Chapter III

Results

1. Effects of Indole Alkaloids on Blood Pressure and Heart Rate in Anaesthetized Rats

Primary screening of the actions of indole alkaloids was performed on three anaesthetized rats by measuring blood pressure and heart rates. The results obtained were shown in the Fig. 3, 4, 5 and 6, which represents one example of the response to each indole alkaloid.

Intravenous injection of I-1 produced dose-related decrease in blood pressure and heart rate (Fig. 3). The decrease in diastolic pressure was more noticable than that of the systolic pressure, making the pulse pressure wider than the control values. In higher dose (e.g. 24 mg/kg B.W.), the contractile properties of the heart became depressed which was manifested by arrhythmia and heartbeat cessation.

Fig. 4 showed that I-2 produced a biphasic responses on the blood pressure comprising an initial transient pressor effect followed by a more pronounced longer-lasting depressor phase. In the initial phase, both systolic and diastolic pressure increased. After 10-20 s of I-2 injection, the blood pressure and heart rate reduced. The alleviation of diastolic pressure was more than systolic pressure so that the pulse pressure became wider than control values. Furthermore, at 12 and 24 mg/kg B.W. of I-2, the respiratory rate was stimulated as indicated by increase in the depth of respiration. At the dose of 48 mg/kg B.W., cardiac contractility was interfered to cessation.

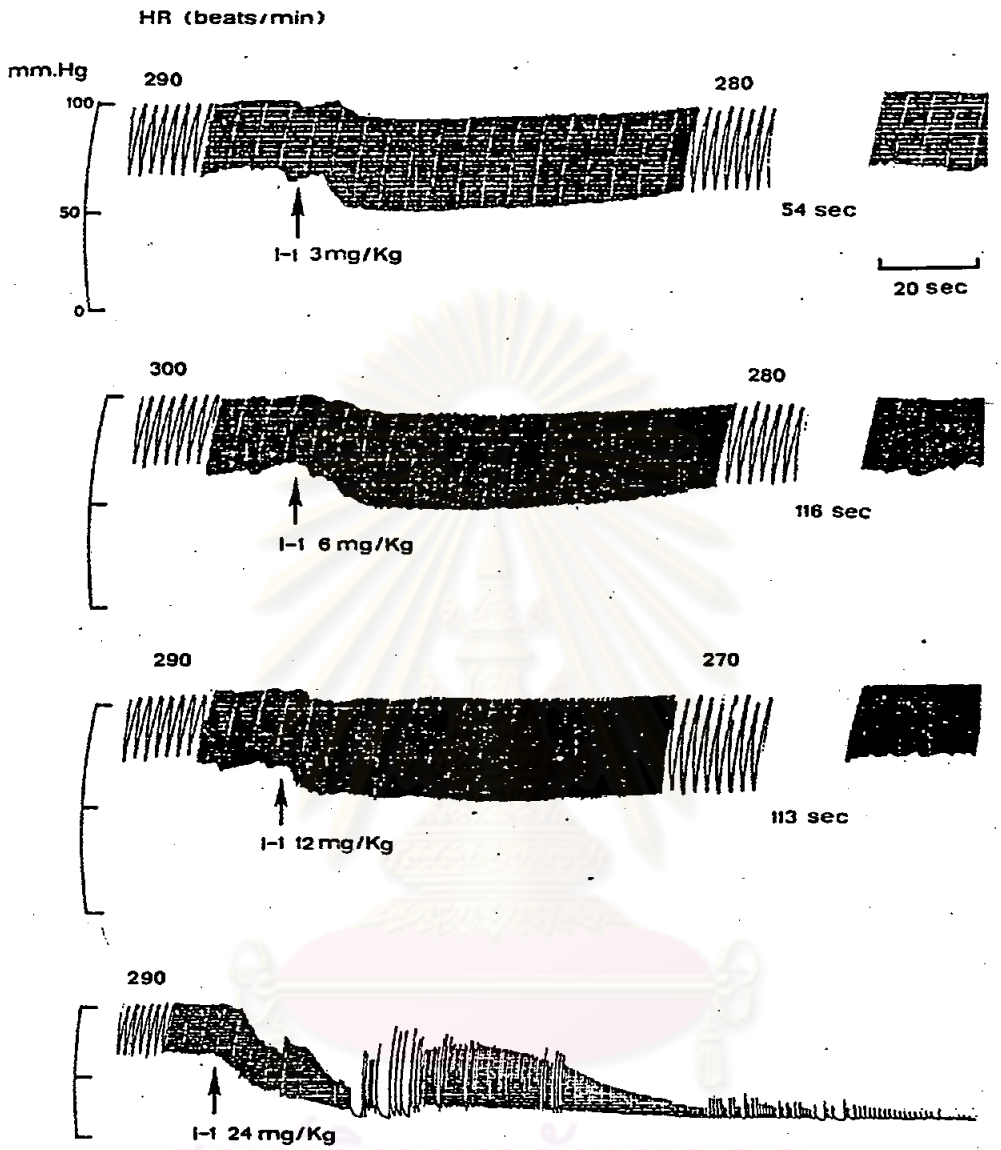

Figure 3. Trace of dose-response relationship for I-1 on blood pressure and heart rate in anaesthetized rat. Intravenous injection of I-1 produced dose-related decrease in blood pressure and heart rate. In this and the following figures 4-6, tracings were obtained from a curvilinear pen recorder; the scales of blood pressure in mm.Hg are shown on the vertical axis; the numbers above the recordings indicate heart rate (beats/min); time scale is shown under the first row of recordings and is identical for all cases and the doses of indole alkaloids shown are expressed in mg/kg B.W. I.V.

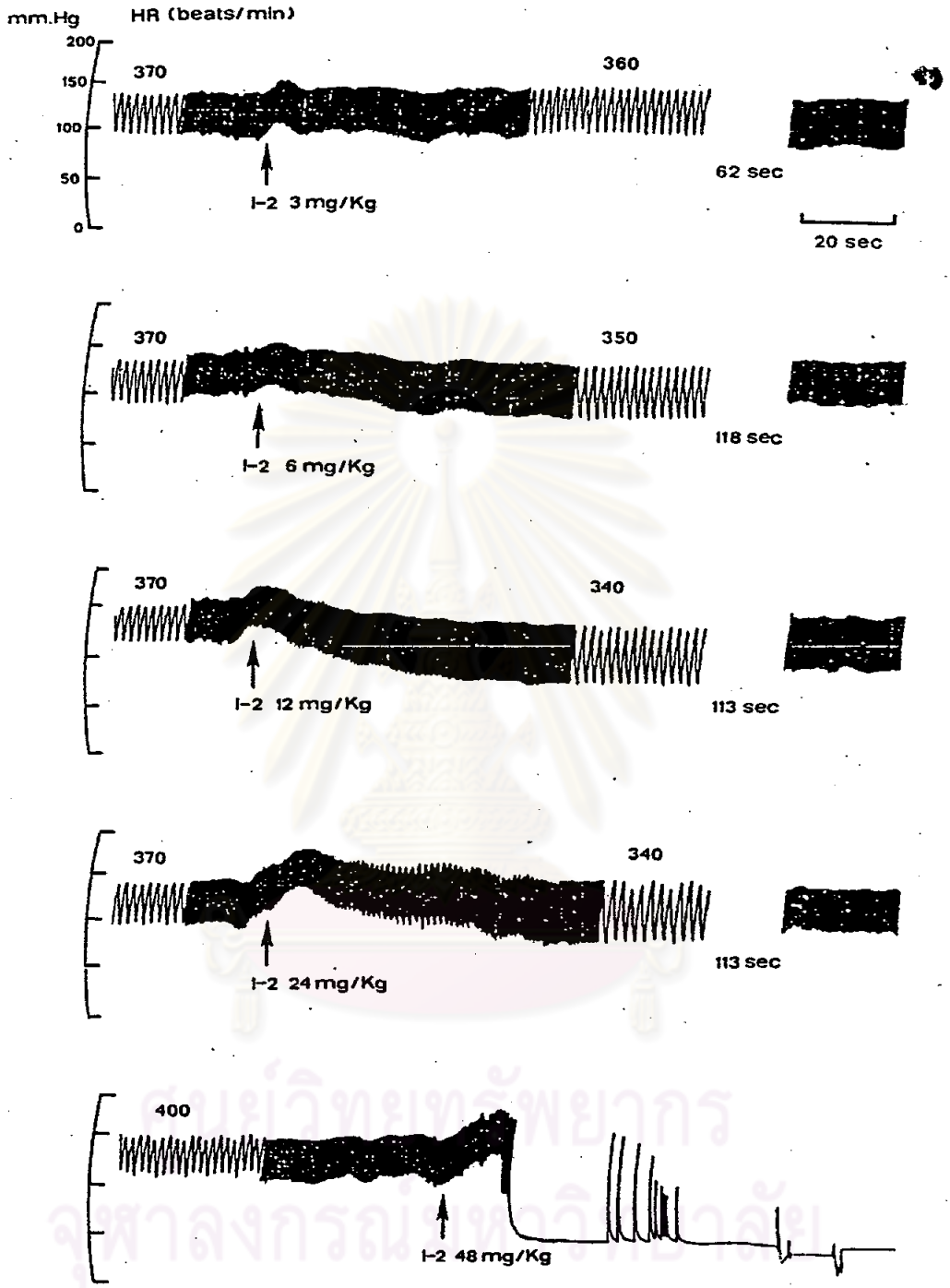

Figure 4. Trace of dose-response relationship for I-2 on blood pressure and heart rate in anaesthetized rat. I-2 produced a biphasic responses on the blood pressure comprising an initial transient pressor effect followed by a more pronounced longer-lasting depressor phase.

