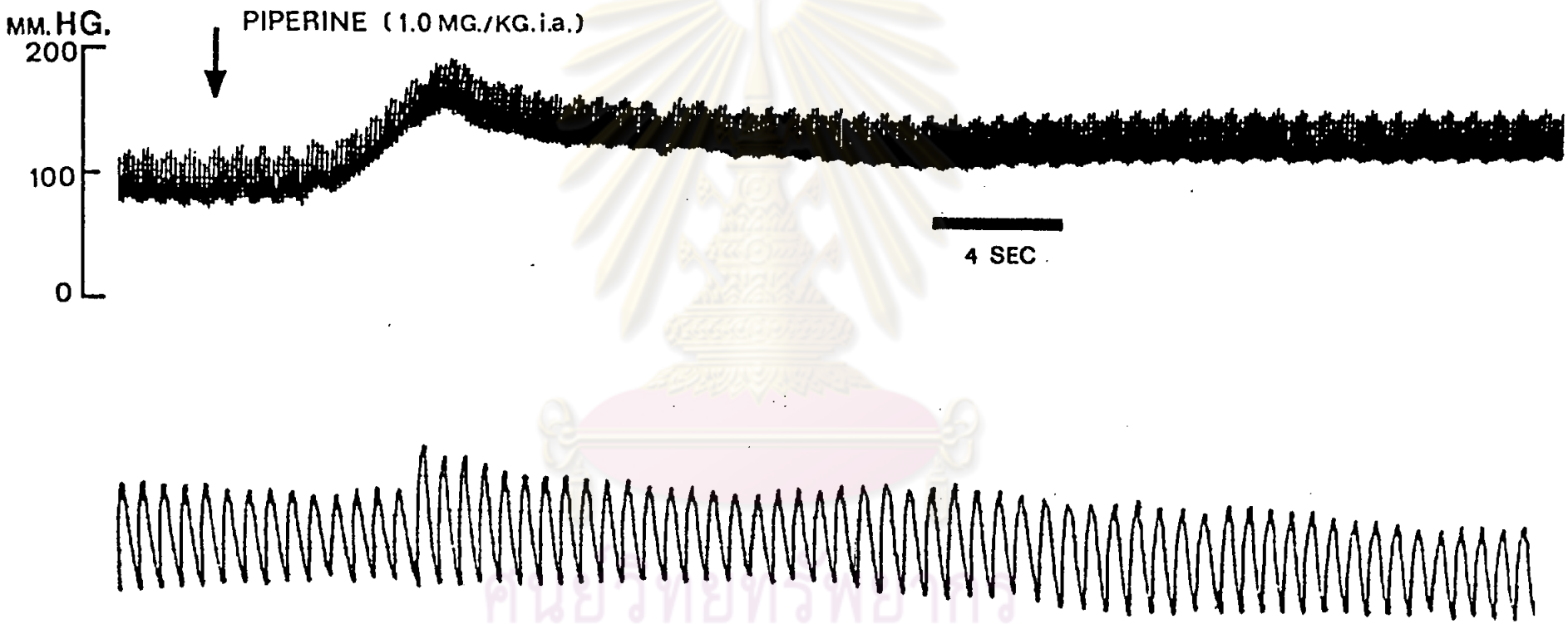

Figure 6 . Effect of intracarotidal piperine on blood pressure and respiration. Readings from top to bottom : blood pressure, respiration.

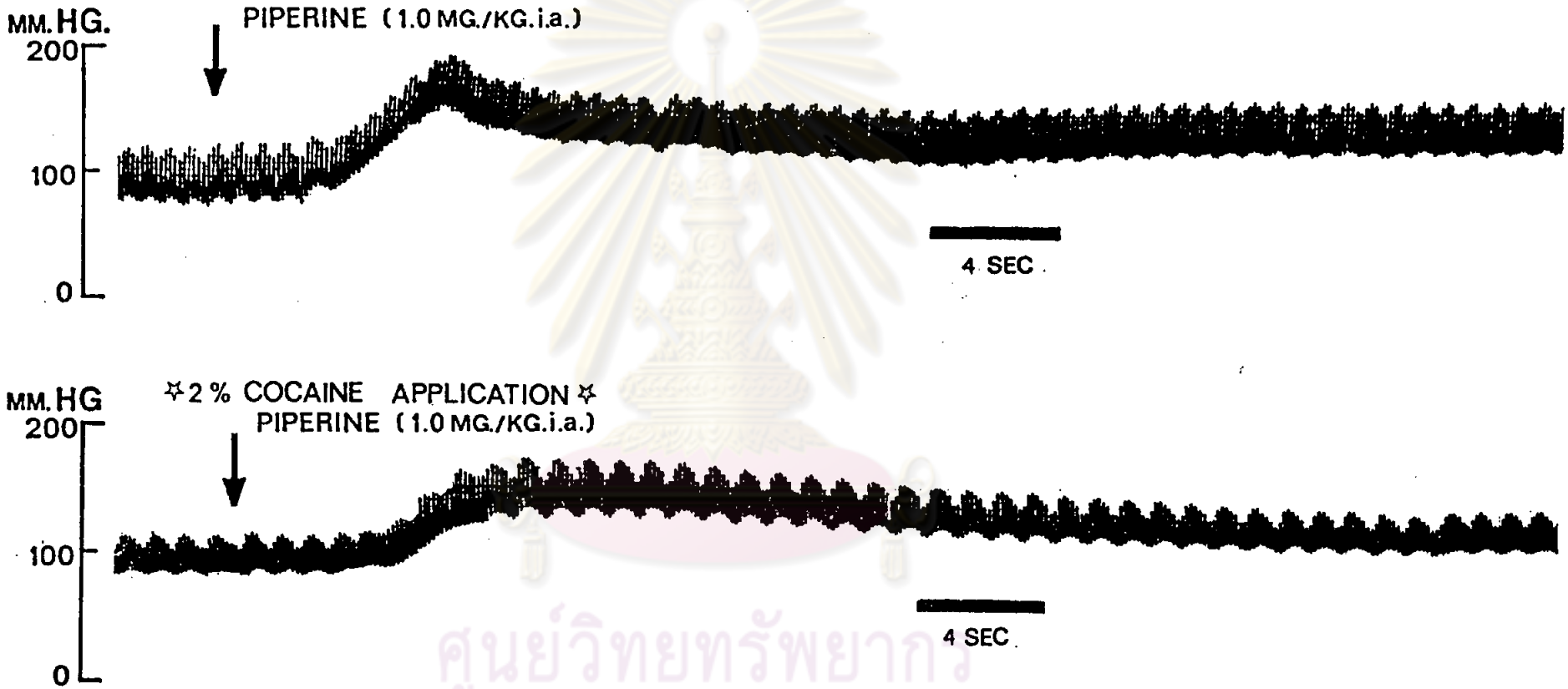

Figure 7. Cocaine application (0.5 ml of 2 %) of the carotid sinus in the 30 th minute of the experiment does not modify the effect of intracarotidal piperine.

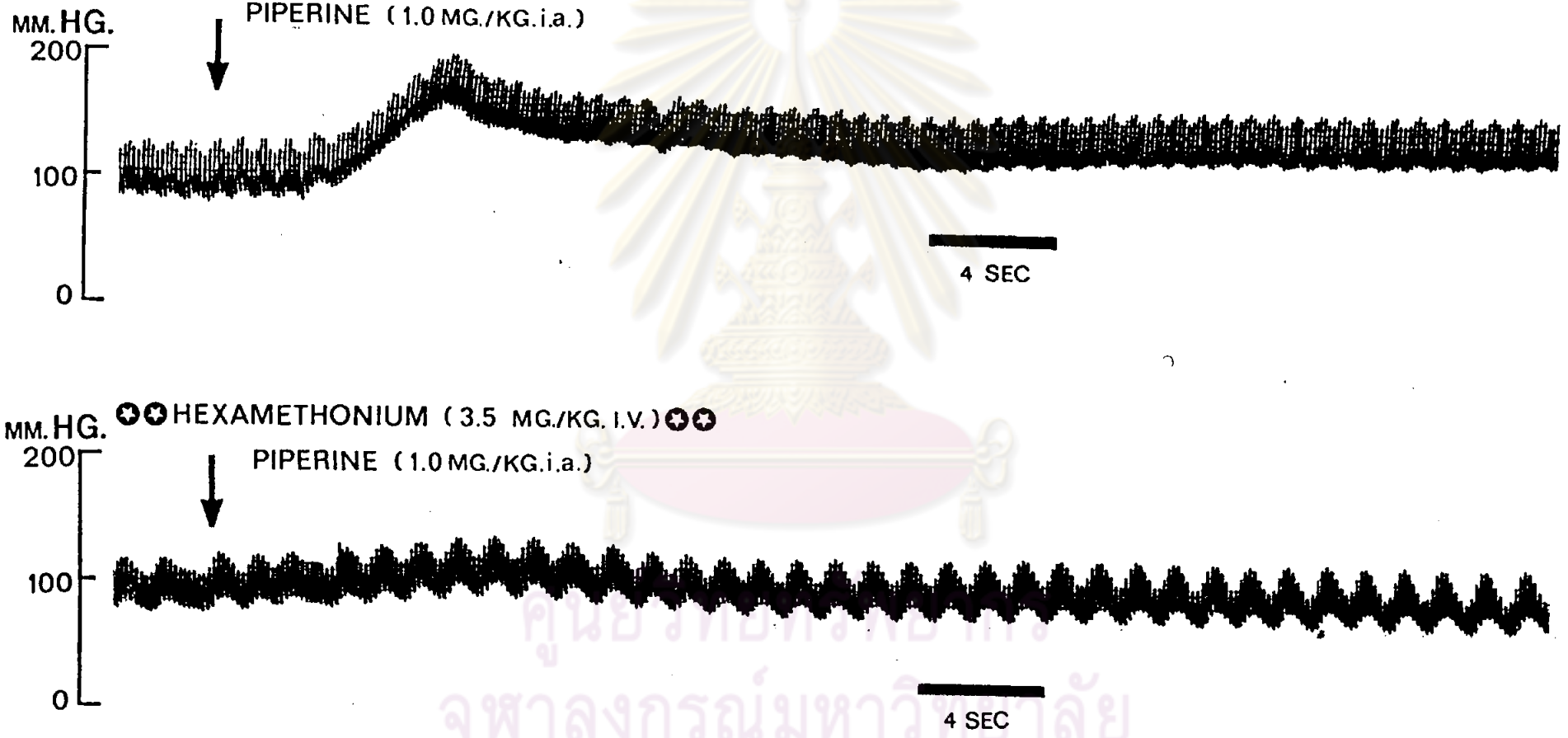

Figure 8 . Records showing the pressor effect of intracarotidal piperine before and after hexamethonium 5 mg/kg blockade 5 min in anesthetized rats.

Substance	No. of Rat	Systolic blood pressure			Diastolic blood pressure			Mean blood pressure		
		Before piperine injection	After piperine injection	% change	Before piperine injection	After piperine injection	% change	Before piperine injection	After piperine injection	% change
Piperine (1 mg/kg i.c.).	7	122±2.38	174±2.32	42.62±2.53	91±2.10	138±2.01	51.65±2.48	101.3±2.4	150.67±2.43	48.69±2.41
Piperine (1 mg/kg i.c.). After hexamethonium (5 mg/kg i.v.).	7	108±2.08	126.3±2.86	16.94±2.52	81±2.12	95±2.09	17.28±2.39	90±1.83	105.43±2.32	17.14±2.41

Table 3. Comparison of the pressor effect of intracarotidal piperine before and after hexamethonium 5 mg/kg blockade , in anesthetized rats (mean±S.E.M.)

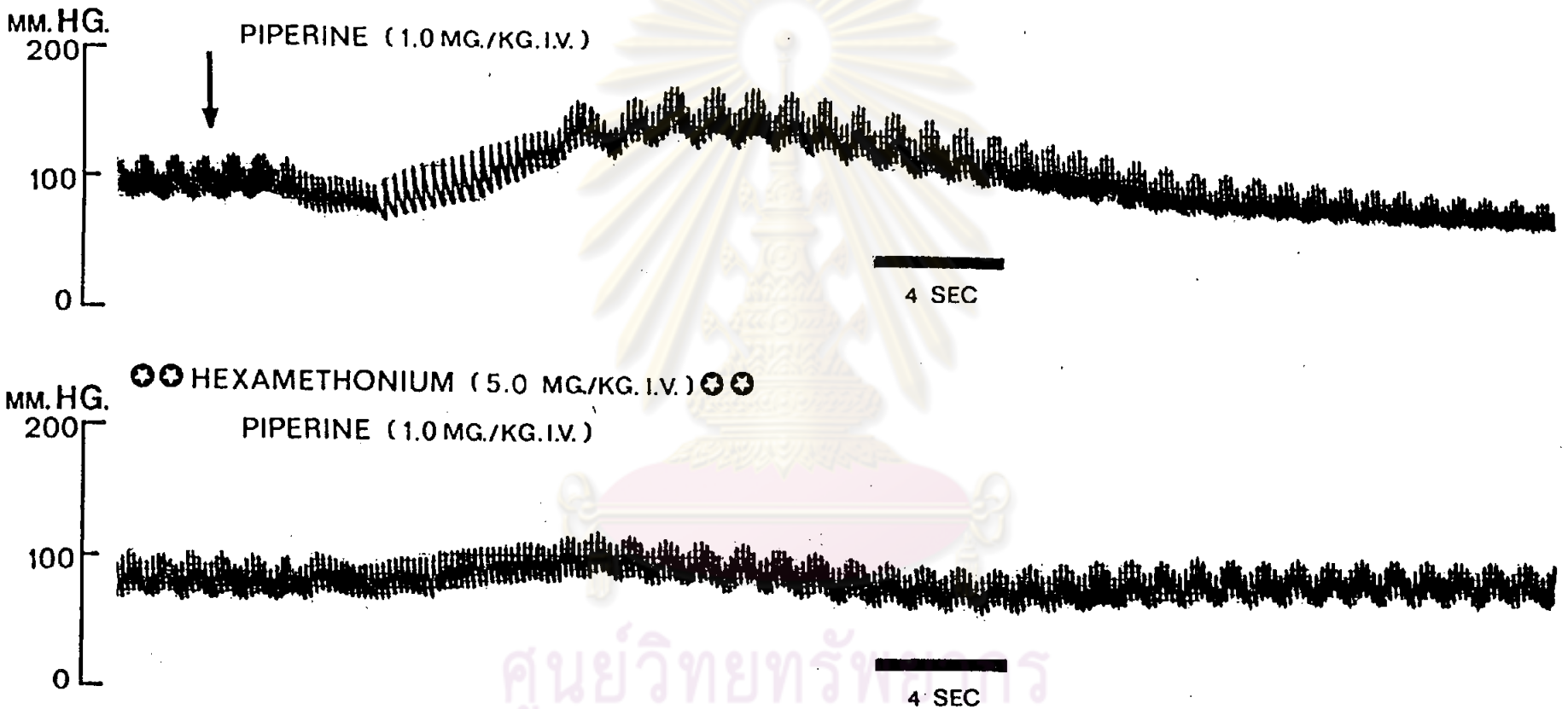

Figure 9. Records showing the pressor effect of intravenous piperine before and after hexamethonium 5 mg/kg blockade for 5 min in vagotomized rats.

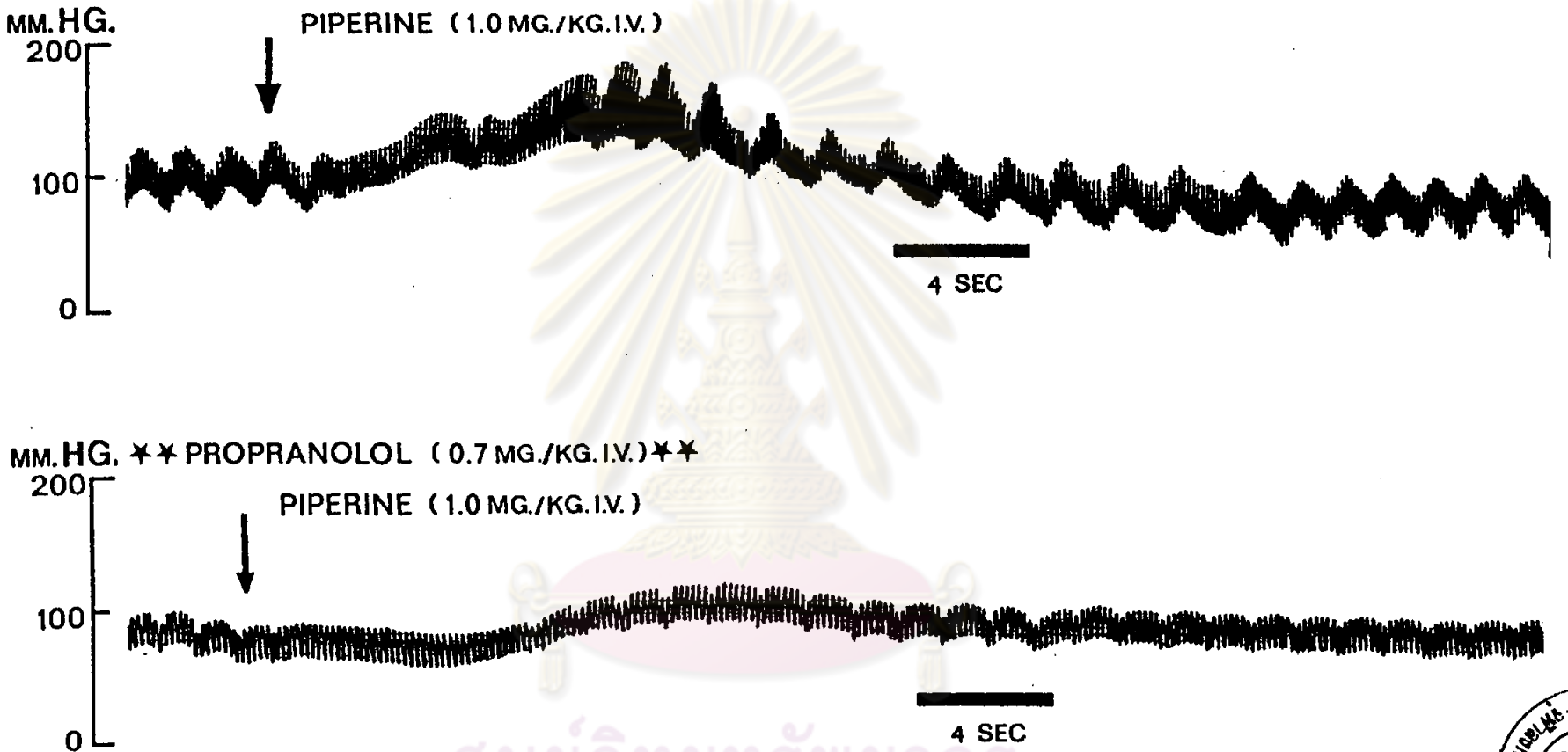

Figure 10. Records showing the pressor effect of intravenous piperine before and after propranolol 0.70 mg/kg blockade for 5 min in vagotomized rats.

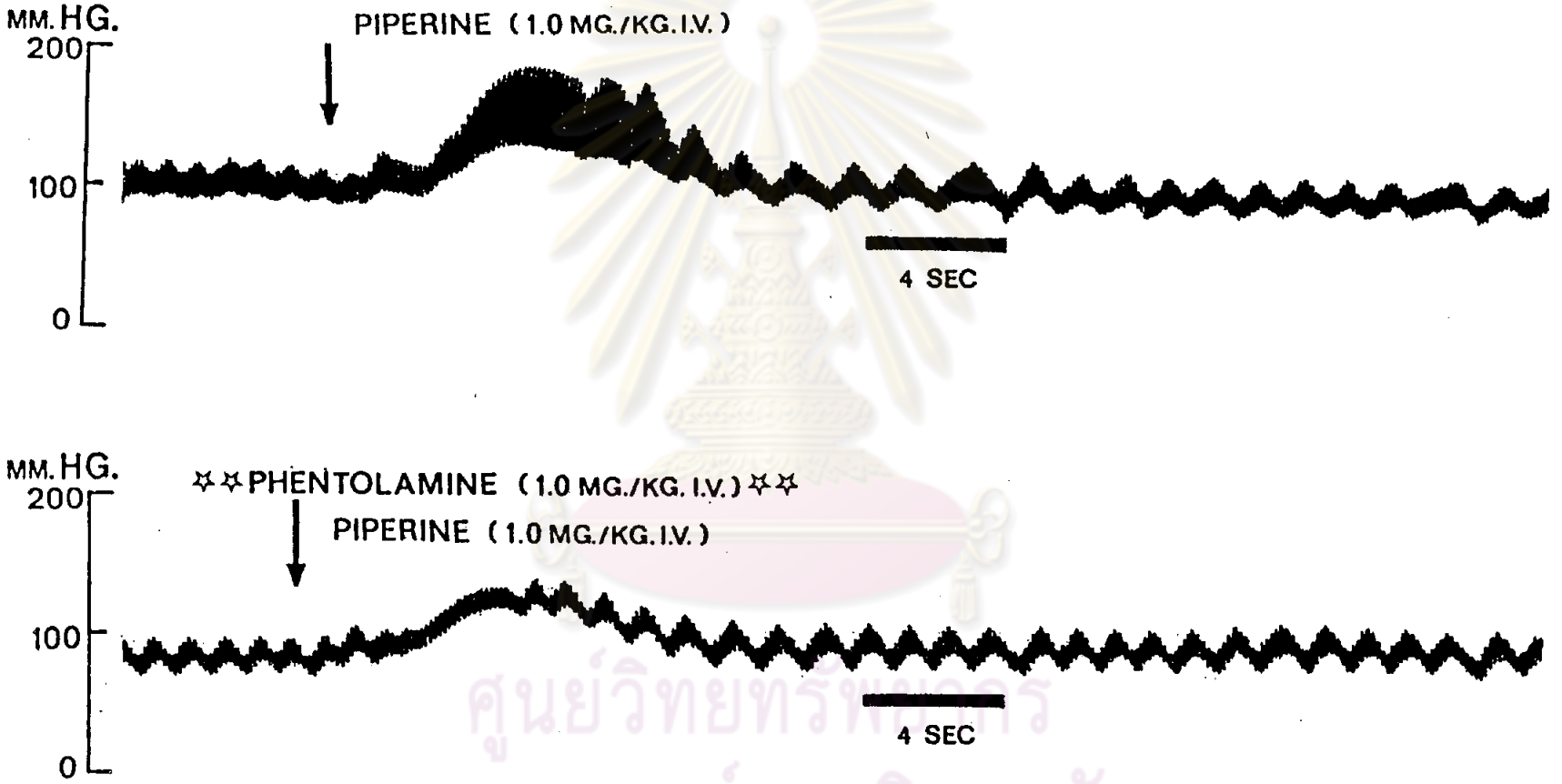

Figure 11. Records showing the pressor effect of intravenous piperine before and after phentolamine 1 mg/kg blockade for 5 min in vagotomized rats.

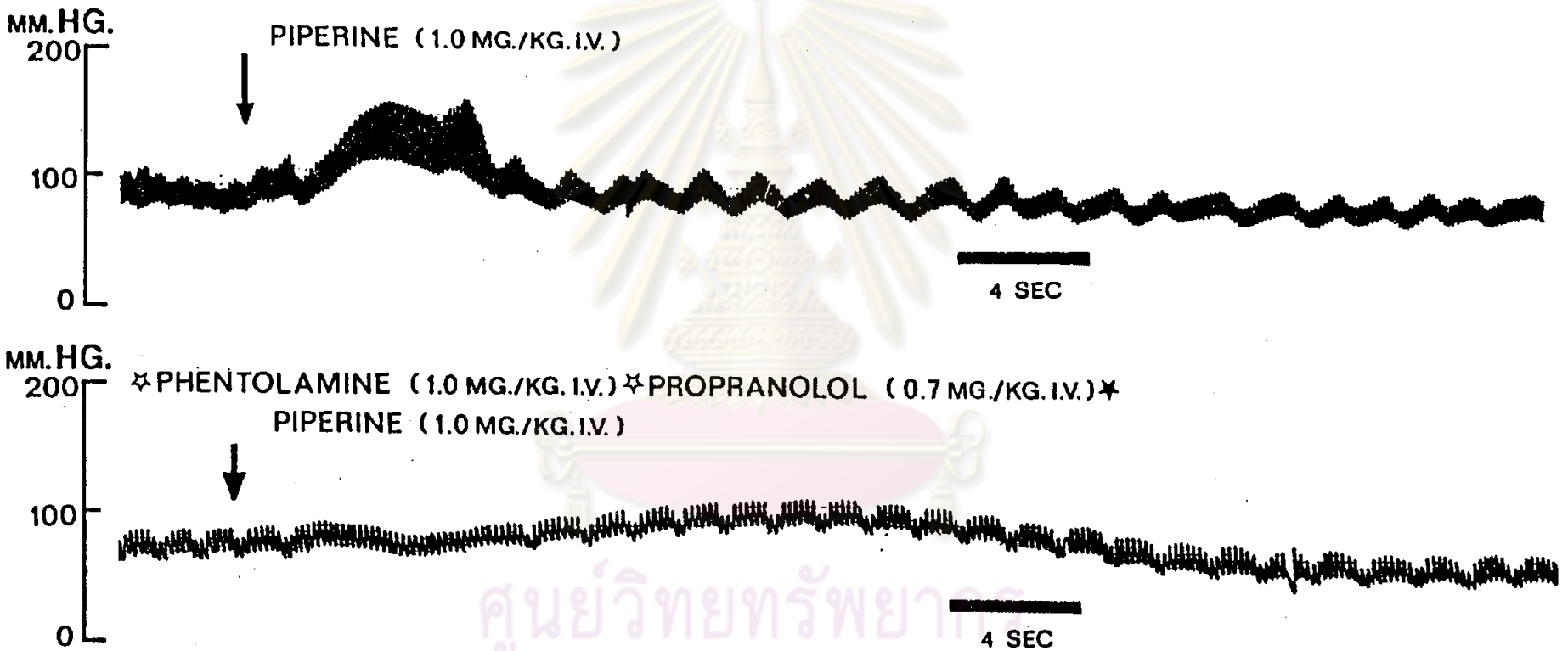

Figure 12. Records showing the pressor effect of intravenous piperine before and after propranolol 0.70 mg/kg and phentolamine 1 mg/kg blockade for 5 min in vagotomized rats.

Substance (mg/kg)	No of Rat	Systolic blood pressure (mm.Hg) After bilateral vagotomy		
		Before piperine injection	After piperine injection	% change
Piperine 1.0	6	118.4±1.56	179.72±2.91	51.79±2.53
Piperine 1.0 After hexamethonium 5.0	6	114.75±2.12	136.5±2.51	19.45±2.59
Piperine 1.0	5	112.28±3.21	170.82±2.19	52.14±2.82
Piperine 1.0 After propranolol 0.7	5	100.63±2.19	121.91±2.92	21.15±2.93
Piperine 1.0	5	116.39±2.66	177.83±2.86	52.79±2.79
Piperine 1.0 After phentolamine 1.0	5	103.23±2.85	143.69±2.49	39.19±2.45
Piperine 1.0	5	102±2.96	164.72±2.94	61.49±3.01
Piperine 1.0 After propranolol 0.7 and phentolamine 1.0	5	85.93±2.83	104.96±2.84	22.96±2.91

Table 4. Comparison of the effects of piperine on systolic blood pressure before and after hexamethonium, propranolol, phentolamine, propranolol and phentolamine blockade in anesthetized rats (mean±S.E.M.).

Substance (mg/kg)	No of Rat	Diastolic blood pressure (mm.Hg) After bilateral vagotomy		
		Before piperine injection	After piperine injection	% change
Piperine 1.0	6	93.8±2.25	137.4±2.32	46.48±2.18
Piperine 1.0 After hexamethonium 5.0	6	90.5±2.18	109.26±2.85	20.72±2.83
Piperine 1.0	5	91.23±2.63	138.62±2.49	51.84±2.91
Piperine 1.0 After propranolol 0.7	5	80.19±2.74	101.19±2.64	26.19±2.86
Piperine 1.0	5	90.26±2.91	134.73±2.91	49.27±2.73
Piperine 1.0 After phentolamine 1.0	5	80.67±2.92	109.54±2.39	35.79±2.52
Piperine 1.0	5	81.45±2.69	126.53±3.21	55.35±3.02
Piperine 1.0 After propranolol 0.7 and phentolamine 1.0	5	66.32±2.81	78.23±3.21	17.96±3.12

Table 5. Comparison of the effects of piperine on diastolic blood pressure befote and after hexamethonium, propranolol, phentolamine, propranolol and phentolamine blockade in anesthetized rats (mean±S.E.M.).

Substance (mg/kg)	No of Rat	Mean blood pressure (mm.Hg) After bilateral vagotomy		
		Before piperine injection	After piperine injection	% change
Piperine 1.0	6	102.67±2.61	151.49±2.12	46.78±2.38
Piperine 1.0 After hexamethonium 5.0	6	98.51±2.08	118.34±2.48	20.09±2.79
Piperine 1.0	5	98.24±2.92	149.29±2.79	51.96±2.85
Piperine 1.0 After propranolol 0.7	5	87±2.78	108.11±2.98	24.26±2.69
Piperine 1.0	5	98.97±2.76	149.09±2.83	50.64±2.98
Piperine 1.0 After phentolamine 1.0	5	88.19±2.84	120.92±2.96	37.11±3.03
Piperine 1.0	5	88.45±2.83	139.26±3.13	57.44±2.99
Piperine 1.0 After propranolol 0.7 and phentolamine 1.0	5	72.86±2.79	87.14±3.09	19.59±2.93

Table 6. Comparison of the effects of piperine on mean blood pressure before and after hexamethonium, propranolol, phenolamine, propranolol and phentolamine blockade in anesthetized rats (mean ± S.E.M.)

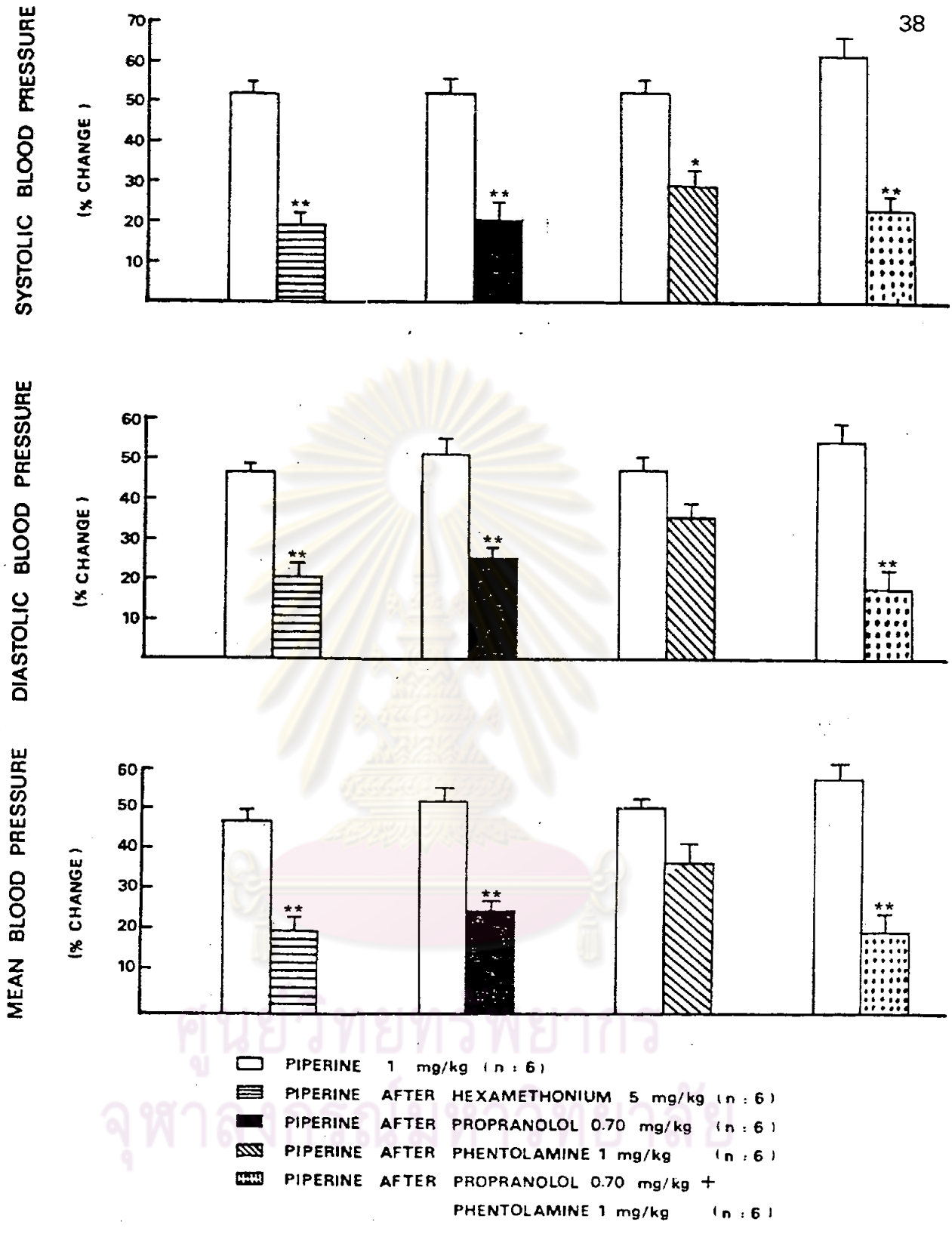

Figure 13. Comparison of the effects of piperine on systolic blood pressure diastolic blood pressure mean blood pressure before and after hexamethonium, propranolol,phentolamine, propranolol and phentolamine blockade in anesthetized rats. Bar graph represent mean percent increase±S.E.M.