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From the Fig. 5, O-1 exerted weak pressor activity which increased both systolic and diastolic pressure in the low dose (3 mg/kg B.W.). At higher doses, blood pressure became decreased with diastolic pressure reduction being more apparent than that of the systolic. Force of contraction was enhanced, and pulse pressure wider. Moreover, O-1 had depressant action on the heart rate. At the higher doses (e.g. 96 mg/kg B.W.) the rate was impaired whereas the forces initially increased. The initial force urgmentation was followed by the depression of both force and rates, which lead to a complete cessation of heartbeat.

Similarly, O-2 had a weak pressor effect at lower doses, while higher doses produced depressor action (Fig. 6). O-2 was also capable of stimulating the respiratory rate at the higher dose. The lethal dose was resemble to O-1.

2. Effects of Indole Alkaloids on Isolated Atria

2.1 Negative Chronotropic Effect

Contractile responses of the right atria when exposed to different concentrations of the four indole alkaloids (2×10^{-6} M - 6.4×10^{-5} M) were measured in Locke's solution for 5 min after the addition of each alkaloid to the bathing fluid. Administration of indole alkaloids in a cumulative regimen caused a concentration dependent reduction of the spontaneous rate of isolated rat atria (Fig. 7). At higher concentrations, cessation of heartbeat was observed with all alkaloids (I-1 > 1.6×10^{-5} M, I-2 > 3.2×10^{-5} M, O-1 and O-2 > 6.4×10^{-5} M). Irregular beats conspiciously occured before the atria became quiescent. Example of records obtained from one experiment is illustrated in Fig. 8 A. The EC 50, i.c. the

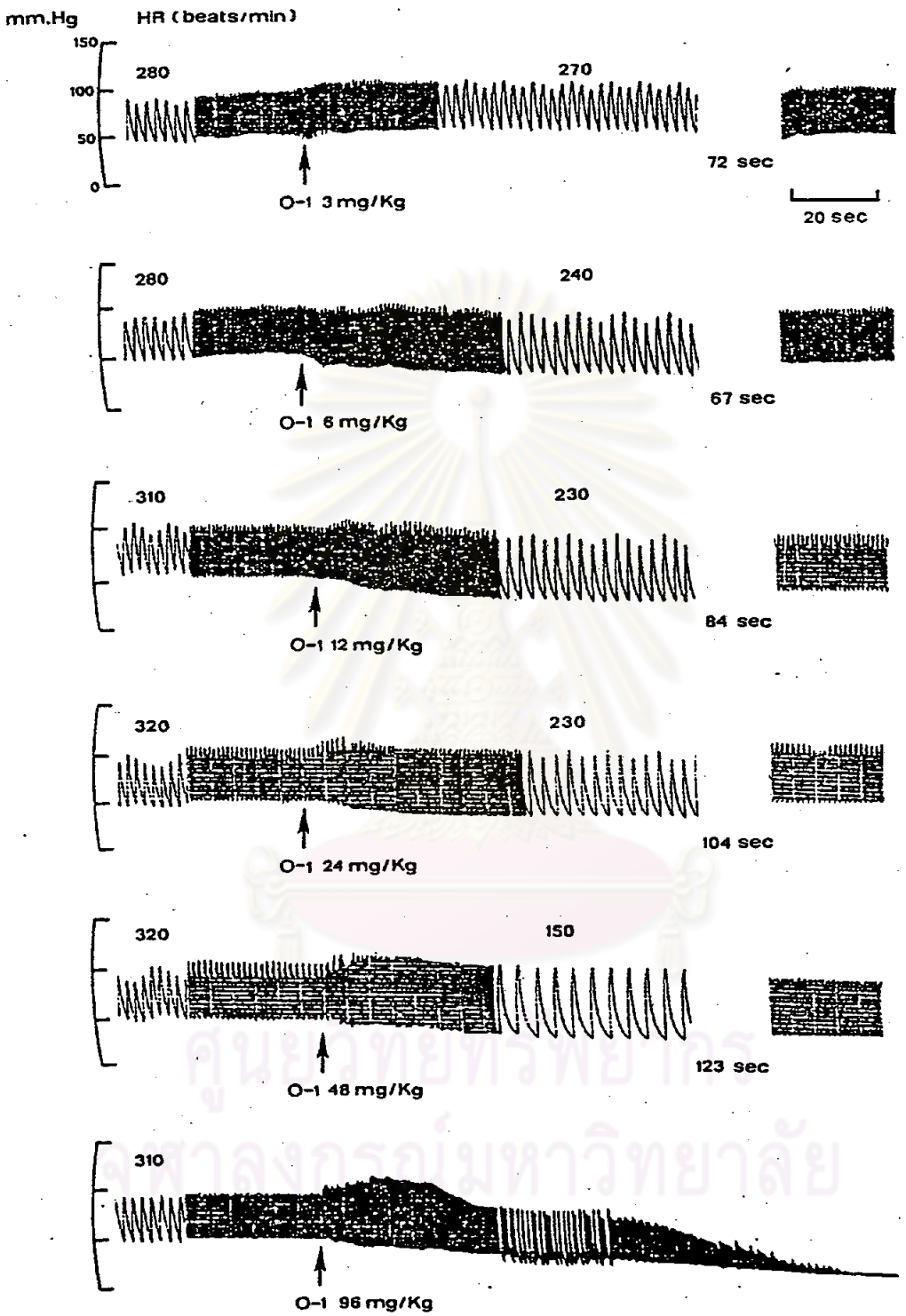

Figure 5. Trace of dose-response relationship for O-1 on blood pressure and heart rate in anaesthetized rat. O-1 produced weak pressor activity at low dose whereas blood pressure and heart rate became decreased in the higher doses.

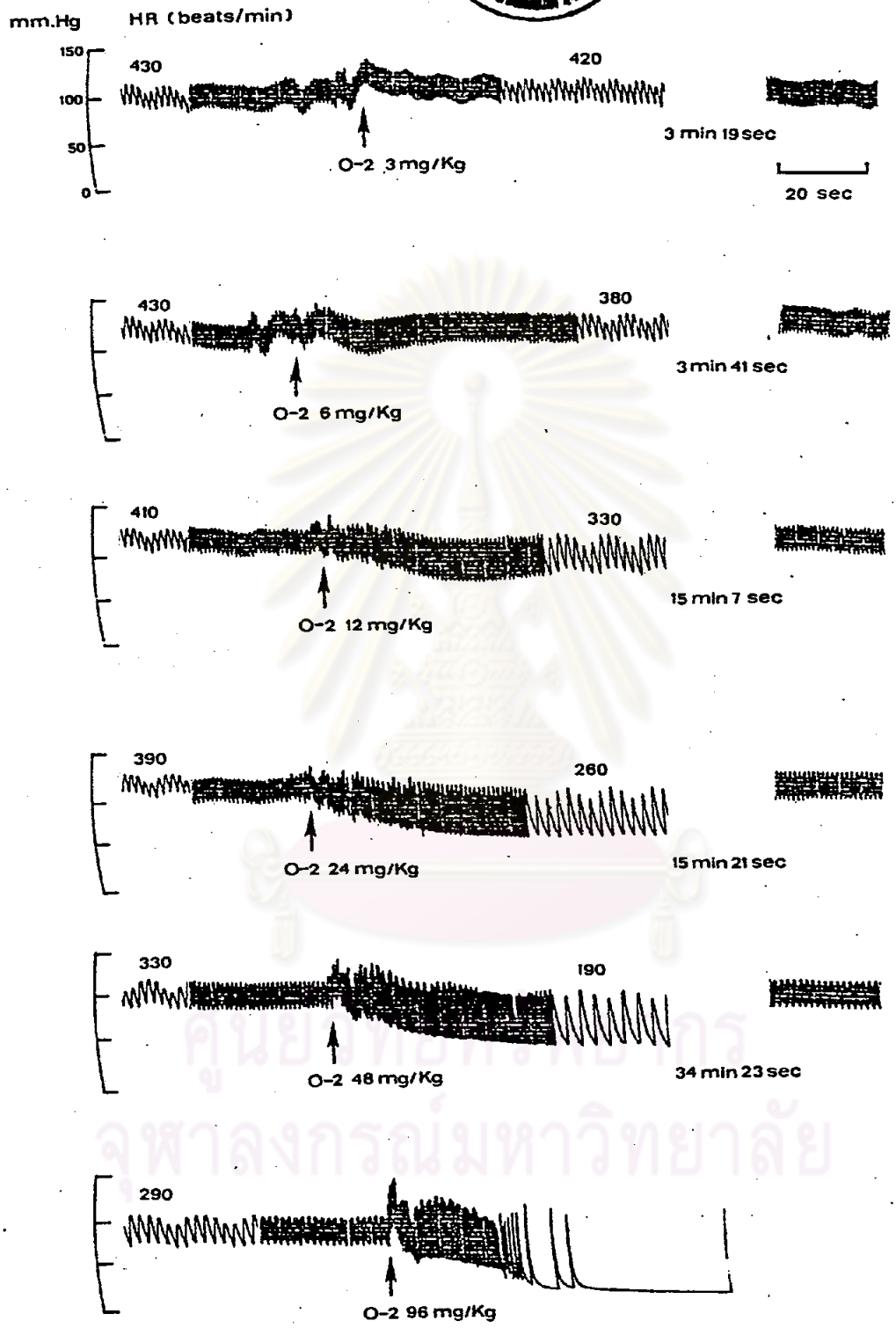

Figure 6. Trace of dose-response relationship for O-2 on blood pressure and heart rate in anaesthetized rat. O-2 had a weak pressor effect at lower dose, while higher doses produced depressor action.

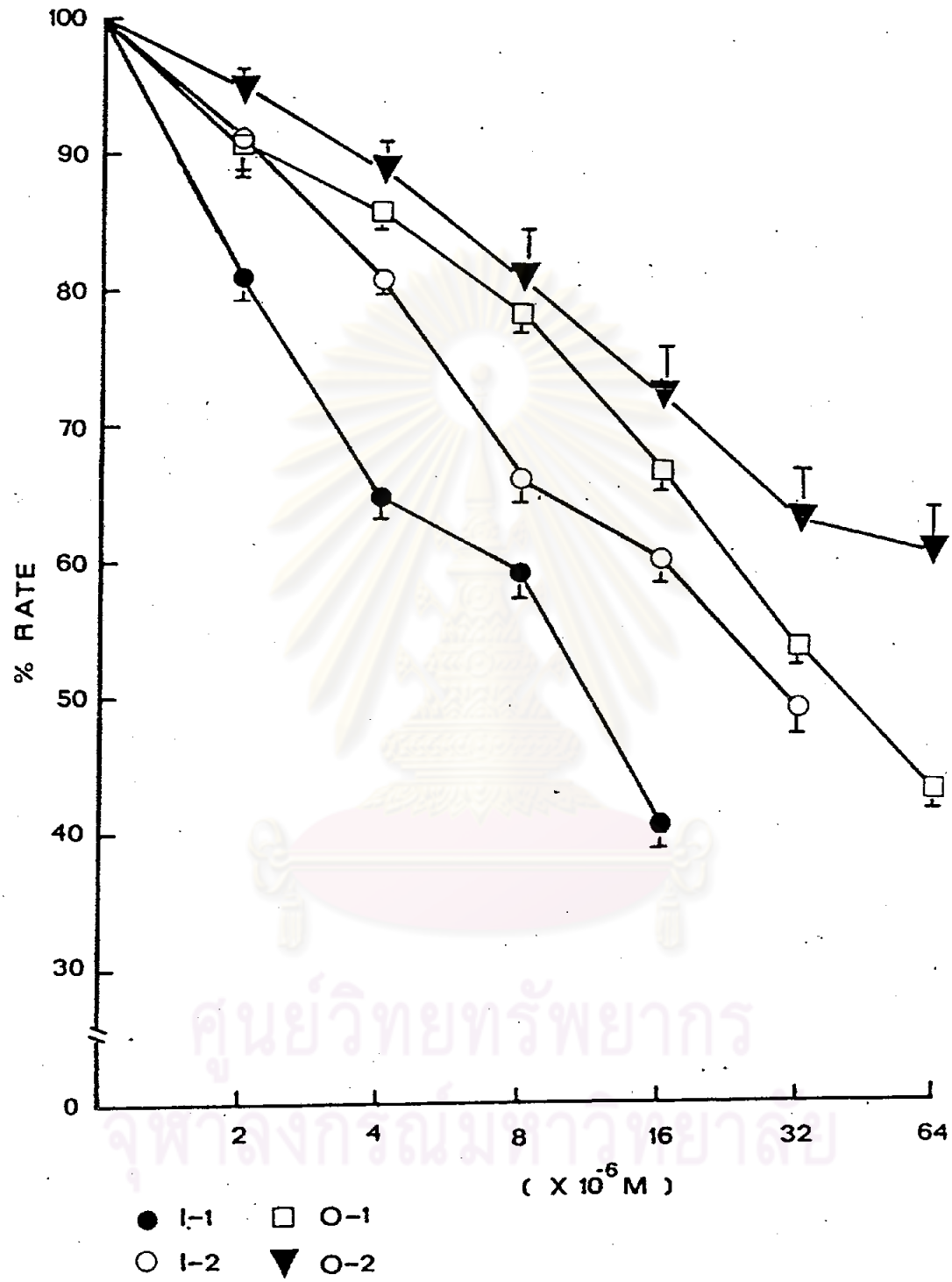

Figure 7. Cumulative log concentration-response curves for the negative chronotropic effect of four indole alkaloids on isolated spontaneously-beating right atrial preparations of the rats. Effects after preincubation during 5 min with indole alkaloids at indicated concentrations. Each point is the mean of at least 10 measurements; vertical lines show standard error of the mean. The ordinate scale is percentage of heart rate; the abscissa scale is the logarithm of the micromolar concentration of indole alkaloids.

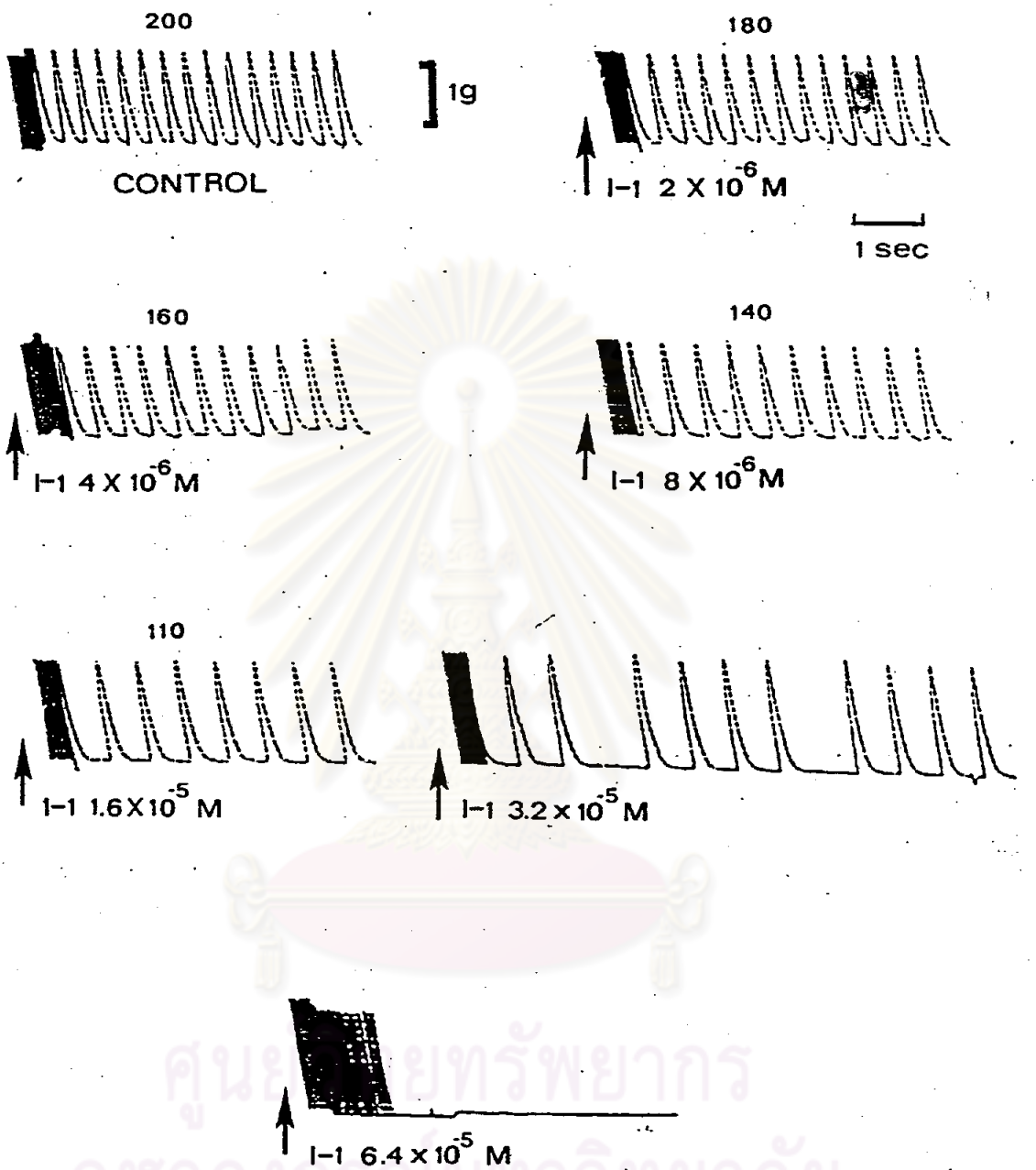

Figure 8. A. Trace of cumulative concentration-response relationship for I-1 in a rat right atrial strip. The record shows the depressive effect of I-1 on atrial rate, arrhythmogenesis and heartbeat cessation. Tracings were obtained from a curvilinear pen recorder. The numbers above the recordings indicate the heart rate (beats/min); vettical line shows tension of 1 gram and time scale is shown under the top panel. The concentrations of I-1 shown are expressed in molar.

concentrations of the alkaloids which produced 50% inhibition of the control spontaneous rates, are summarized in Table 1. It can be seen from Table 1 that I-1 was the most potent alkaloid sample producing 50% inhibition of cardiac contraction with as a low concentration as 12.1×10^{-6} M.