$^{*}P < 0.05$ $^{**}P < 0.01$

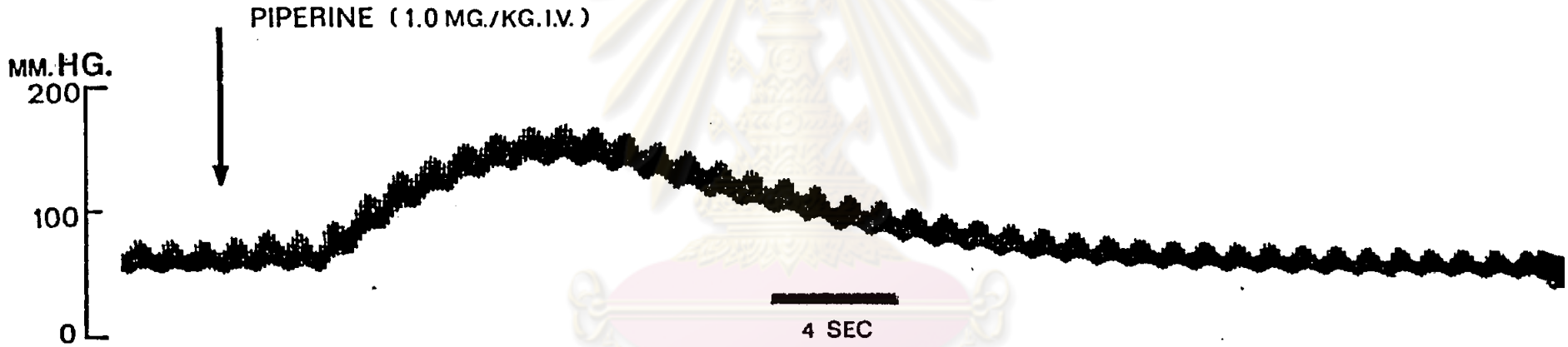

Figure 14. Effect of 1 mg/kg of intravenous piperine on blood pressure in pithed rats.

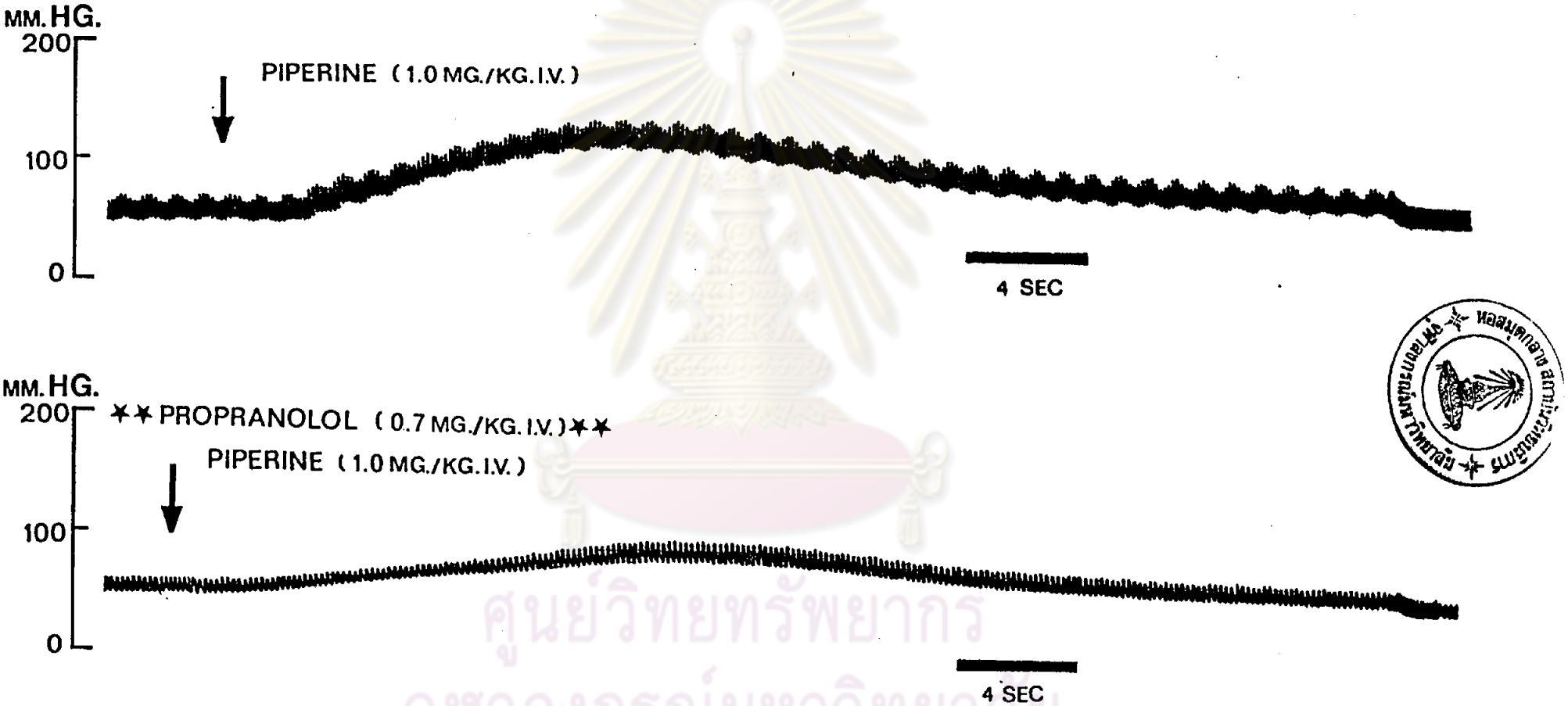

Figure 15. Records showing the pressor effect of intravenous piperine before and after propranolol 0.70 mg/kg blockade for 5 min in pithed rats.

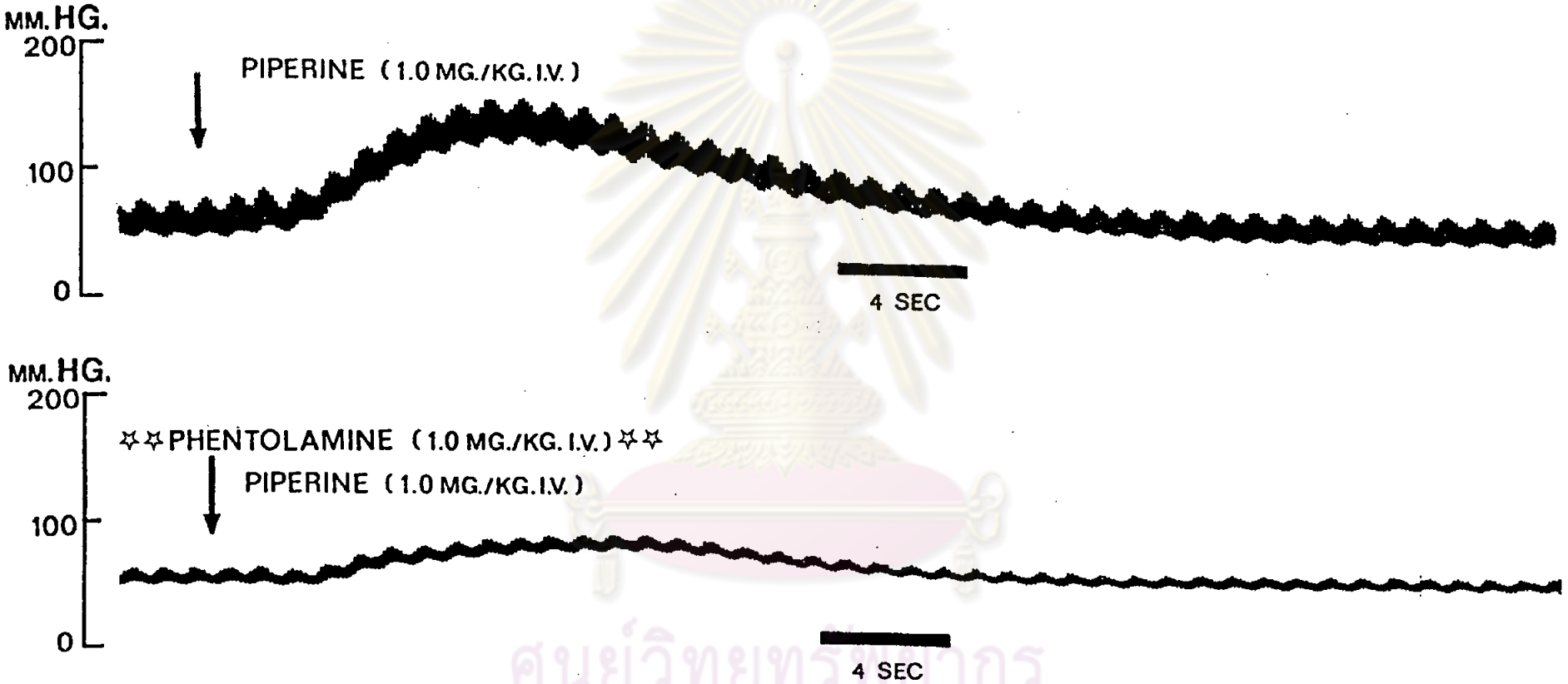

Figure 16. Records showing the pressor effect of intravenous piperine before and after phentolamine 1 mg/kg blockade for 5 min in pithed rats.

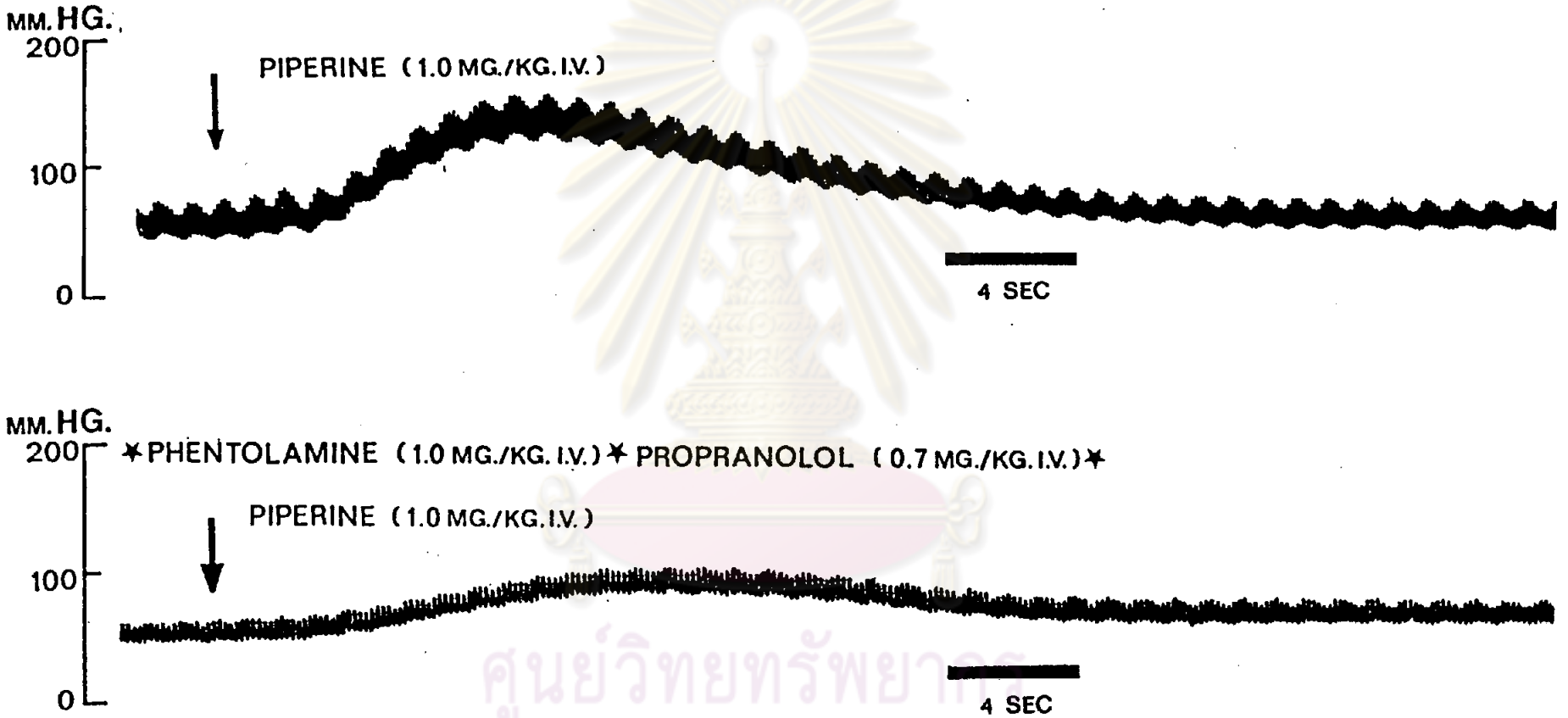

Figure 17. Records showing the pressor effect of intravenous piperine before and after propranolol 0.70 mg/kg and phentolamine 1 mg/kg blockade for 5 min in pithed rats.

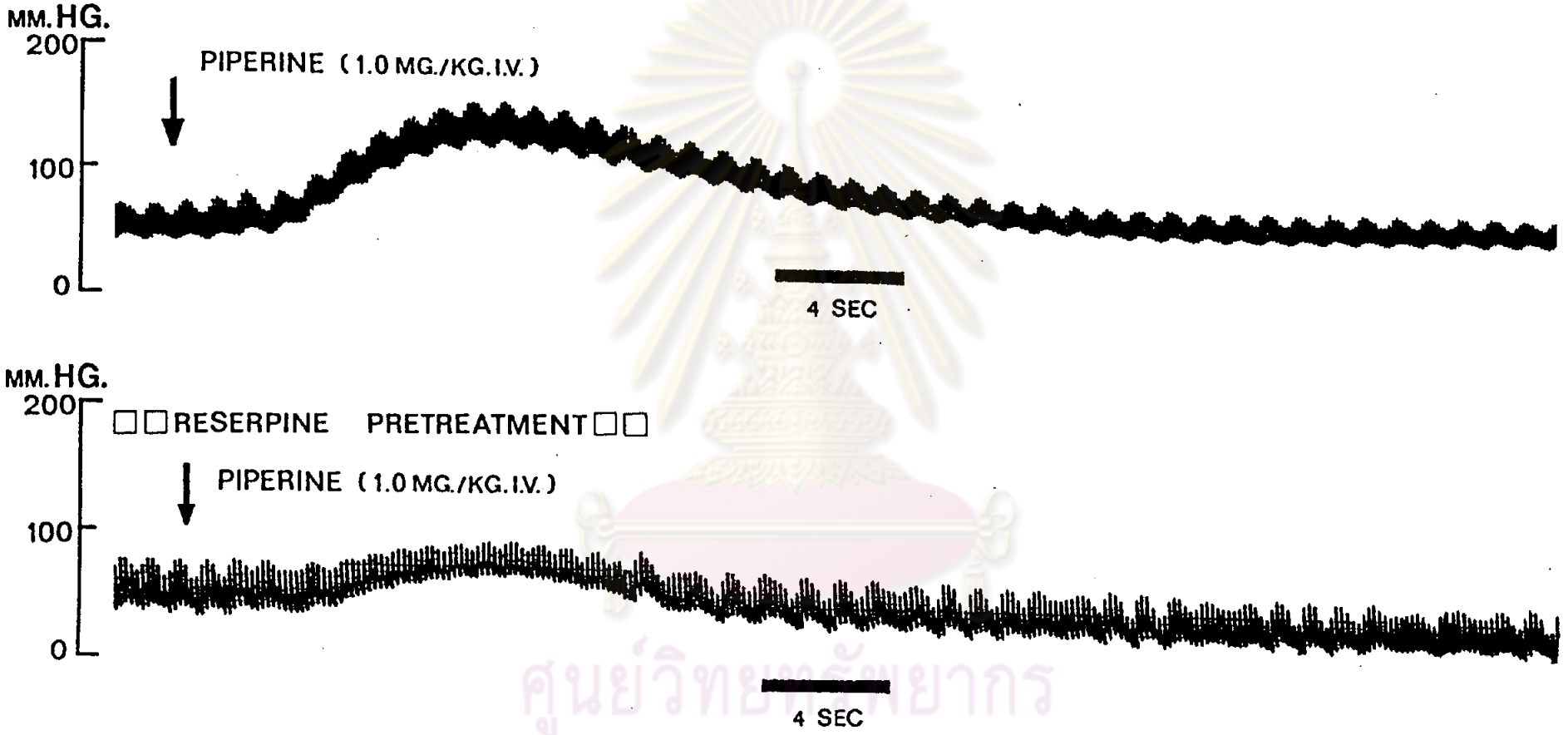

Figure 18. Records showing the pressor effect of intravenous piperine in pithed rats and reserpine-pretreated pithed rats.