2.2 Negative Inotropic Effect.

Similarly, the three alkaloids excluding 0-2 caused a concentration - dependent depression of myocardial contractile tension in the electrically paced left atrial preparations. Mean cumulative concentration - response curves for the negative inotropic effect of alkaloids is shown in Fig. 8 B, 9 . Alkaloids also induced arrhythmogenesis and heartbeat cessation at the higher concentrations (I-1 $>$ 8×10^{-6} M, I-2 $>$ 1.6×10^{-5} M and 0-1 $>$ 3.2×10^{-5} M). Table 2 summarizes the EC 50 of the three alkaloids tested.

2.3 Effect on Relative Refractoriness (Negative Dromotropic Effect).

Most of indole alkaloids caused a concentration - dependent reduction on the maximum stimulation frequency that guinea - pig left atria could follow (Fig. 10). The concentrations required to reduce maximum driving frequency by 50% are given in Table 3. Untreated preparation showed very little change in responsiveness over the same time period of trial.

2.4 Effects of a Cholinergic Blocking Drug (Atropine) on Negative Chronotropic Action of Indole Alkaloids

The negative chronotropic effects of indole alkaloids on spontaneously beating right atrial preparation was not antagonized by prior administration of atropine (2×10^{-6} M) to the bath fluid, as shown in Fig. 11, 12, 13 and 14. This concentration of atropine

INDOLE ALKALOIDS	EC 50 (x 10^{-6} M)
I-1	12.1
I-2	28.6
O-1	48.23
O-2	70.75

Table 1. Concentrations of indole alkaloids (x 10^{-6} M) which produced 50% inhibition (EC 50) of rat isolated spontaneously-beatinging right atria. From this and the following table 2-3, the EC 50 values were calculated from linear regression lines which intrapolate or extrapolate from the scales.

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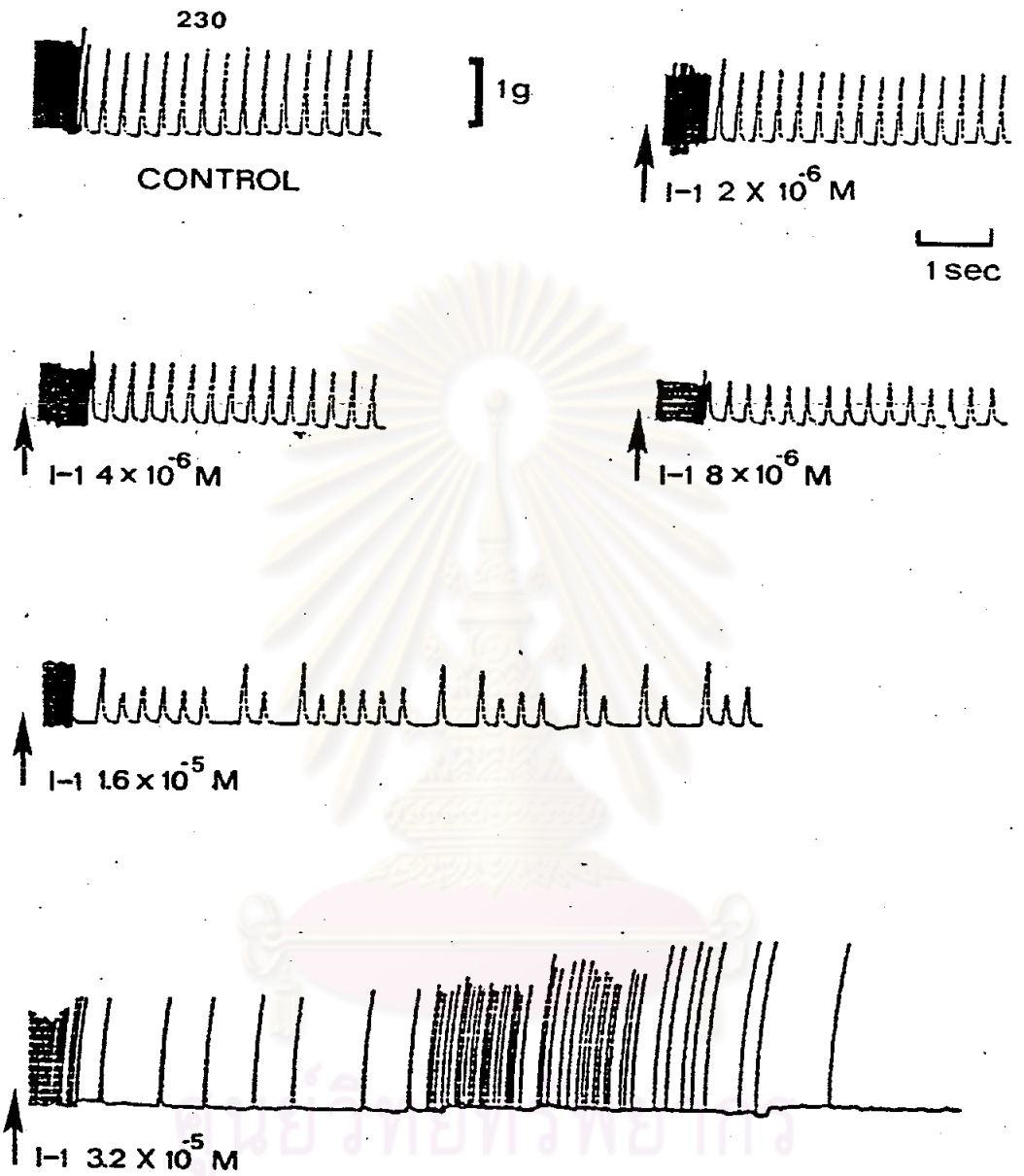

Figure 8. B. Trace of cumulative-concentration-response relationship for I-1 in a rat electrically-driven left atrial strip. The records show the depressive effect of I-1 on contractile tension of the heart, arrhythmogenesis and heartbeat cessation. Tracings were obtained from a curvilinear pen recorder. The calibration of 1 gram tension is shown on the vertical line and time scale under the top panel. The concentrations of I-1 shown are expressed in molar.

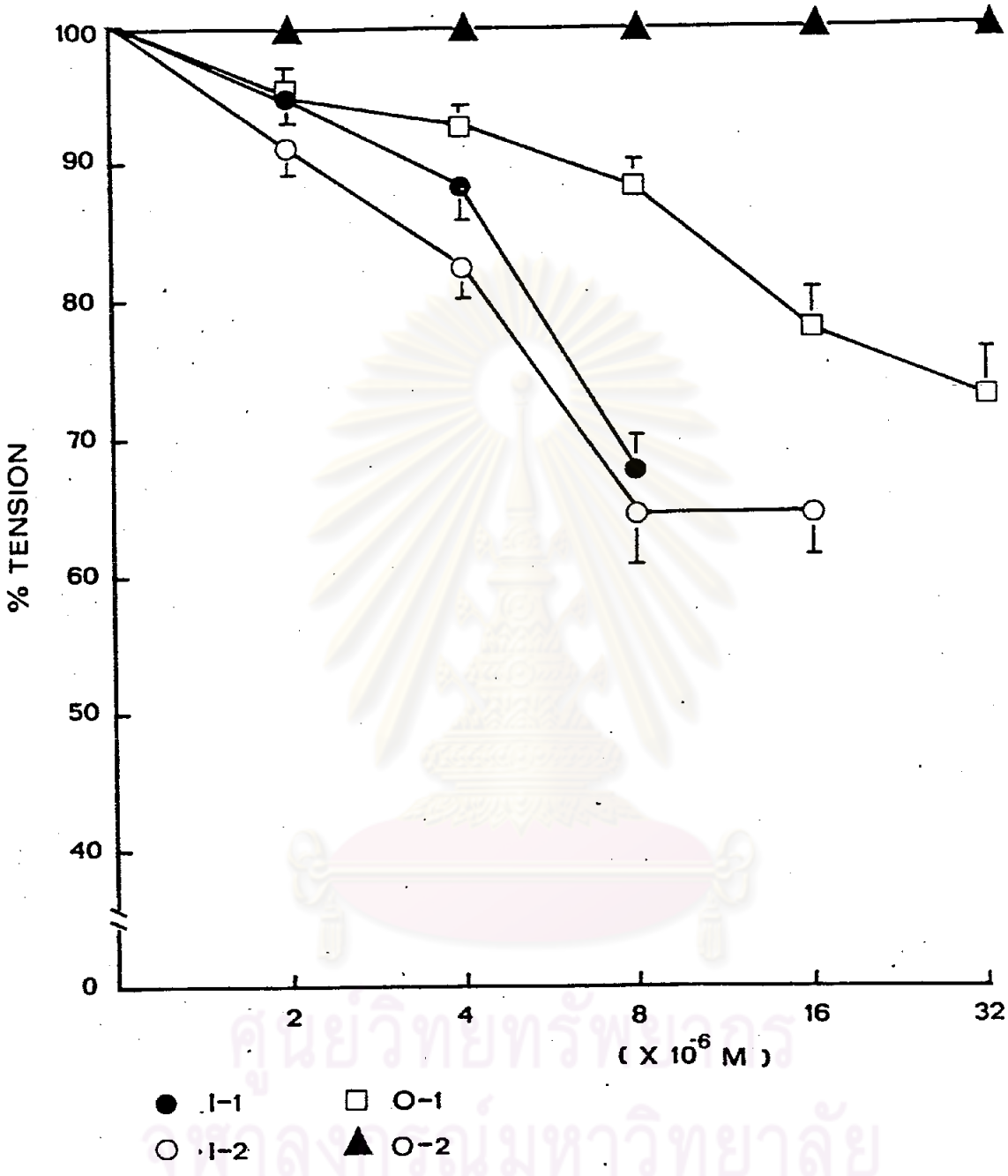

Figure 9. Cumulative log concentration-response curves for the negative inotropic effect of four indole alkaloids on isolated electrically-driven left atrial preparations of the rats. Effects after preincubation during 5 min with indole alkaloids at indicated concentrations. Each point is the mean of at least 10 measurements; vertical lines show standard error of the mean. The ordinate scale is percentage of contractile tension; the abscissa scale is the logarithm of the micromolar concentration of indole alkaloids.

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INDOLE ALKALOIDS	EC 50 ($\times 10^{-6}$ M)
I-1	11.9
I-2	21.4
O-1	59.4

Table 2. Concentrations of indole alkaloids ($\times 10^{-6}$ M) which produced 50% inhibition (EC 50) of rat isolated electrically-driven left atria.

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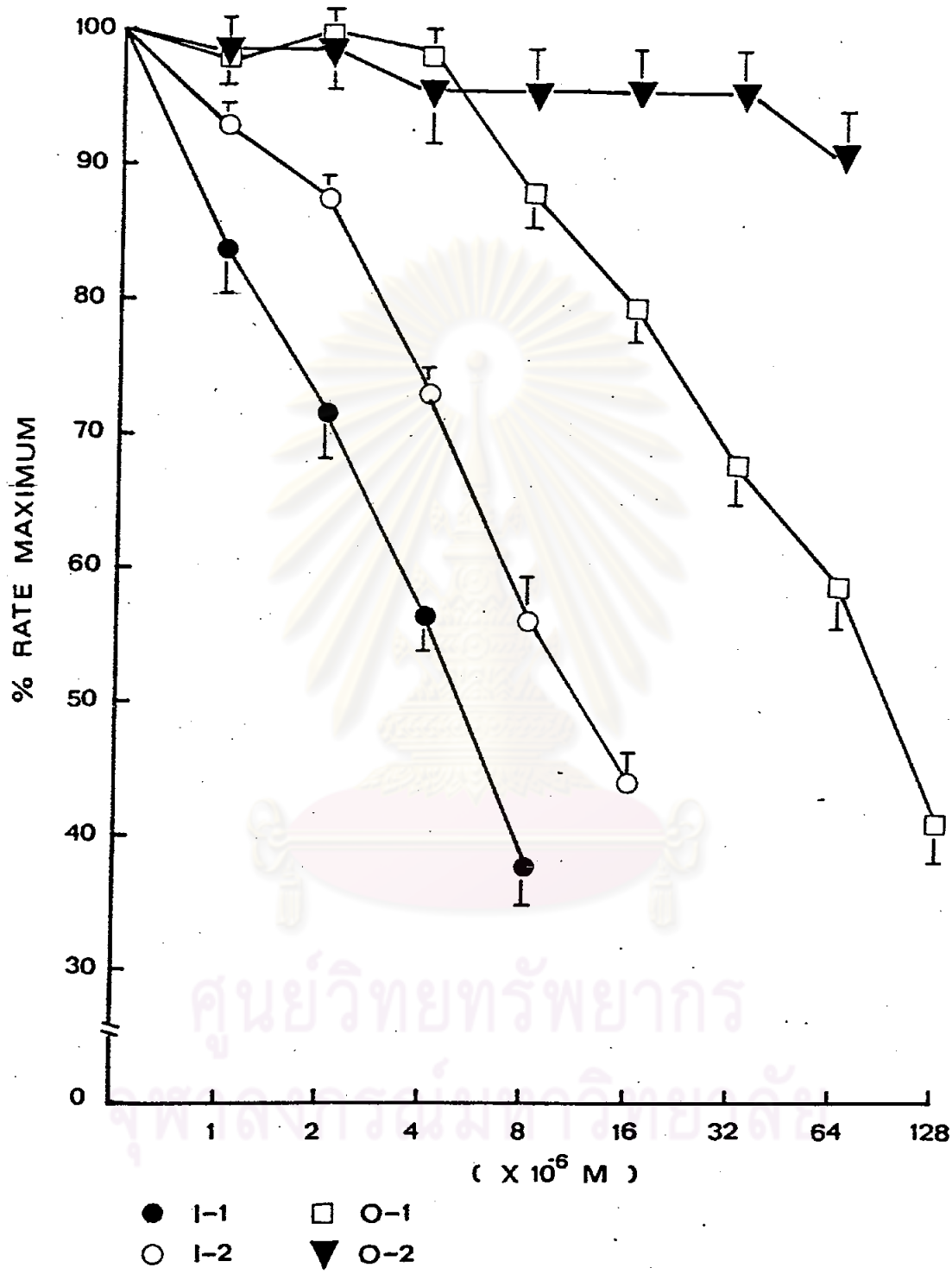

Figure 10. Cumulative log concentration-response curves for the negative dromotropic effect of four indole alkaloid on isolated electrically-driven left atrial preparations of the guinea-pigs. Effects after preincubation during 5 min with indole alkaloids at indicated concentrations. Each point is the mean of at least 3 measurements; vertical lines show standard error of the mean. The ordinate scale is percentage of maximal rate that guinea-pigs left atrias could follow; the abscissa scale is the logarithm of the micromolar concentration of indole alkaloids.

INDOLE ALKALOIDS	EC 50 (M)
I-1	5.74×10^{-6}
I-2	1.3×10^{-5}
O-1	1.16×10^{-4}
O-2	4.66×10^{-4}

Table 3. Concentrations of indole alkaloids (M) which reduced maximum driving requency by 50% (EC 50) that guinea-pig left atria could follow.

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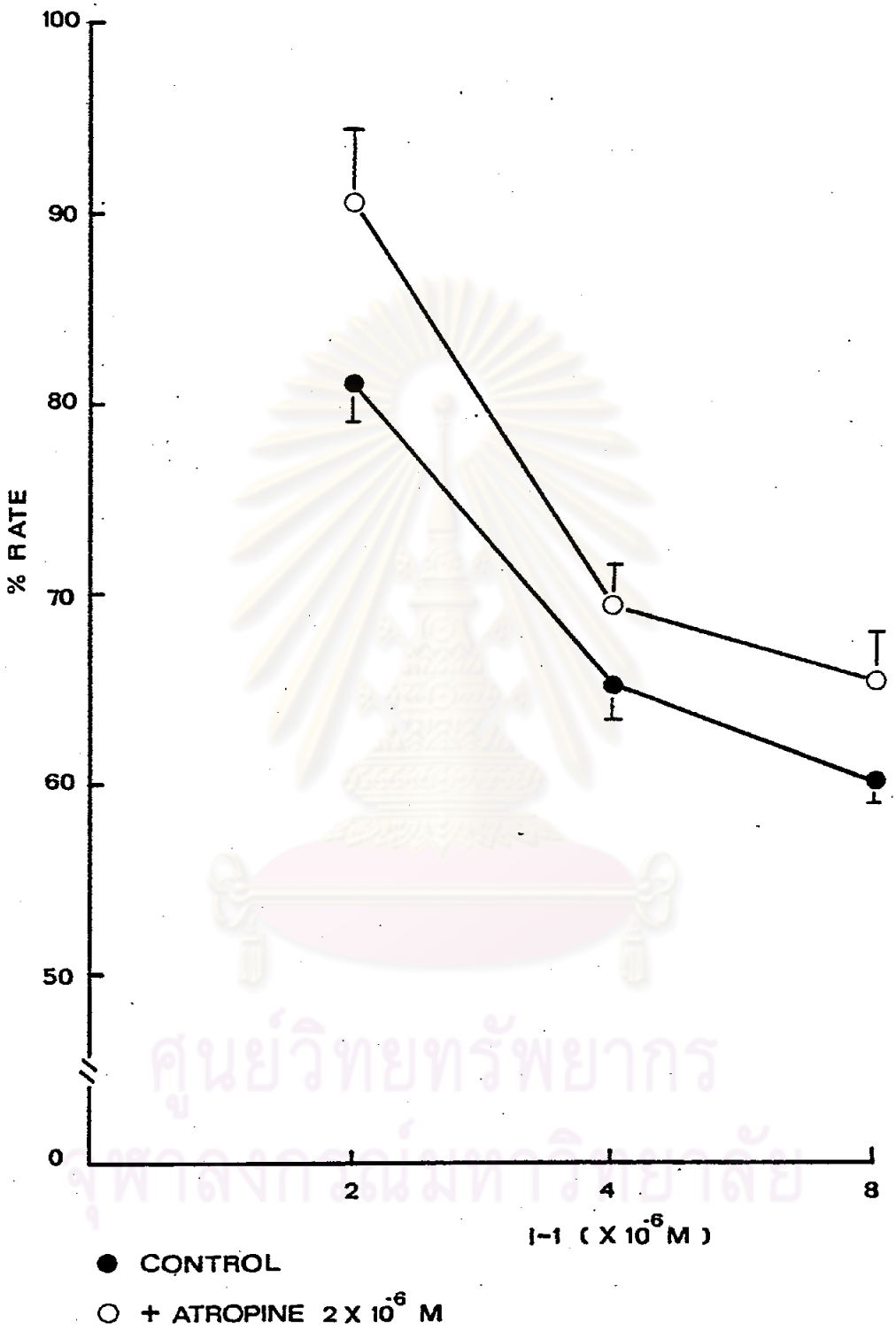