Substance (mg/kg)	No. of Rat	Systolic blood pressure (mm.Hg)		
		Before piperine injection	After piperine injection	% change
Piperine 1.0	6	59.8±1.20	124.2±1.32	108.52±1.39
Piperine 1.0 After propranolol 0.7	6	50.00±0.48	90.6±1.28	81.18±1.16
Piperine 1.0	6	58.00±1.38	132.89±1.96	128.18±1.02
Piperine 1.0 After phentolamine 1.0	6	43.00±1.41	74.5±1.81	74.15±1.31
Piperine 1.0	6	57.8±1.28	125.2±1.22	116.06±1.08
Piperine 1.0 After propranolol 0.7 and phentolamine 1.0	6	46.2±1.44	74.91±2.34	63.78±1.29
Piperine 1.0	6	58.00±1.38	132.89±1.96	129.12±1.41
Piperine 1.0 After reserpine pretreatment	6	65.83±1.83	73.00±1.24	9.16±1.53

Table 7. Comparison of the effects of piperine on systolic blood pressure before and after propranolol 0.70 mg/kg, phentolamine 1 mg/kg, propranolol 0.70 mg/kg and phentolamine 1 mg/kg, reserpine pretreatment in pithed rats. (mean±S.E.M.).

Substance (mg/kg)	No. of Rat	Diastolic blood pressure (mm.Hg)		
		Before piperine injection	After piperine injection	% change
Piperine 1.0	6	41.2±1.01	101.2±0.98	146.71±0.89
Piperine 1.0 After propranolol 0.2	6	36.00±0.52	70.00±1.12	95.12±0.94
Piperine 1.0	6	38.67±1.52	109.00±1.48	182.17±1.25
Piperine 1.0 After phentolamine 1.0	6	31.8±1.23	57.4±1.35	81.43±1.44
Piperine 1.0	6	39.2±1.17	101.2±1.24	159.13±1.23
Piperine 1.0 After propranolol 0.7 and phentolamine 1.0	6	34.4±1.81	60.14±2.18	74.89±2.79
Piperine 1.0	6	38.67±1.52	109.00±1.48	181.72±1.52
Piperine 1.0 After reserpine pretreatment	6	48.33±1.99	55.67±1.42	15.12±1.45

Table 8. Comparison of the effects of piperine on diastolic blood pressure before and after propranolol 0.70 mg/kg, phentolamine 1 mg/kg, propranolol 0.70 mg/kg and phentolamine 1 mg/kg, reserpine pretreatment in pithed rats. (mean±S.E.M.).

Substance (mg/kg)	No. of Rat	Mean blood pressure (mm.Hg)		
		Before piperine injection	After piperine injection	% change
Piperine 1.0	6	47.06±1.14	108.87±1.01	132.89±1.21
Piperine 1.0 After propranolol 0.7	6	40.67±0.41	76.54±1.36	88.11±1.28
Piperine 1.0	6	45.01±1.29	116.76±1.56	159.58±1.09
Piperine 1.0 After phentolamine 1.0	6	36.13±1.32	63.1±1.85	75.47±1.49
Piperine 1.0	6	45.4±1.12	109.22±1.44	141.13±1.34
Piperine 1.0 After propranolol 0.7 and phentolamine 1.0	6	45.4±1.31	64.92±1.49	62.38±1.24
Piperine 1.0	6	45.11±1.11	115.89±1.54	156.91±1.34
Piperine 1.0 After reserpine pretreatment	6	54.06±1.76	61.45±1.39	12.98±1.26

Table 9. Comparison of the effects of piperine on mean blood pressure before and after propranolol 0.70 mg/kg, phentolamine 1 mg/kg, propranolol 0.70 mg/kg and phentolamine 1 mg/kg, reserpine pretreatment in pithed rats. (mean±S.E.M.).

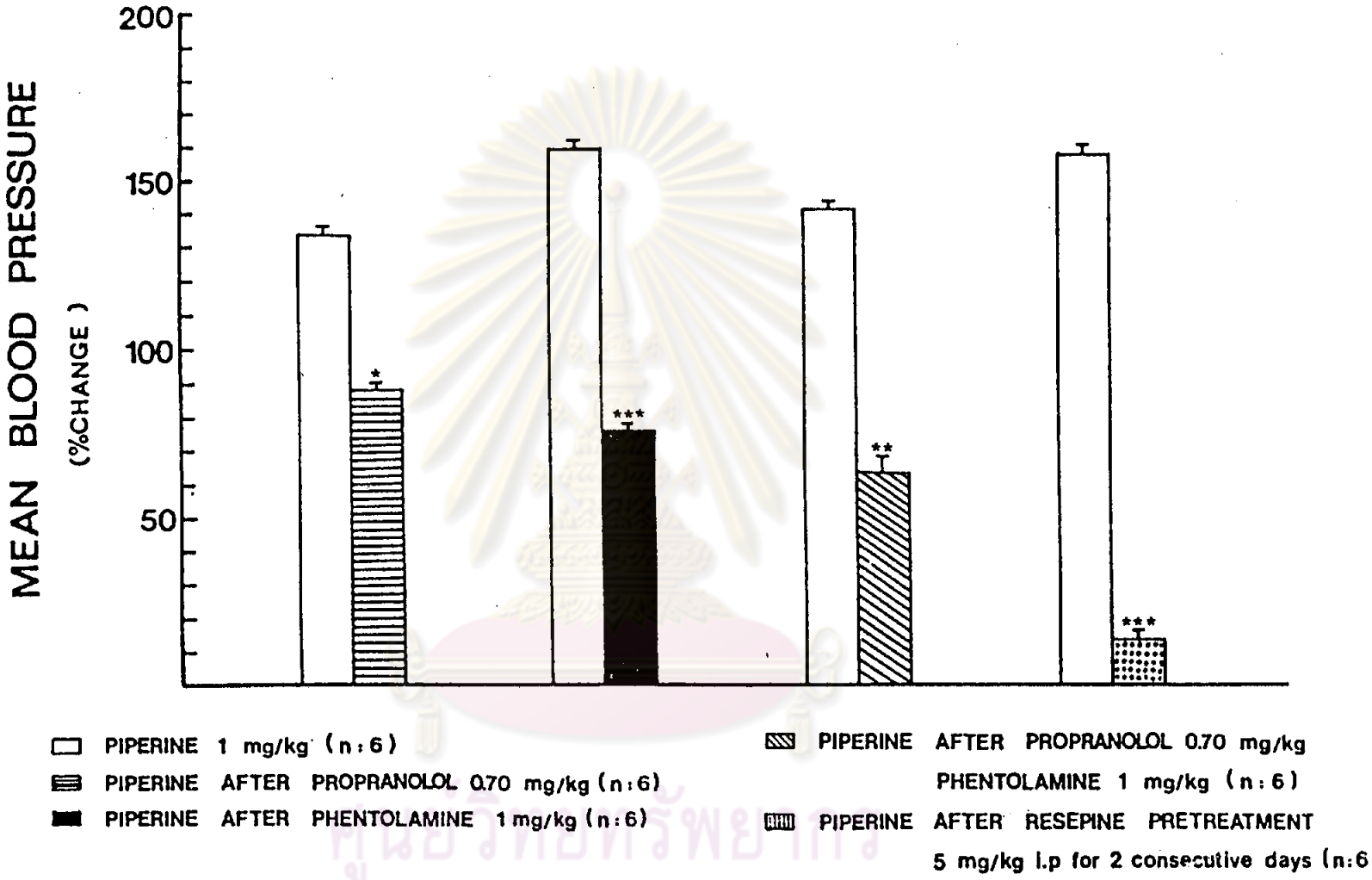

Figure 21. Comparison of the effects of piperine on mean blood pressure before and after propranolol, phentolamine, propranolol plus phentolamine, reserpine pretreatment in pithed rats. Bar graph represent mean percent increase±S.E.M. *P < 0.01 **P < 0.005 ***P < 0.001

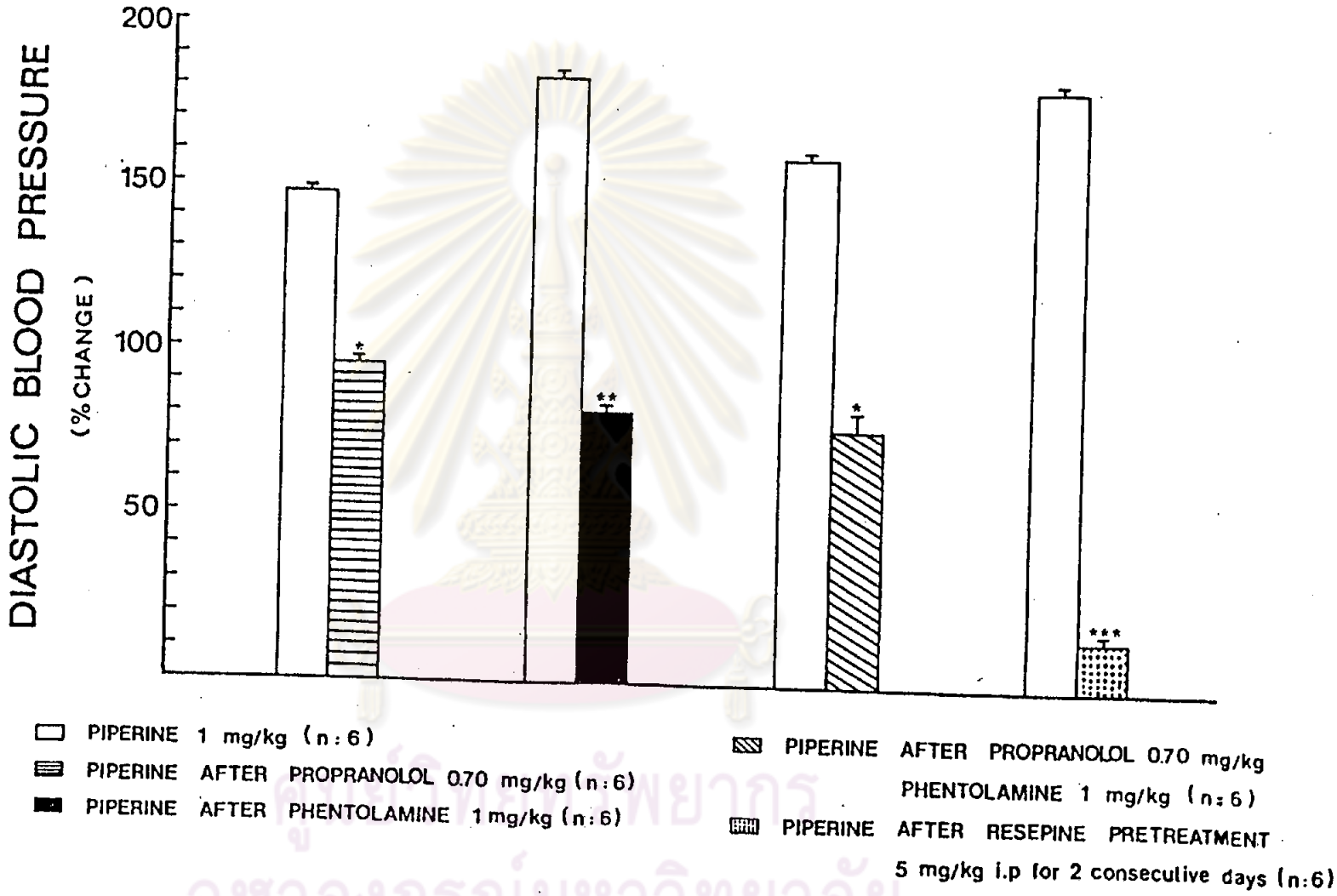

Figure 20. Comparison of the effects of piperine on diastolic blood pressure before and after propranolol, phentolamine, propranolol plus phentolamine, reserpine pretreatment in pithed rats. Bar graph represent mean percent increase±S.E.M. *P < 0.01 **P < 0.005 ***P < 0.001

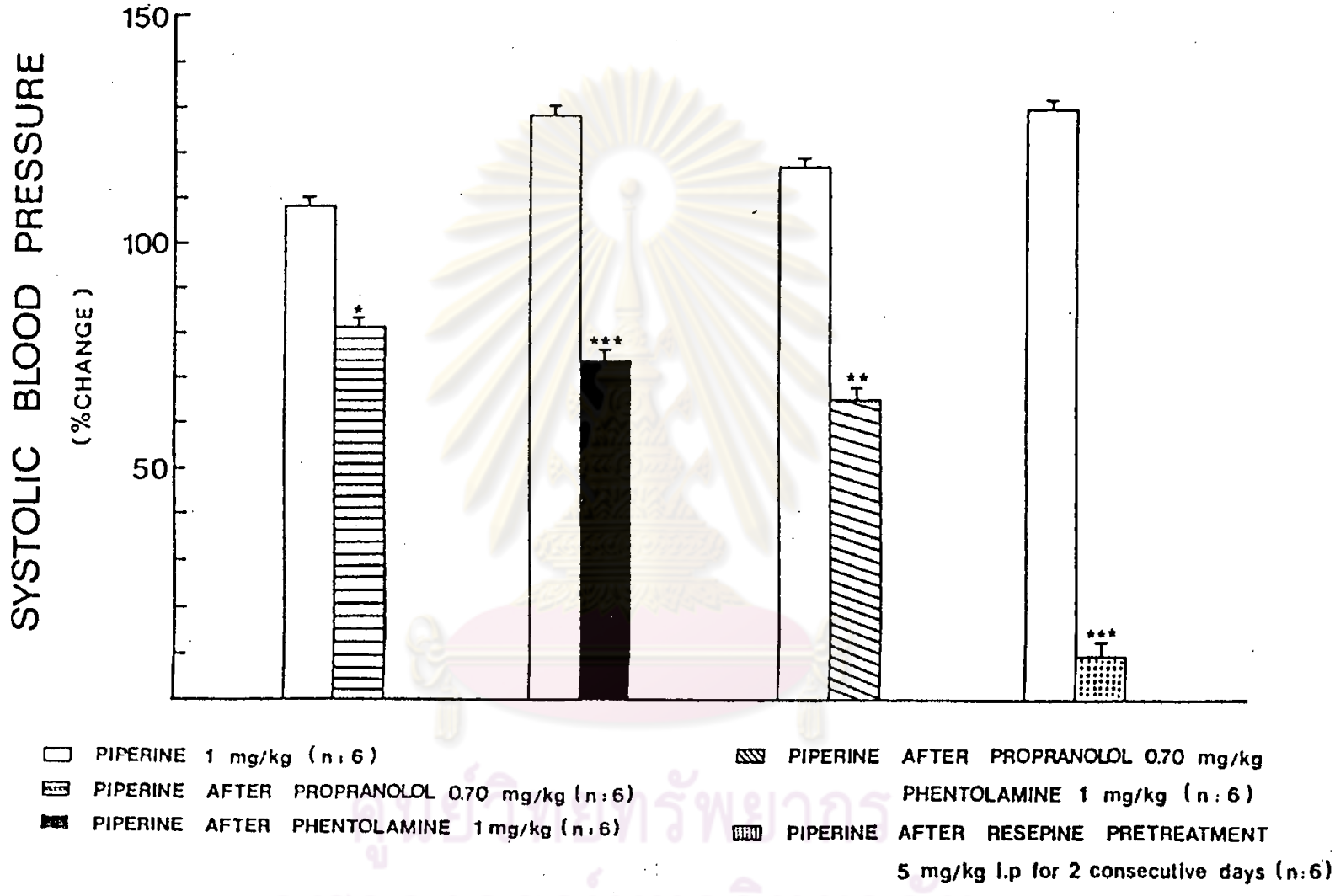

Figure 19. Comparison of the effects of piperine on systolic blood pressure before and after propranolol, phentolamine, propranolol plus phentolamine, reserpine pretreatment in pithed rats. Bar graph represent mean percent increase±S.E.M. * $P < 0.025$ ** $P < 0.01$ *** $P < 0.001$

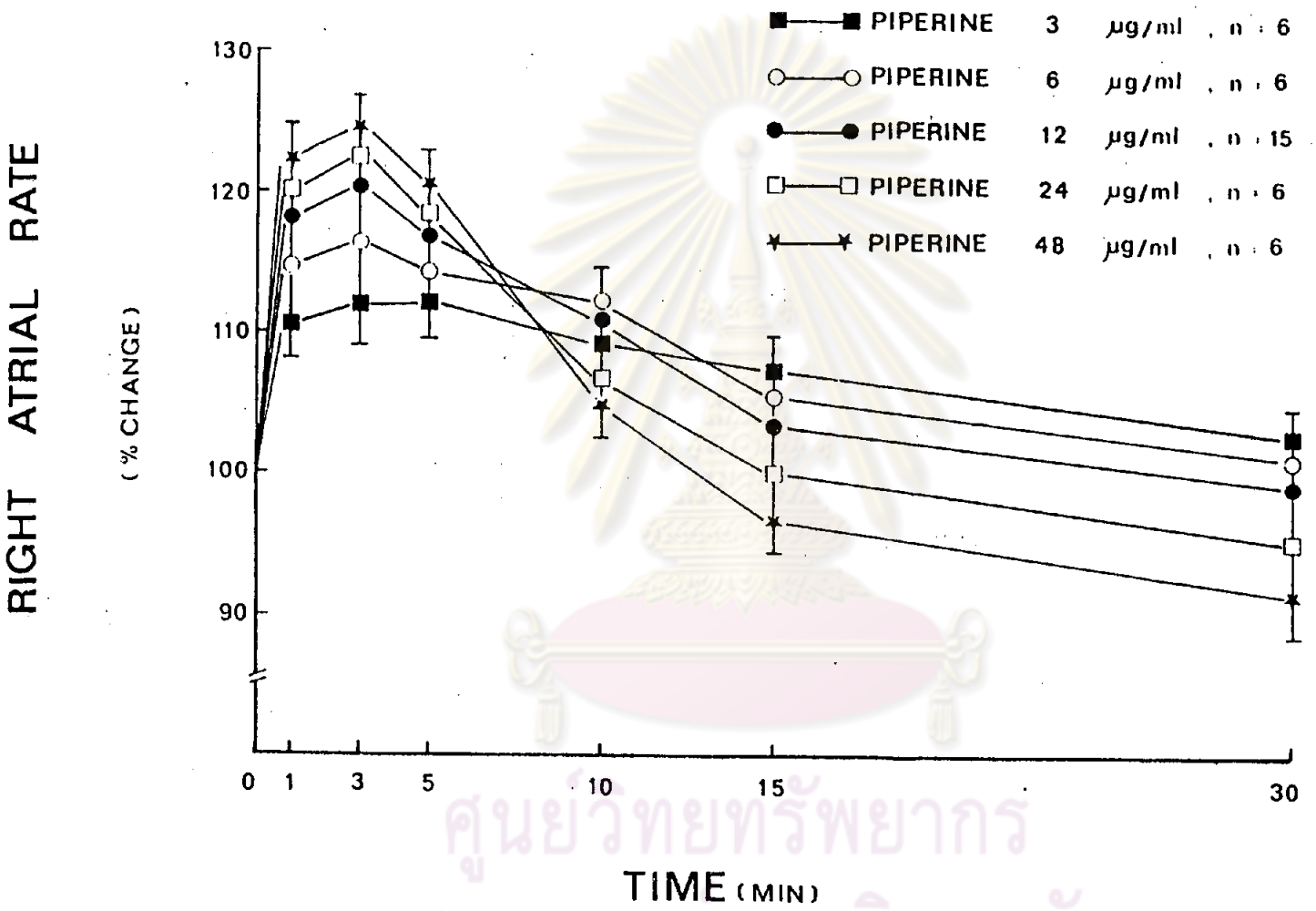

Figure 22. Chronotropic effect of piperine (3, 6, 12, 24 and 48 ug/ml) at various time intervals for 30 minutes.

Graph was represented in term of % control (mean±S.E.M.).

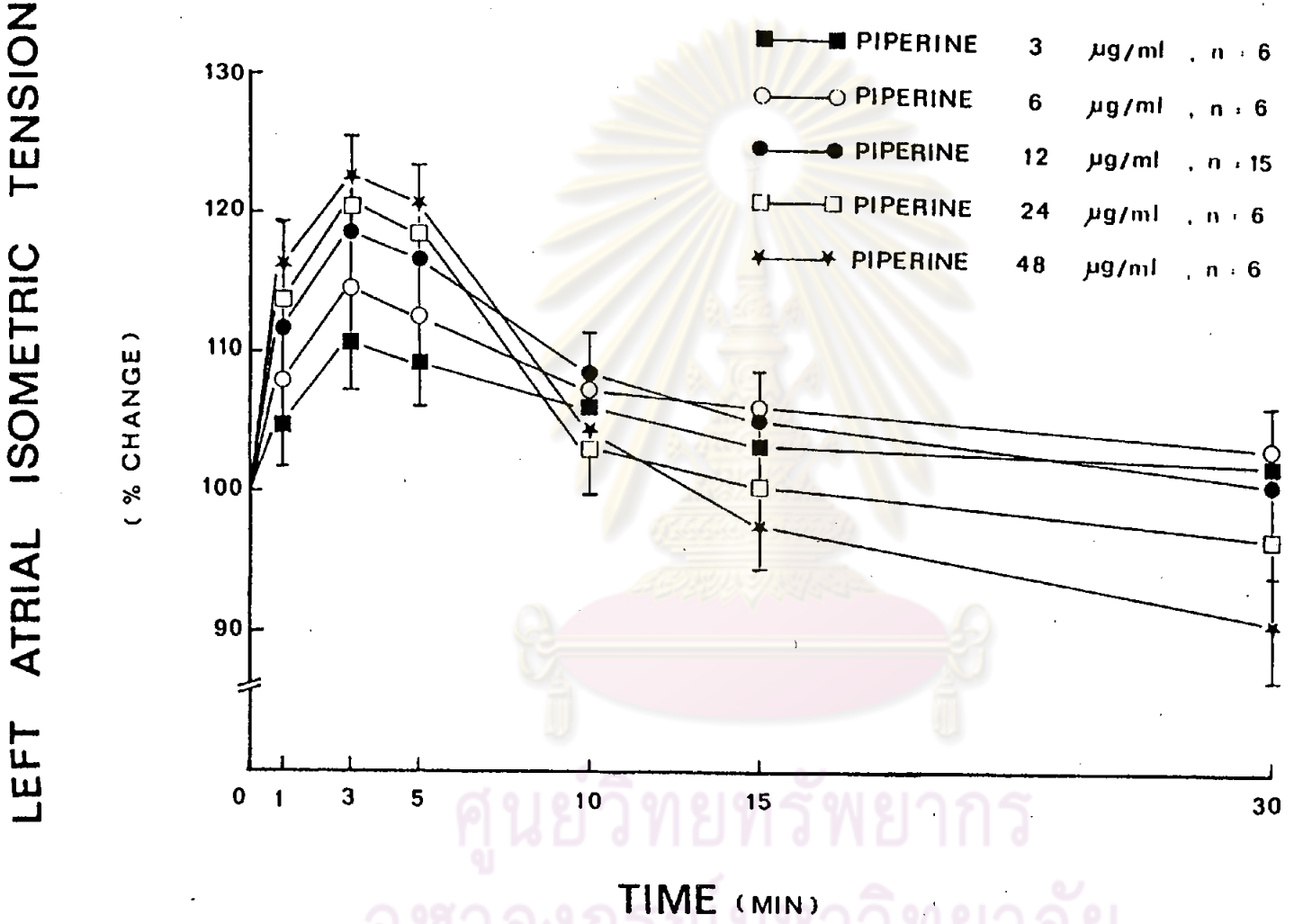

Figure 23. Inotropic effect of piperine (3, 6, 12, 24 and 48 ug/ml) at various time intervals for 30 minutes.

Graph was represented in term of % control (mean±S.E.M.).

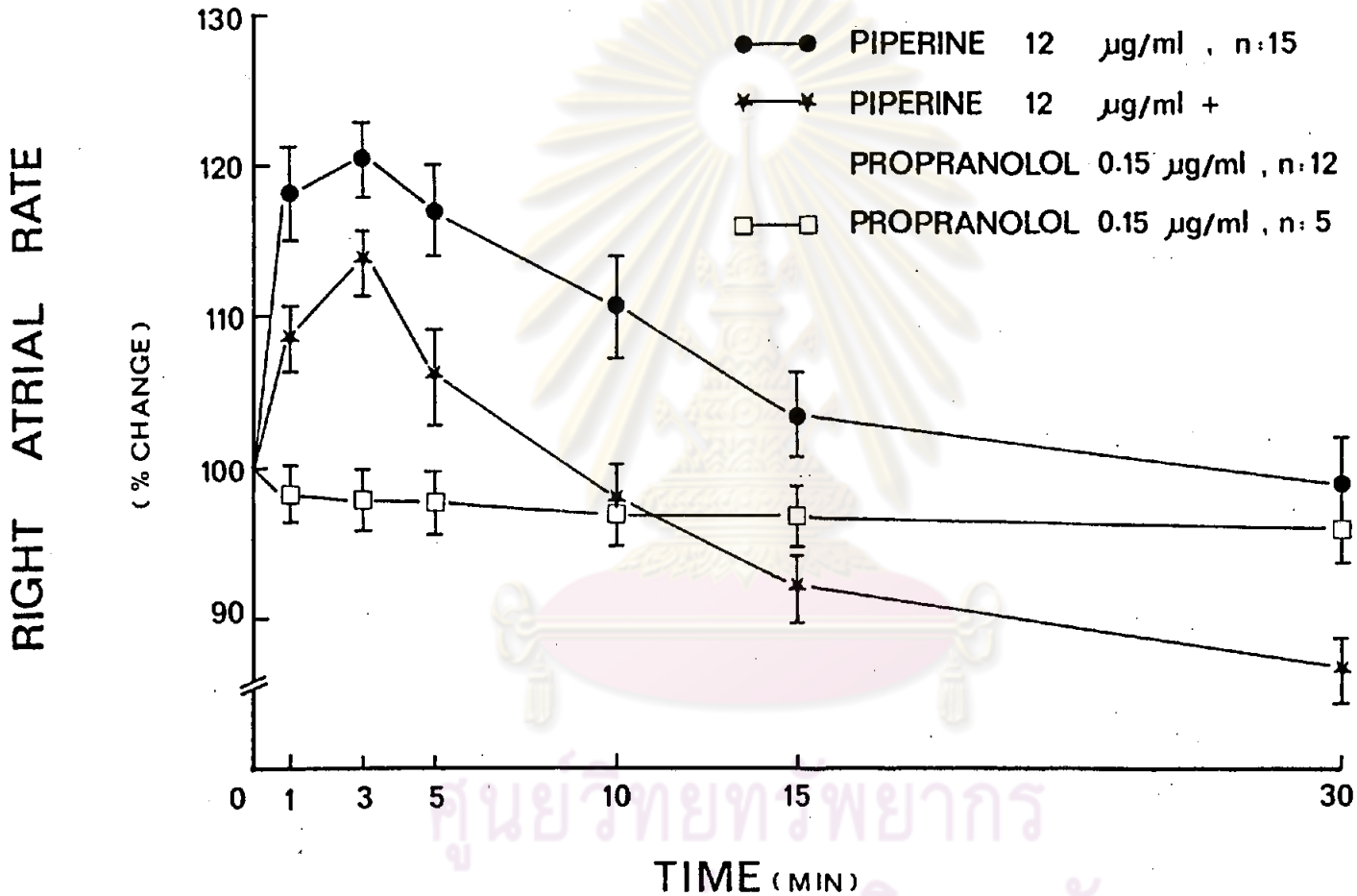

Figure 24. Effect of propranolol (0.15 ug/ml) on dose-effect curve of piperine. Graph was represented in term of % control (mean±S.E.M.).

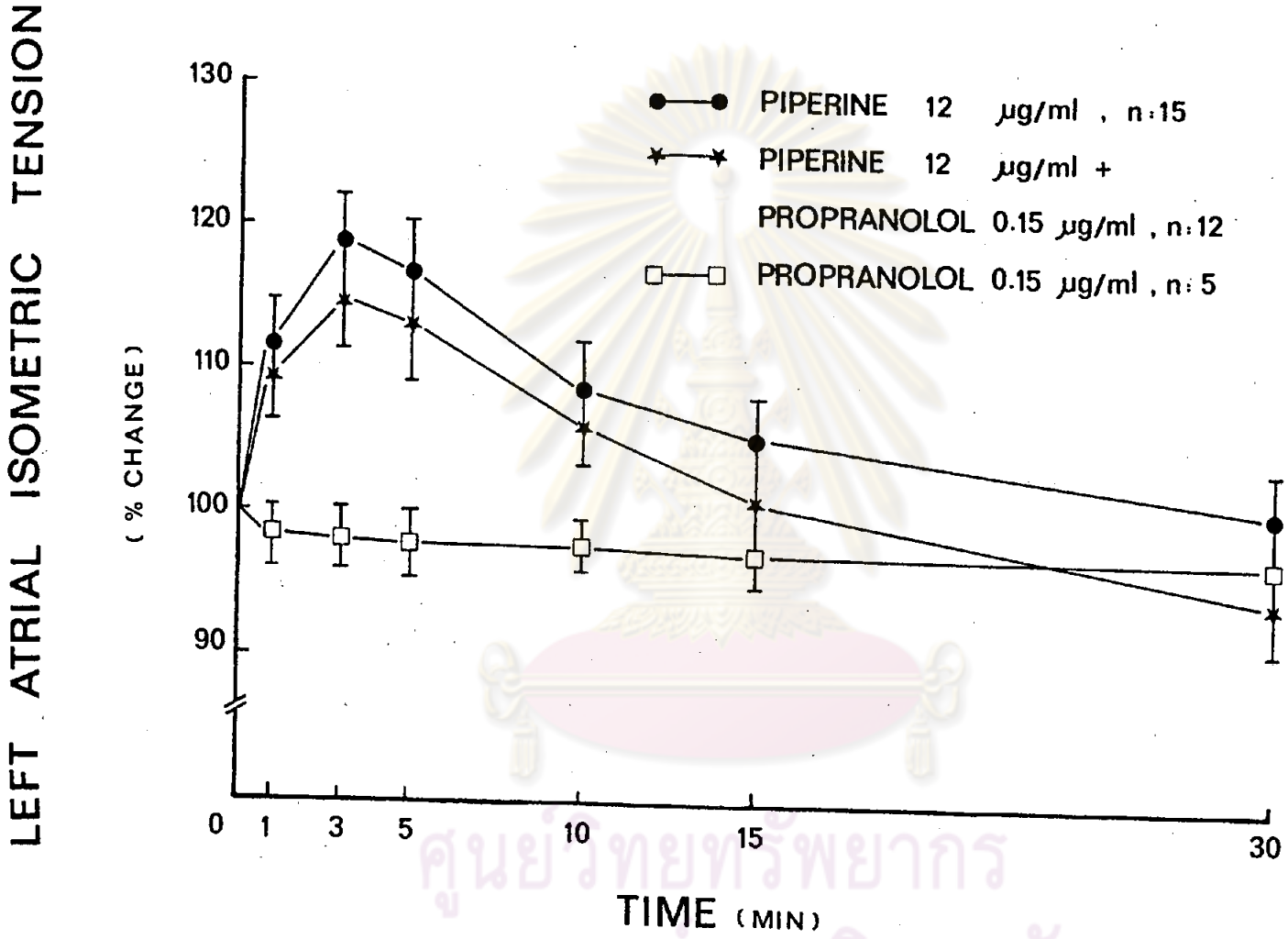

Figure 25. Effect of propranolol (0.15 ug/ml) on dose-effect curve of piperine.

Graph was represented in term of % control (mean±S.E.M.).