Figure 11. Log concentration-response curves for the inhibition of negative chronotropic effect of I-1 by Atropine in the rat right atrial preparations. In this and the following figures 12-15, the control curves are shown by solid symbols; the open symbols indicate responses in the presence of 2×10^{-6} M atropine; vertical lines show standard error of the mean; the ordinate scale is percentage of heart rate; the abscissa scale is the logarithm of the micromolar concentration. Each point is the mean of 3 responses. None of the points in this figure is significantly different. ($P > 0.1$)

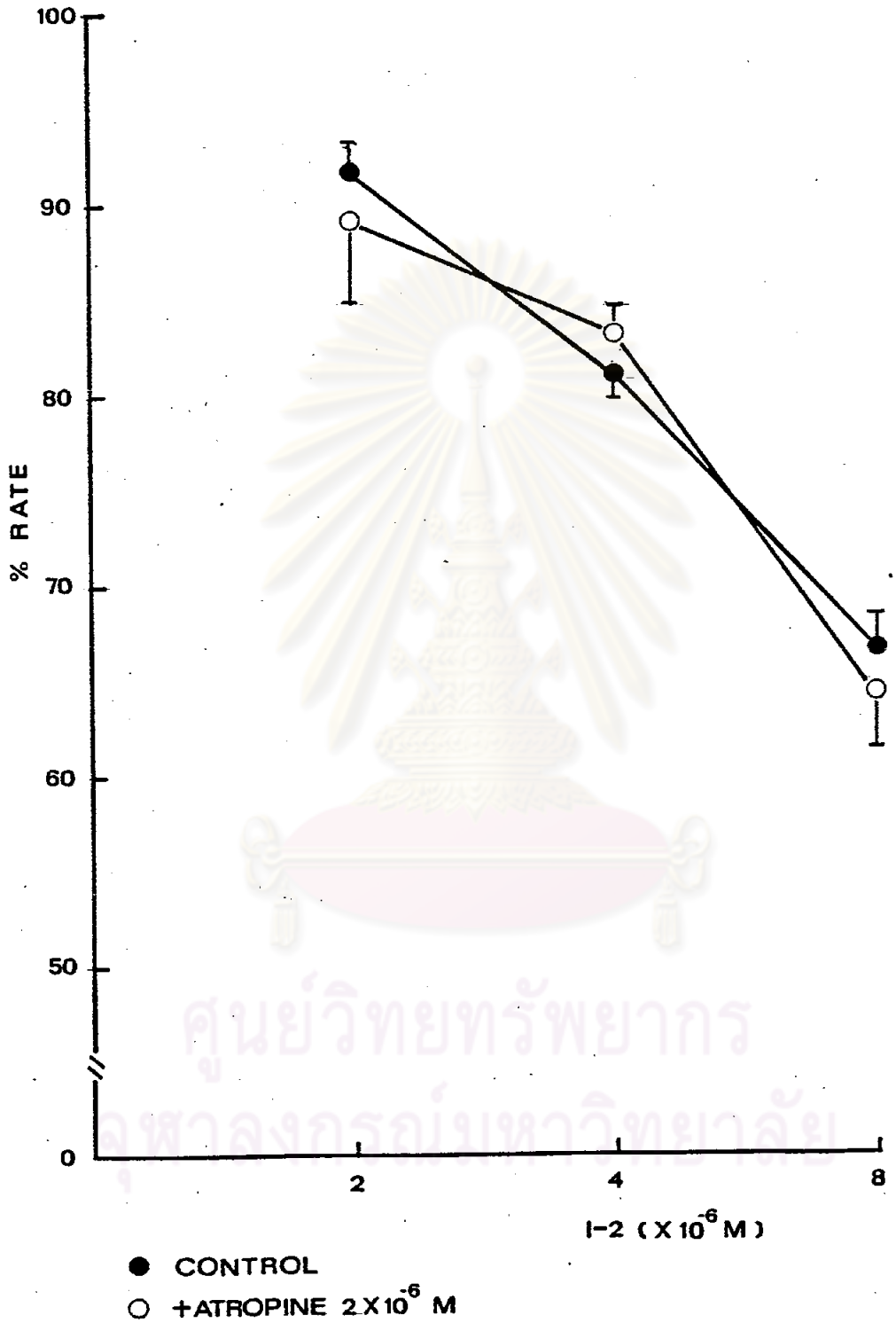

Figure 12. Log concentration-response curves for the inhibition of negative chronotropic effect of I-2 by atropine in the rat right atrial preparations. Each point is the mean of 3 responses. None of the points is significantly different. ($P > 0.4$)

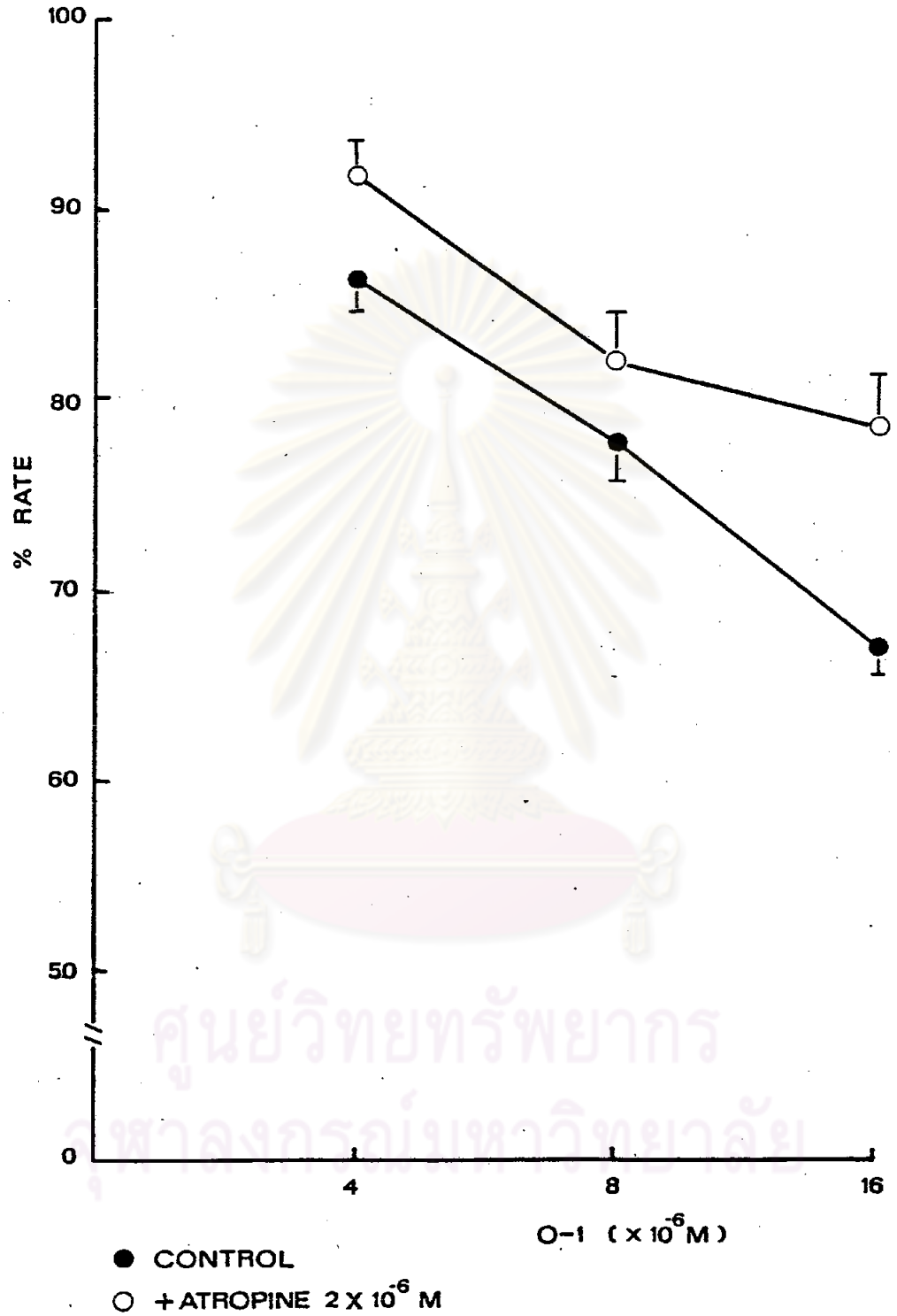

Figure 13. Log concentration-response curves for the inhibition of negative chronotropic effect of O-1 by atropine in the rat right atrial preparations. Each point is the mean of 3 responses. None of the points is significantly different. ($P > 0.2$)