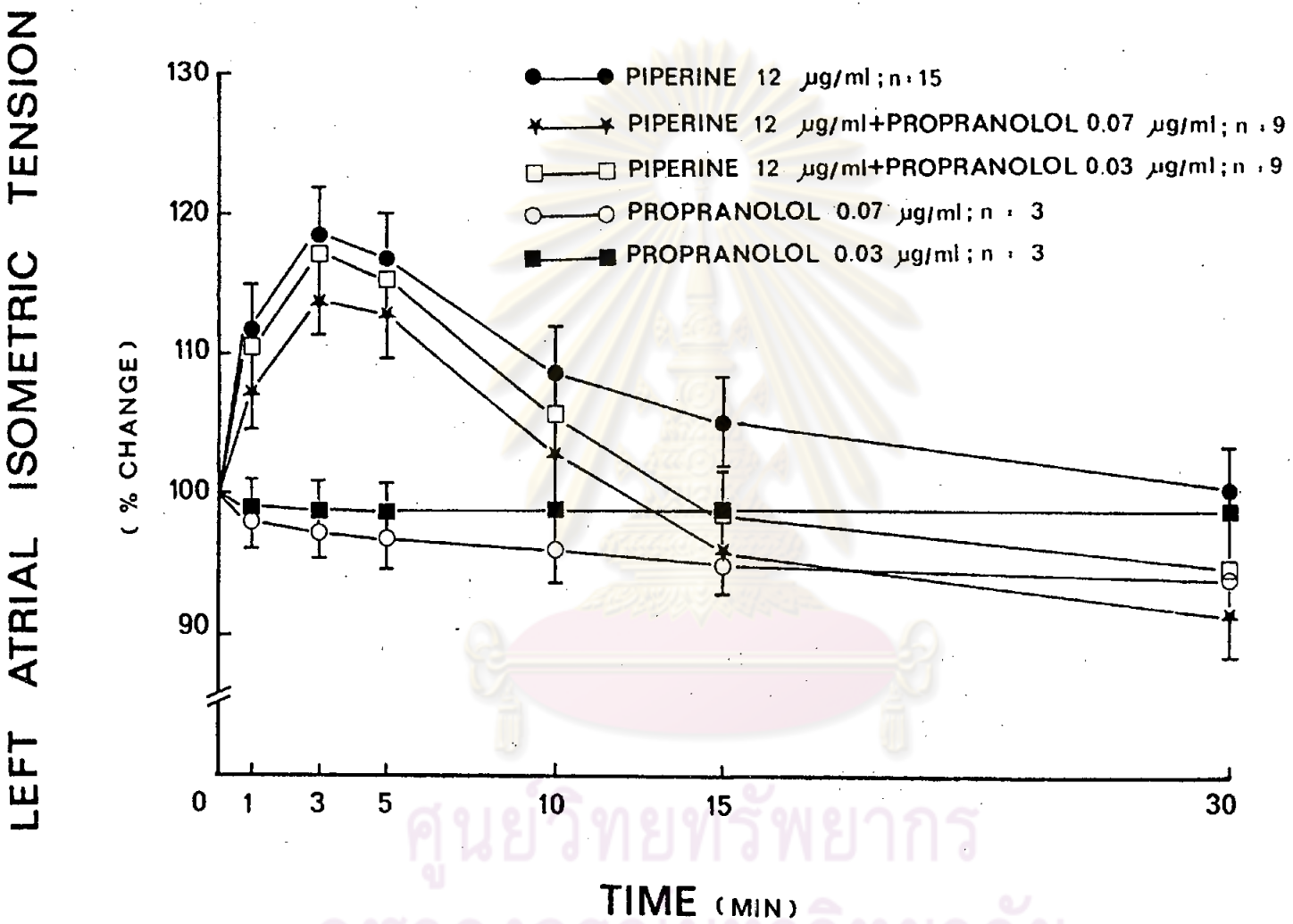

Figure 26. Effect of propranolol (0.03 and 0.07 ug/ml) on dose-effect curve of piperine.

Graph was represented in term of % control (mean±S.E.M.).

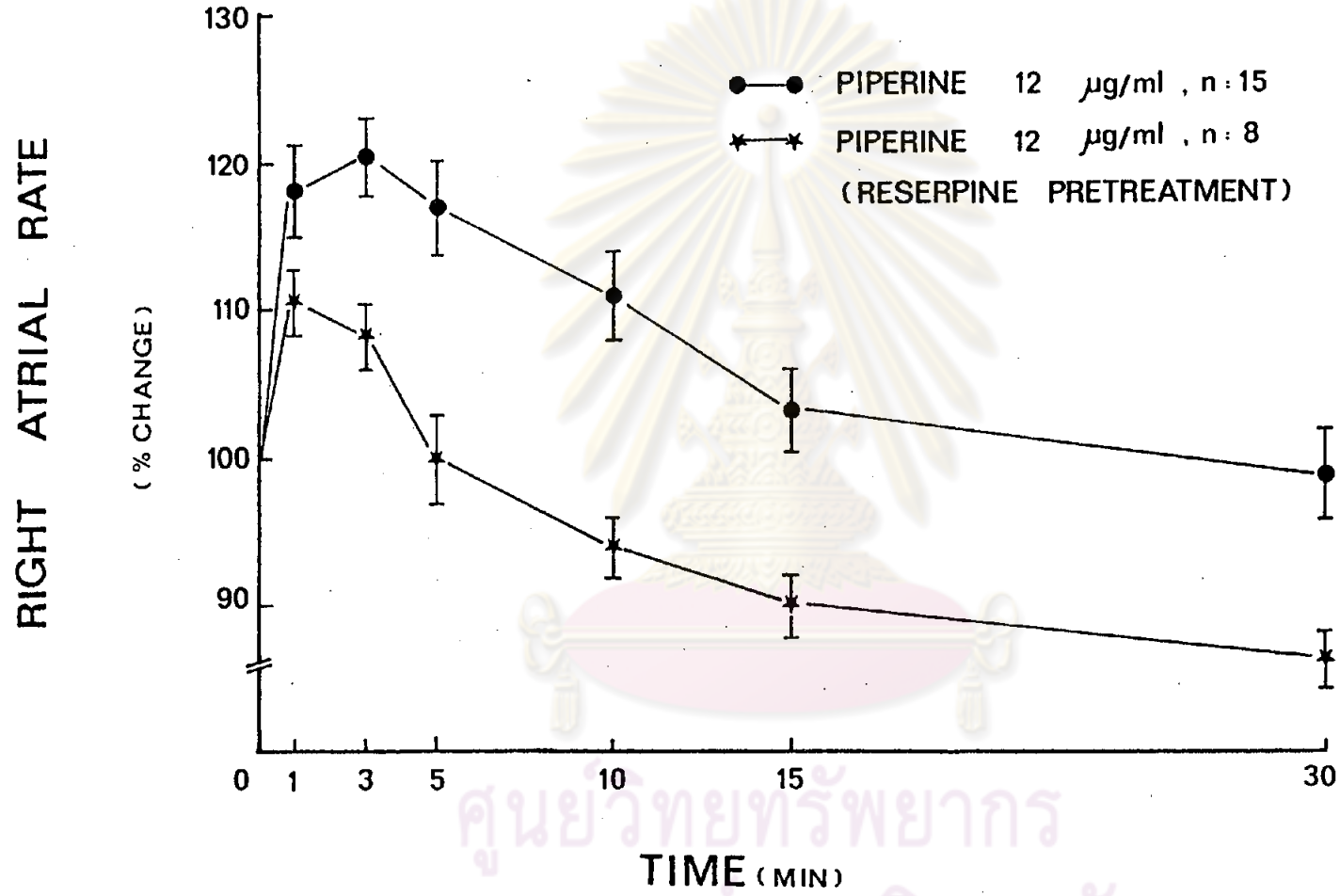

Figure 27. Effect of reserpine pretreatment on dose-effect curve of piperine. Graph was represented in term of % control (mean±S.E.M.).

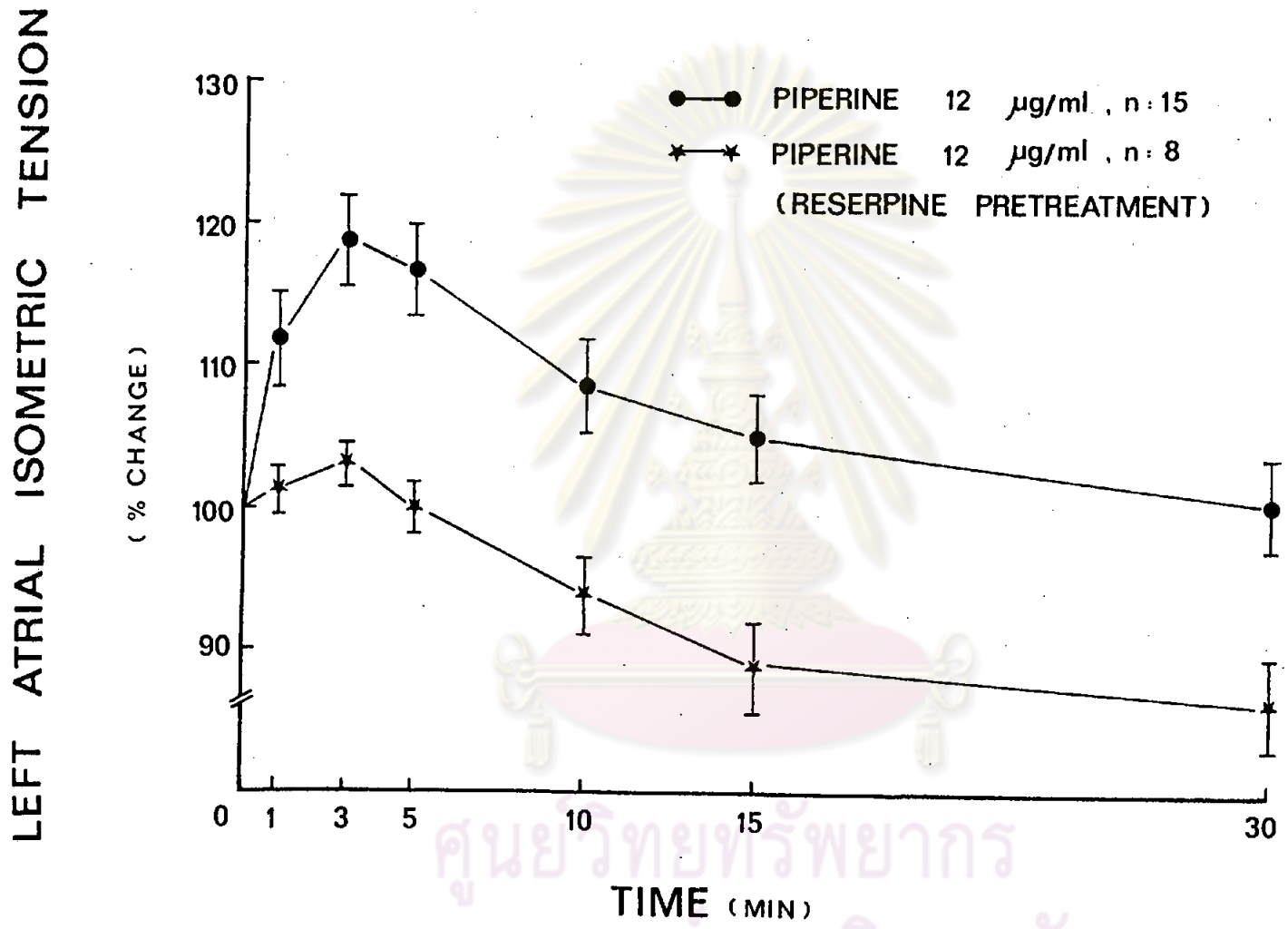

Figure 28. Effect of reserpine pretreatment on dose-effect curve of piperine. Graph was represented in term of % control (mean±S.E.M.).

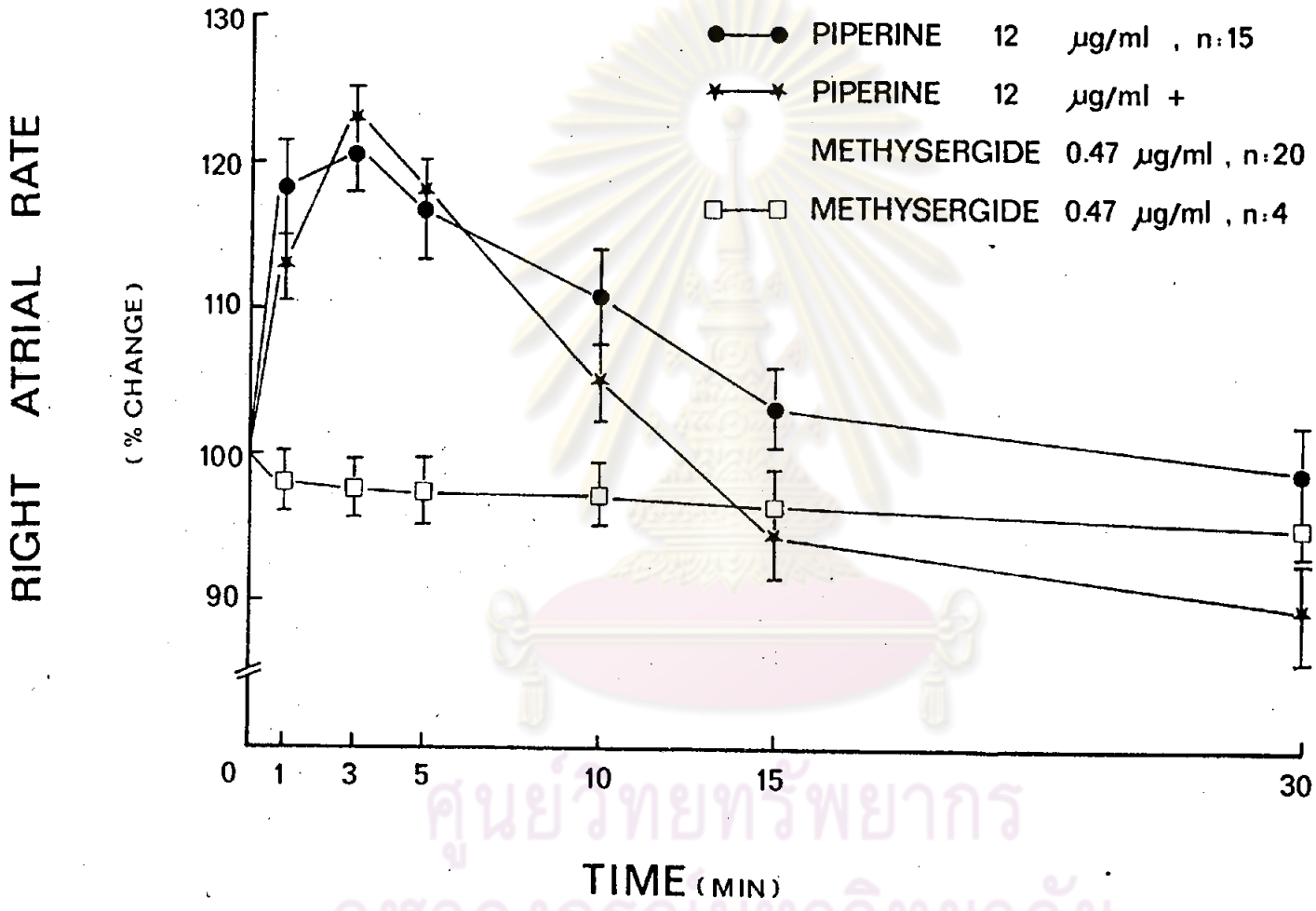

Figure 29. Effect of methysergide (0.47 ug/ml) on dose-effect curve of piperine. Graph was represented in term of % control (mean±S.E.M.).

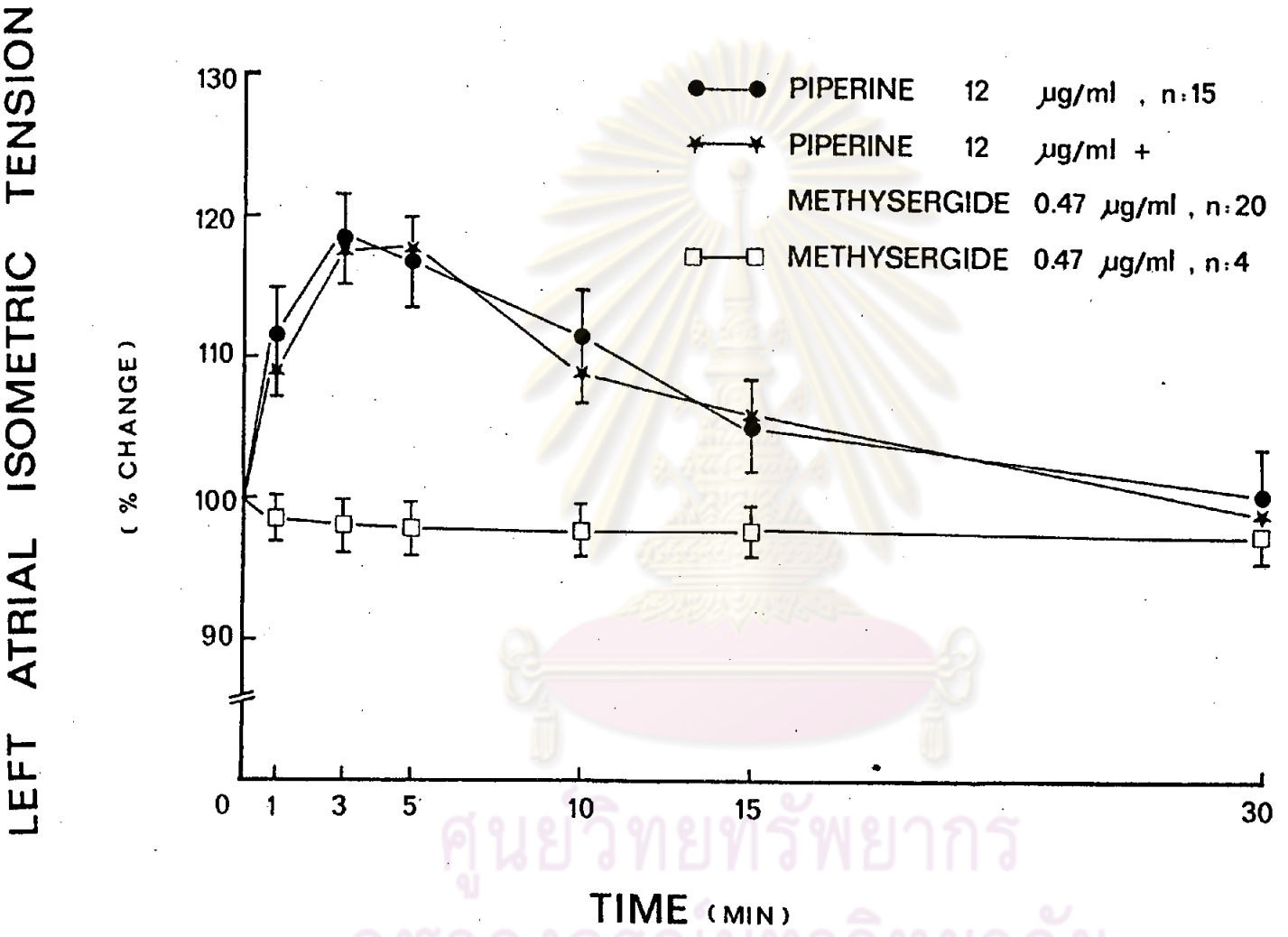

Figure 30. Effect of methysergide (0.47 ug/ml) on dose-effect curve of piperine. Graph was represented term of % control (mean±S.E.M.).

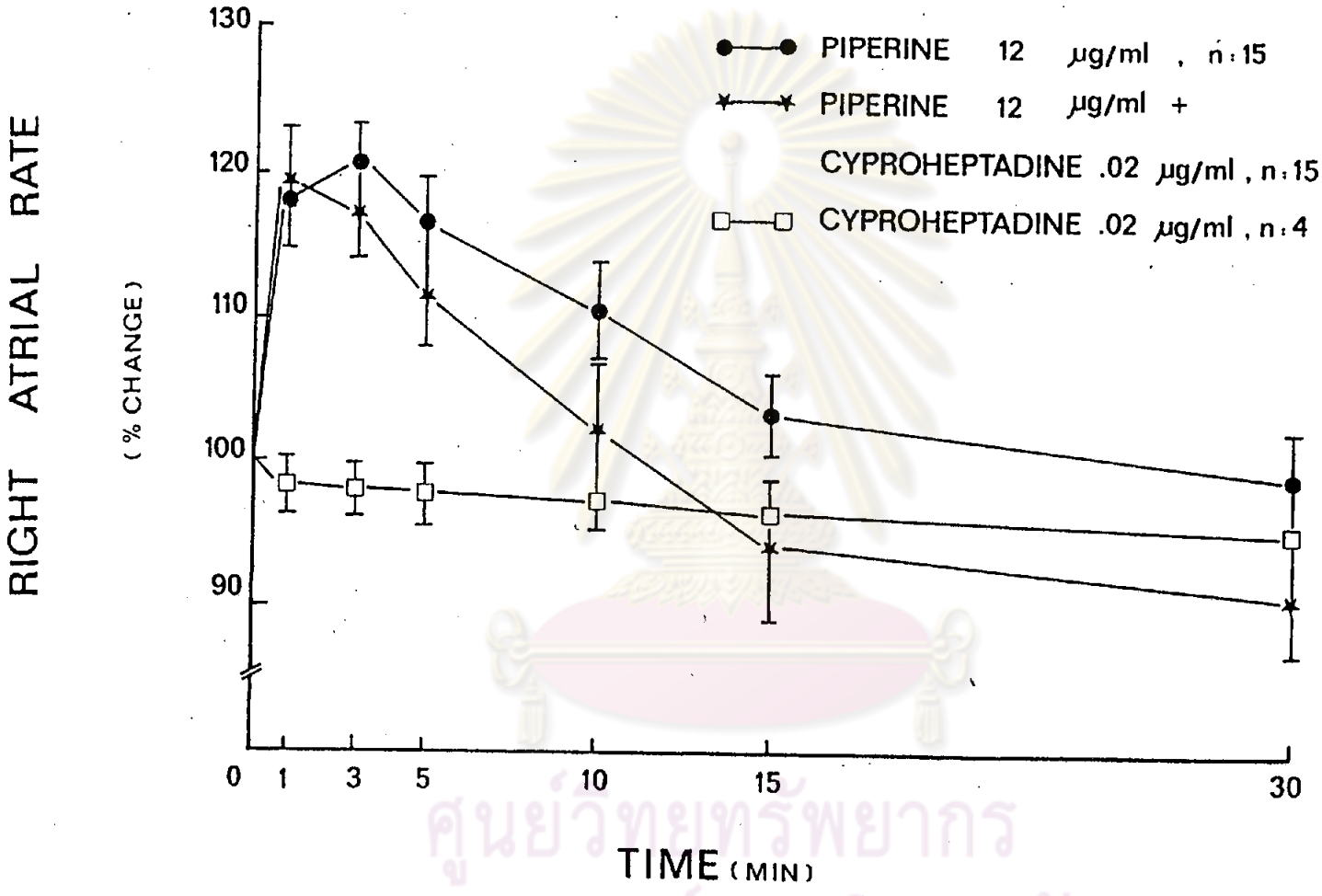

Figure 31. Effect of cyproheptadine (0.02ug/ml) on dose-effect curve of piperine.

Graph was represented in term of % control (mean±S.E.M.).

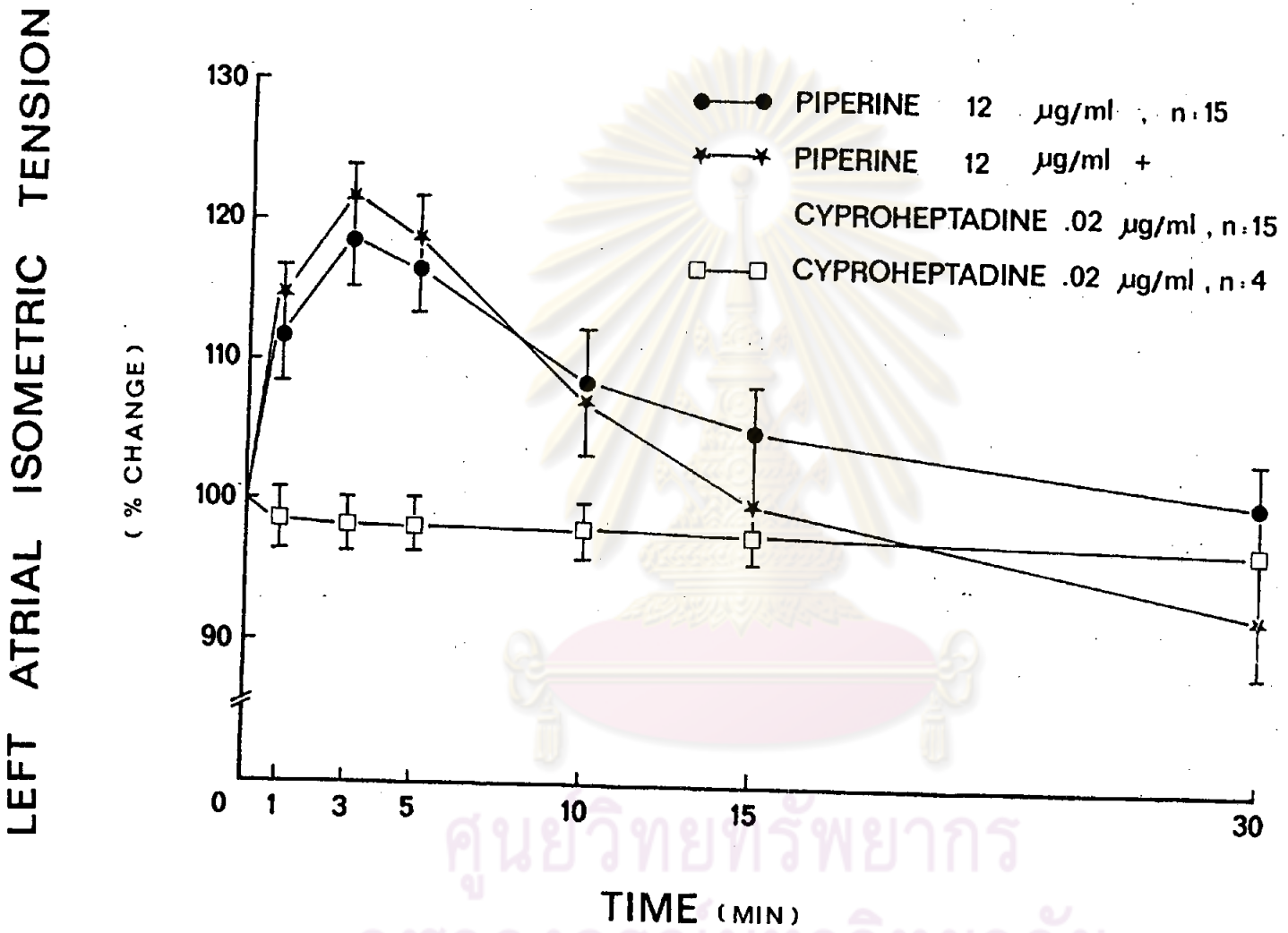

Figure 32. Effect of cyproheptadine (0.02 ug/ml) on dose-effect curve of piperine.

Graph was represented in term of % control (mean±S.E.M.).

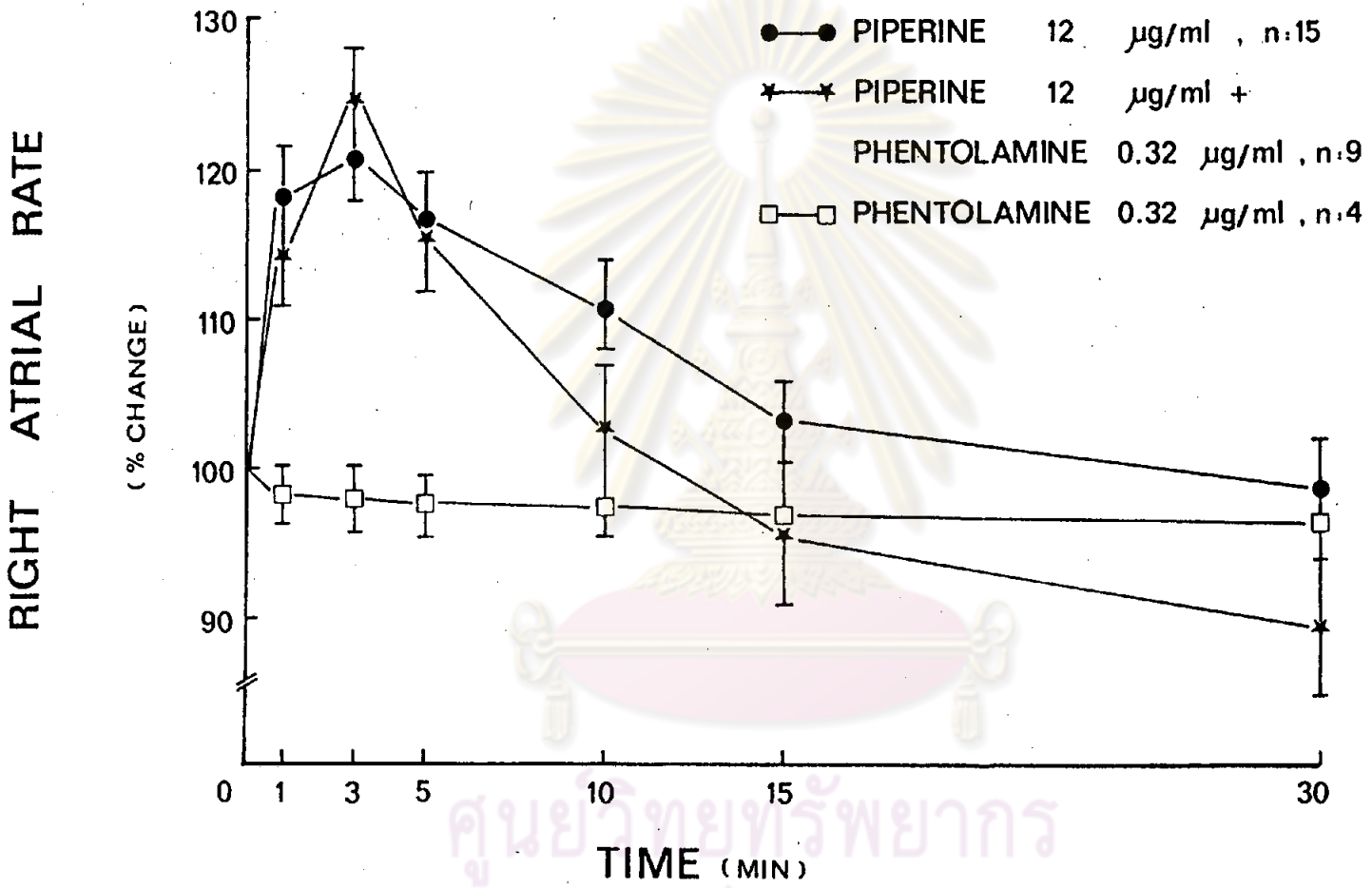

Figure 33. Effect of phentolamine (0.32 ug/ml) on dose-effect curve of piperine.

Graph was represented in term of % control (mean±S.E.M.).

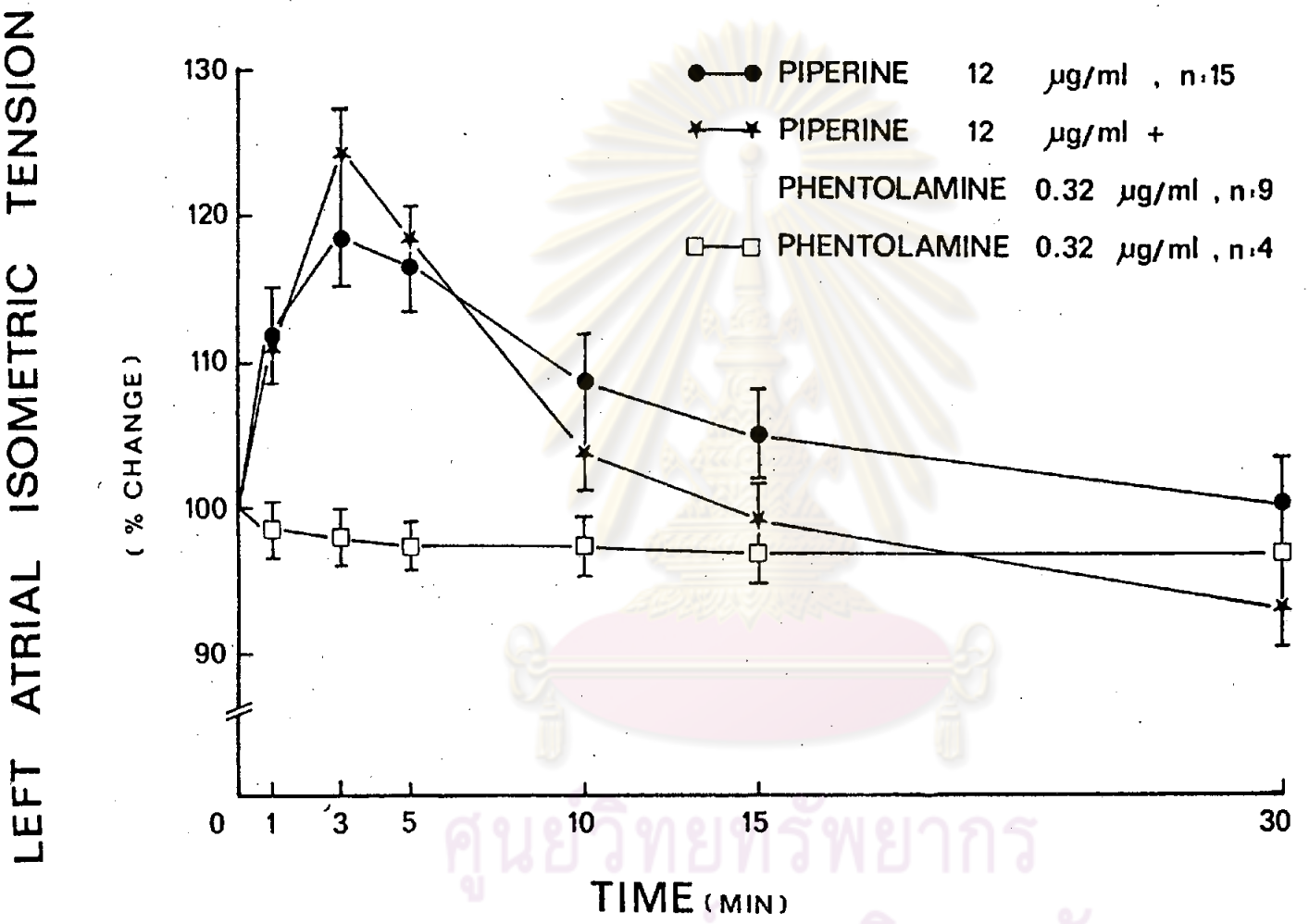

Figure 34. Effect of phentolamine (0.32 ug/ml) on dose-effect curve of piperine.