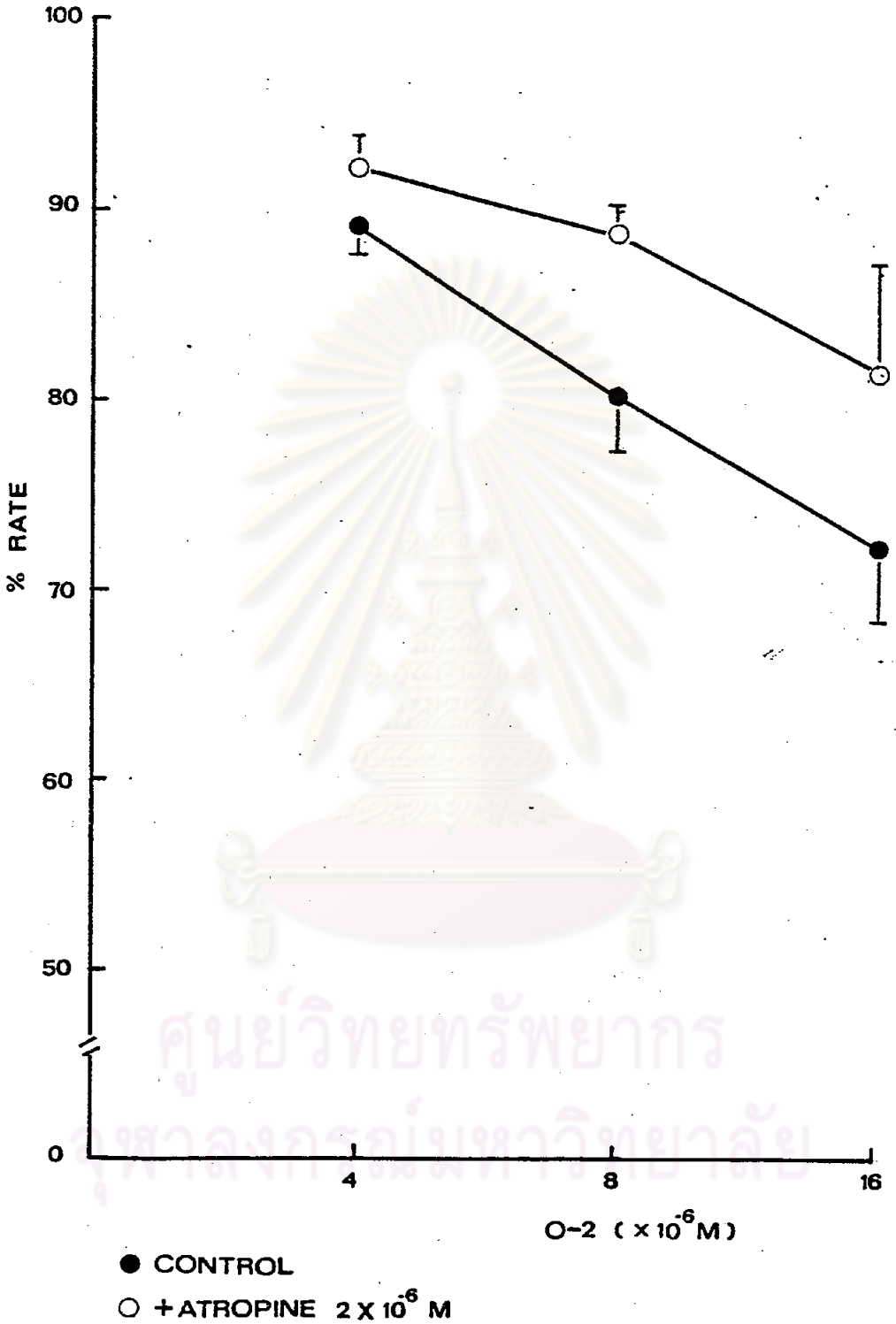

Figure 14. Log concentration-response curves for the inhibition of negative chronotropic effect of O-2 by atropine in the rat right atrial preparations. Each point is the mean of 3 responses. None of the points is significantly different. ($P > 0.1$)

could abolish the negative chronotropic effect of acetylcholine (0.5 - 2 M), which was considerably more potent than the four alkaloids in depressing the heart contraction (Fig. 15).

2.5 Effects of Indole Alkaloids on Positive Chronotropic Action of Adrenaline

Adrenaline, a β-stimulating catecholamine, had direct positive chronotropic and inotropic effect on the heart. In order to investigate whether these alkaloids could modify these effects of adrenaline on isolated atria, adrenaline was added to the right atria in the cumulative dose regimen (0.15 - 2.4 M). As shown in Fig. 16, 17, 18 and 19, adrenaline produced dramatic increase in atrial rate. When added 5 min following pretreatment of the preparation with indole alkaloids (I-1 and I-2 1.6×10^{-5} M, O-1 and O-2 3.2×10^{-5} M), it was apparent that adrenaline still retained its positive chronotropic effects.

2.6 Effects of Indole Alkaloids on Positive Chronotropic Action of Isoproterenol

Application of Isoproterenol, a β_1 adrenoceptor agonist, to the bath fluid produced increases in both rate and force of contraction of the guinea - pig right atrial preparation. The positive chronotropic effect of isoproterenol was not antagonized by indole alkaloids as suggested by Fig. 20, 21, 22 and 23, in which it is shown that concentration - response curves of isoproterenol with and without the presence of the tested alkaloids were not significantly different.

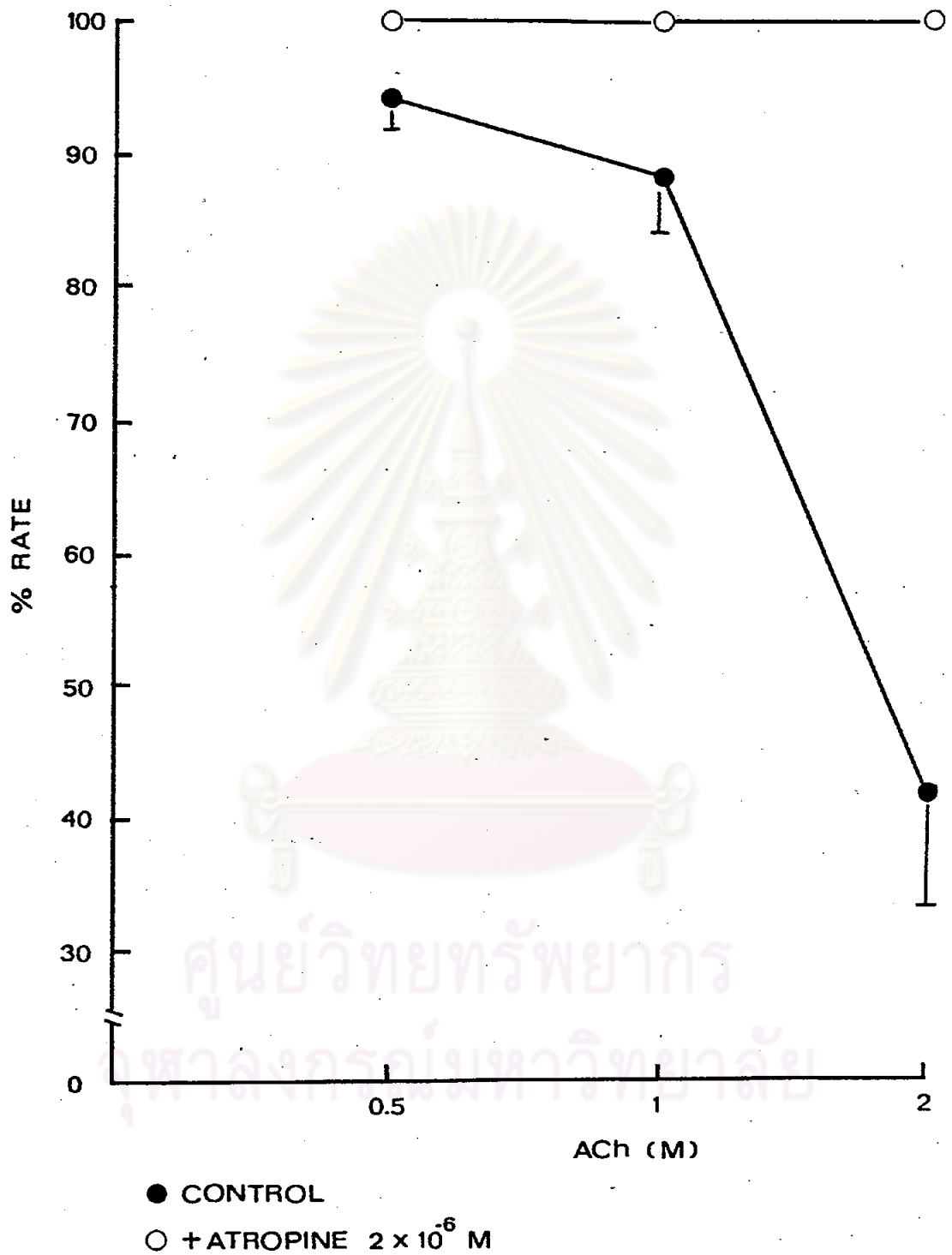

Figure 15. Log concentration-response curves for the inhibition of negative chronotropic effect of acetylcholine by atropine in the rat right atrial preparations. Each point is the mean of 3 responses. All points are significantly different on control and atropine curve ($P < 0.001$; Student's t test).