Graph was represented in term of % control (mean±S.E.M.).

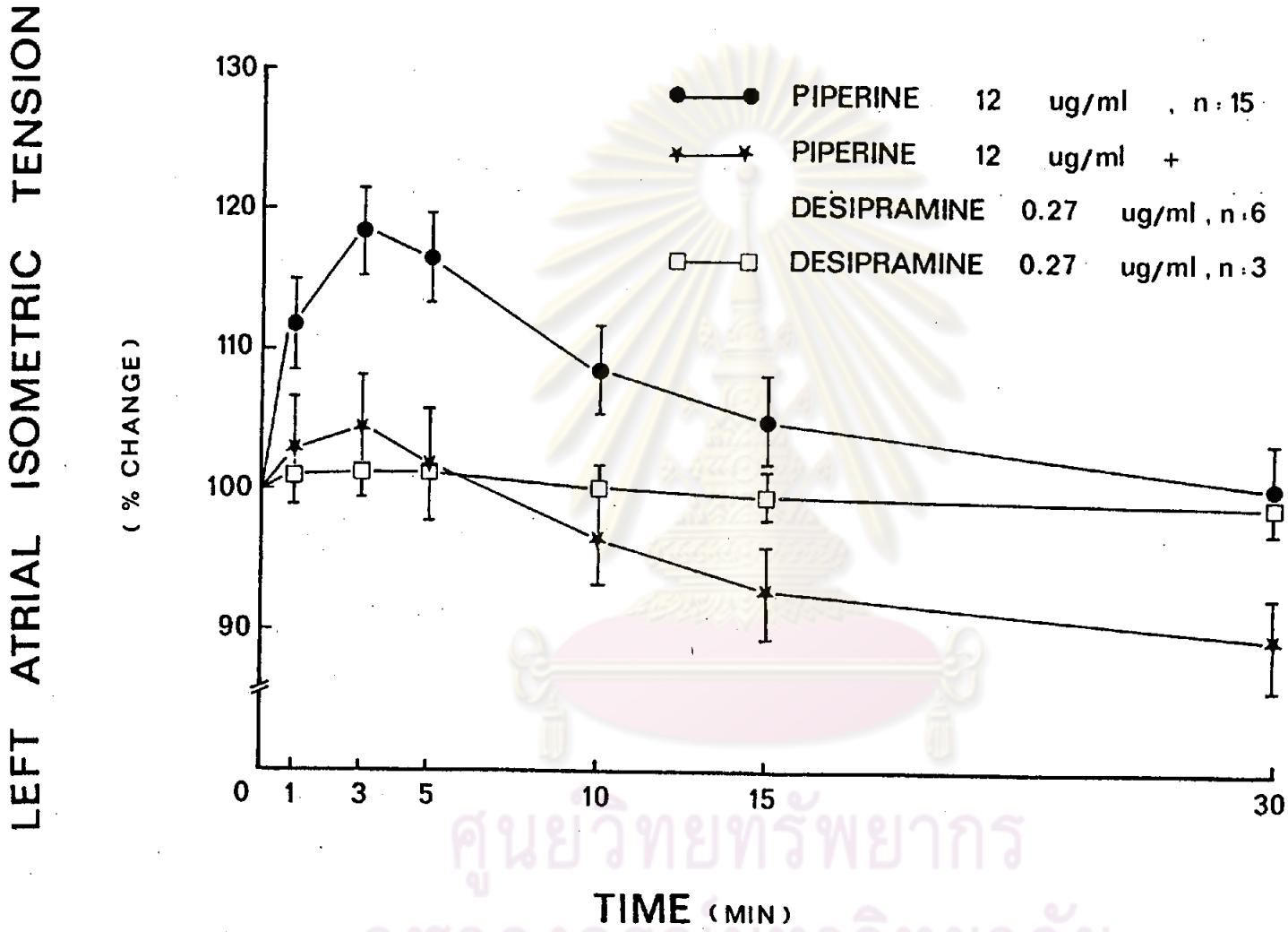

Figure 36. Effect of desipramine (0.27 ug/ml) on dose-effect curve of piperine.

Graph was represented in term of % control (mean±S.E.M.).

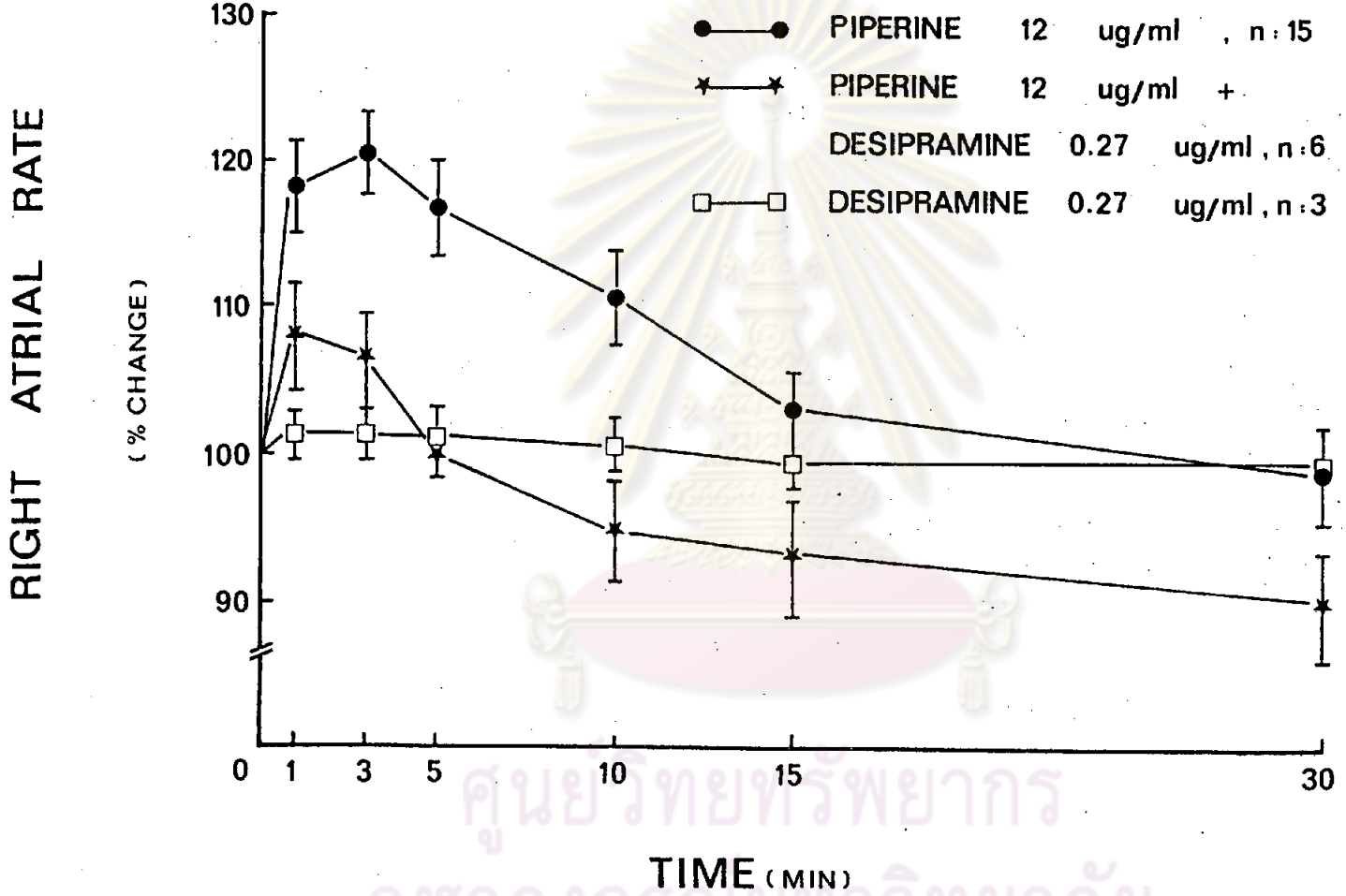

Figure 35. Effect of desipramine (0.27 ug/ml) on dose-effect curve of piperine.

Graph was represented in term of % control (mean±S.E.M.).

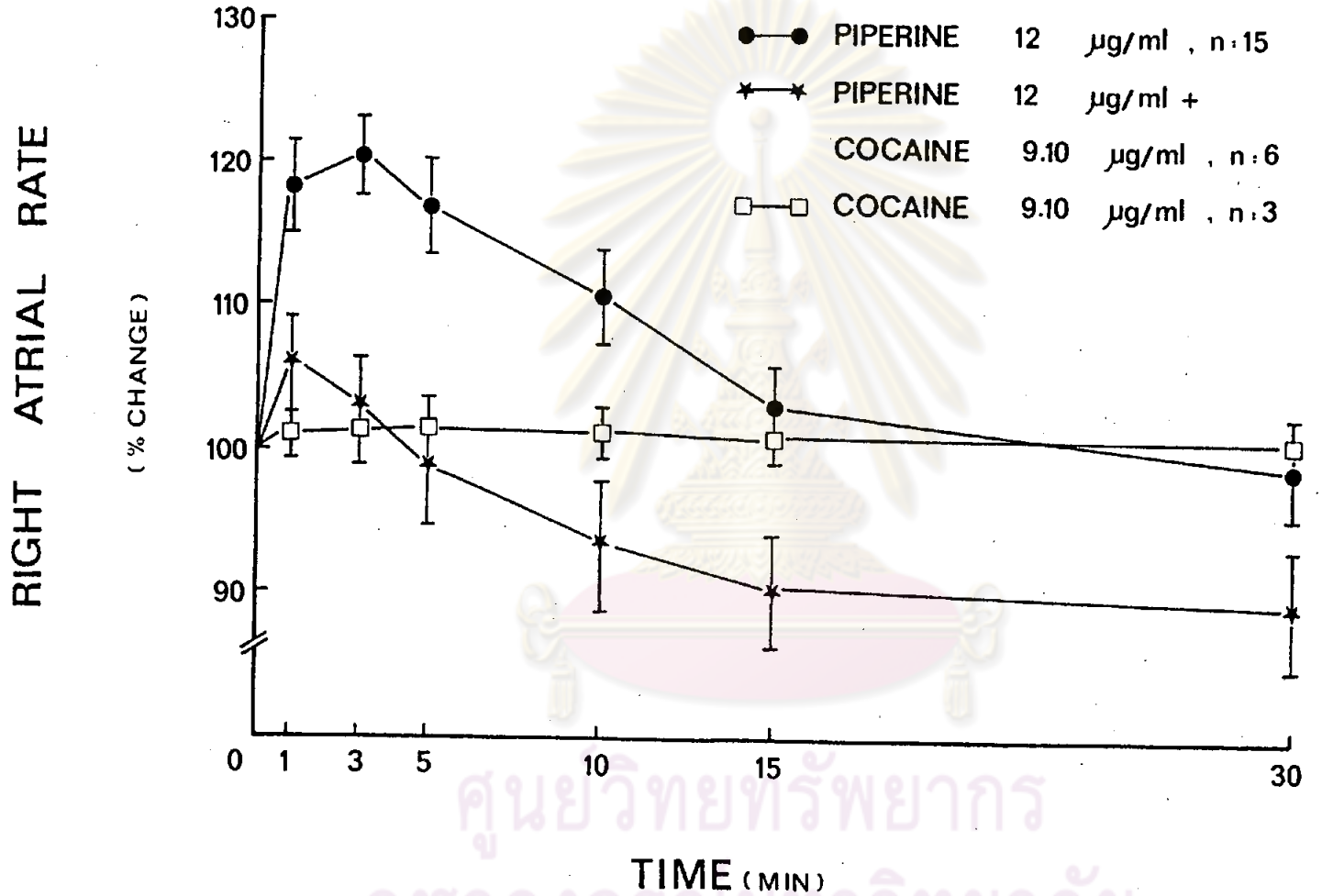

Figure 37. Effect of cocaine (9.1 ug/ml) on dose-effect curve of piperine. Graph was represented in term of % control (mean±S.E.M.).

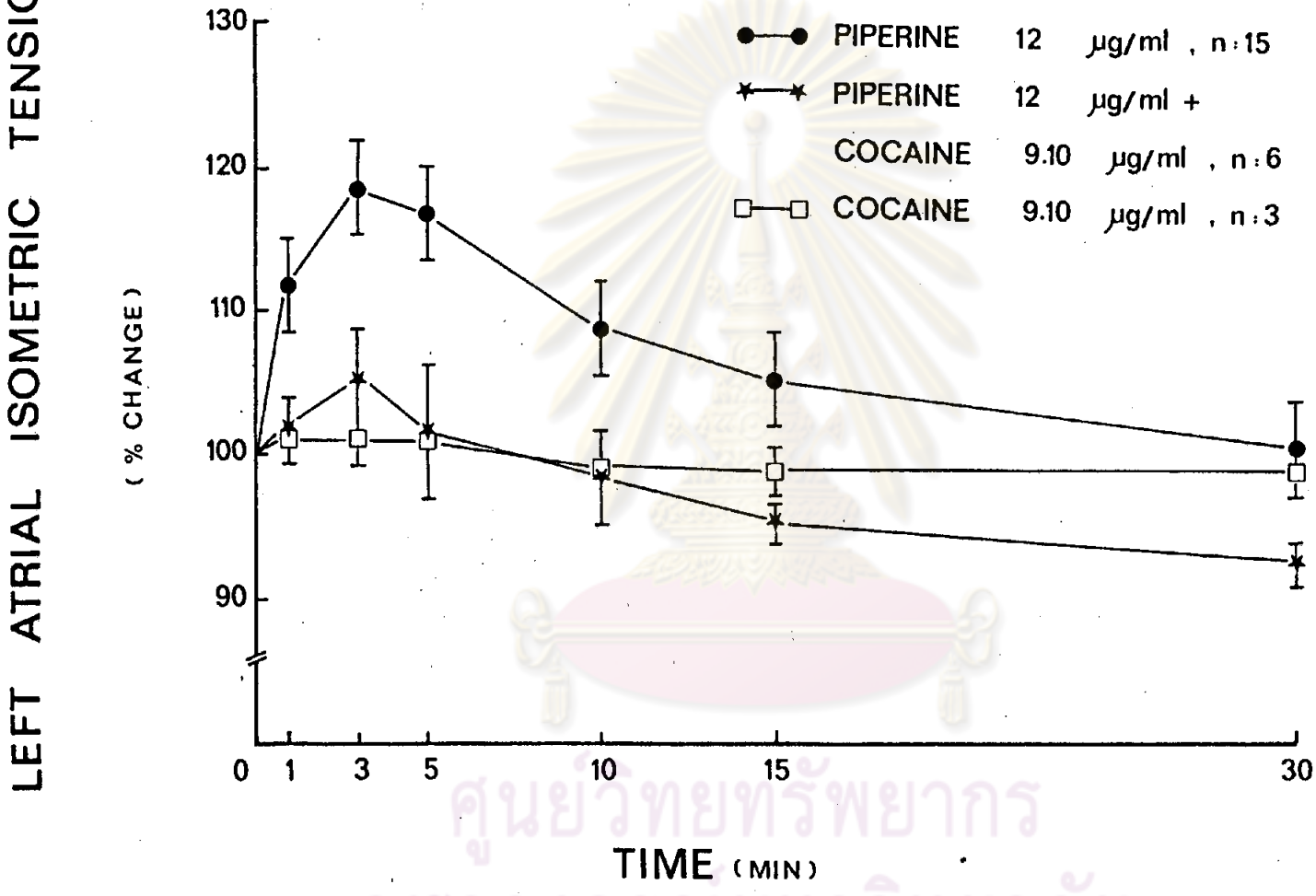

Figure 38. Effect of cocaine (9.1 ug/ml) on dose-effect curve of piperine. Graph was represented in term of % control (mean+S.E.M.).