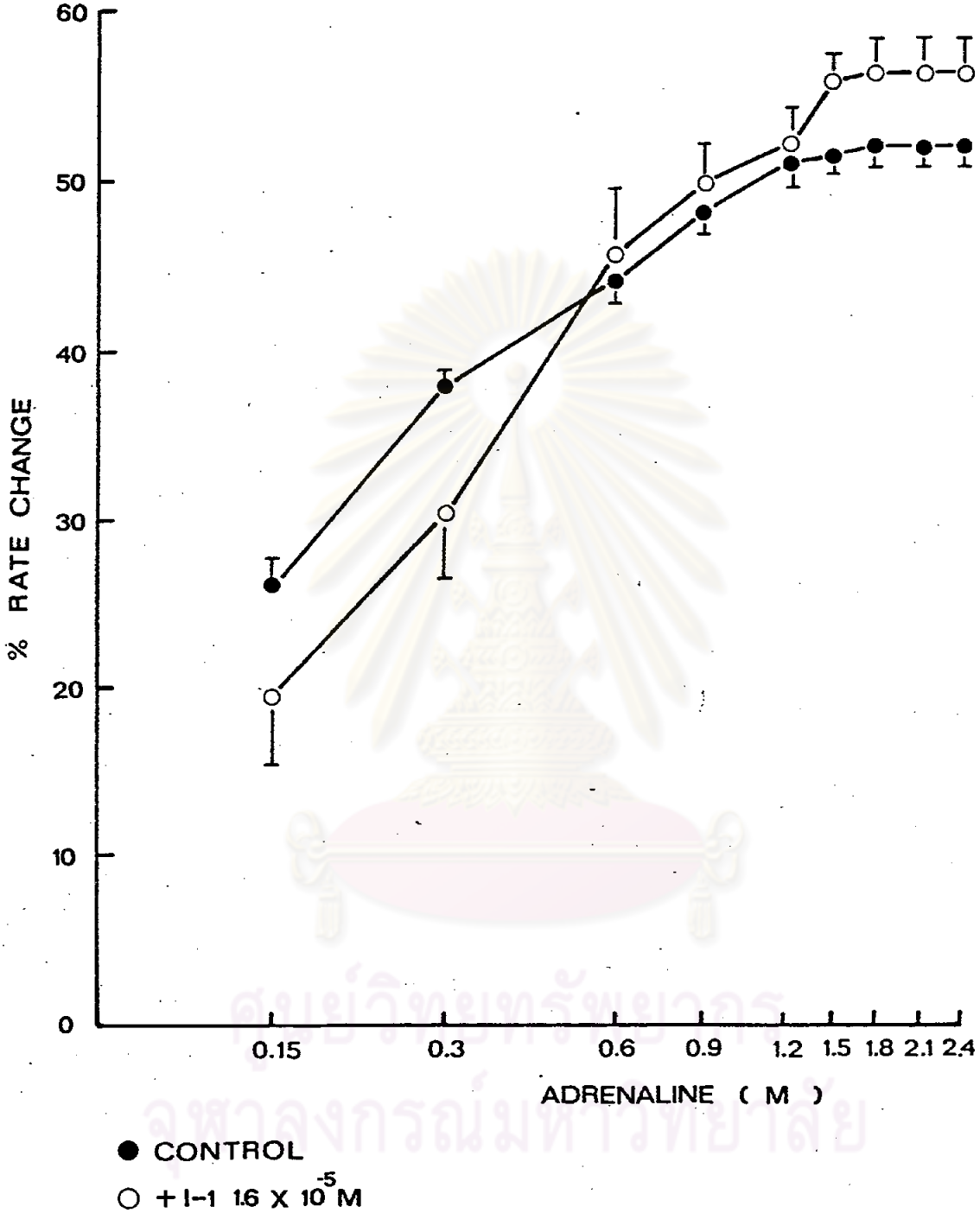

Figure 16. Log concentration-response curves for the inhibition of positive chronotropic effect of adrenaline by I-1 in the rat right atrial strips. In this and the following figures 17-27, the control curves are shown by solid symbols; the open symbols indicate responses in the presence of indole alkaloids; vertical lines show standard error of the mean; the ordinate scale is percentage change in heart rate; the abscissa scale is the logarithm of the molar concentration. Each point is the mean of at least 5 responses. None of the points in this figure is significantly different. ($P > 0.4$)

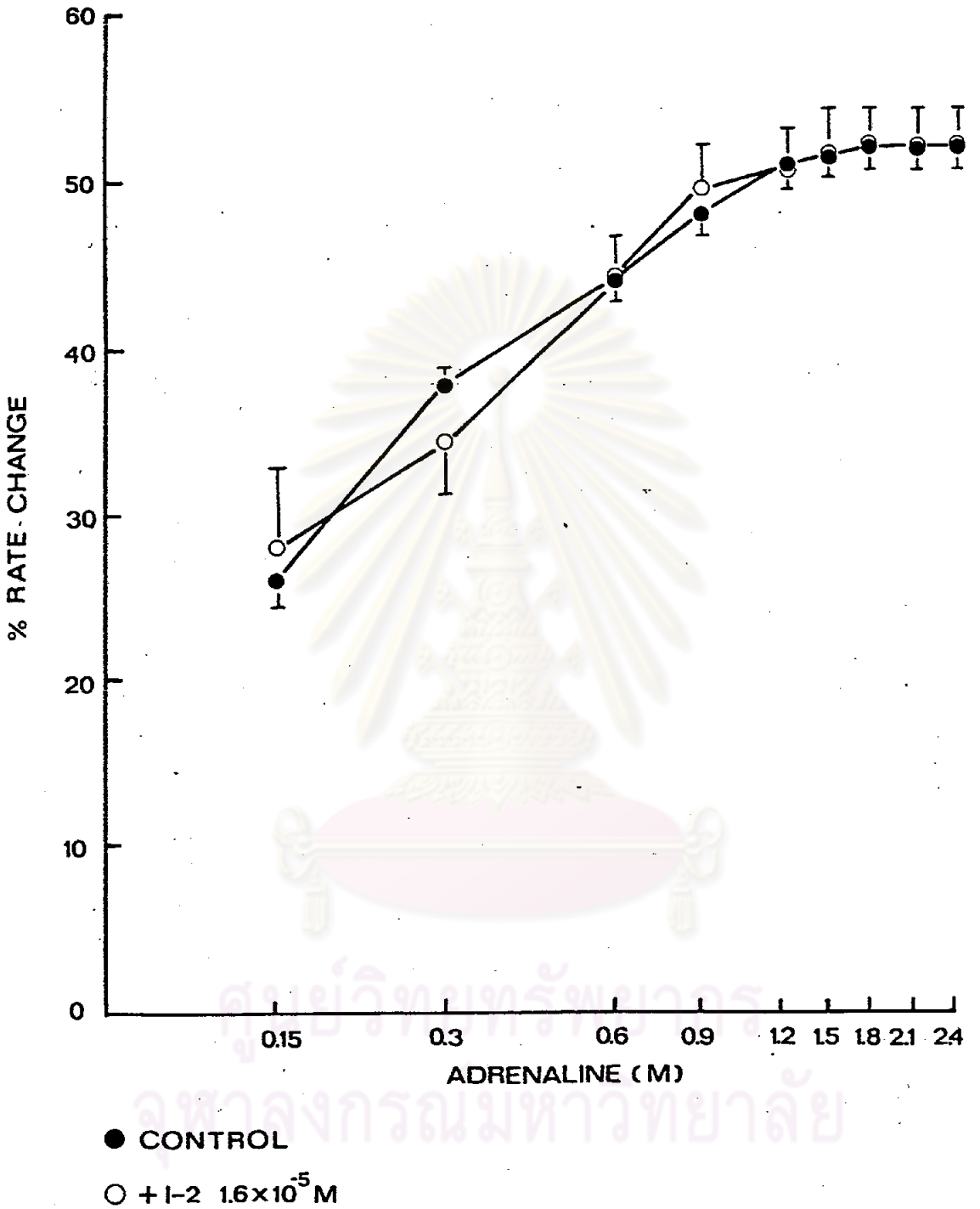

Figure 17. Log concentration-response curves for the inhibition of positive chronotropic effect of adrenaline by I-2 in the rat right atrial strips. Each point is the mean of at least 5 responses. None of the points in this figure is significantly different. ($P > 0.4$)

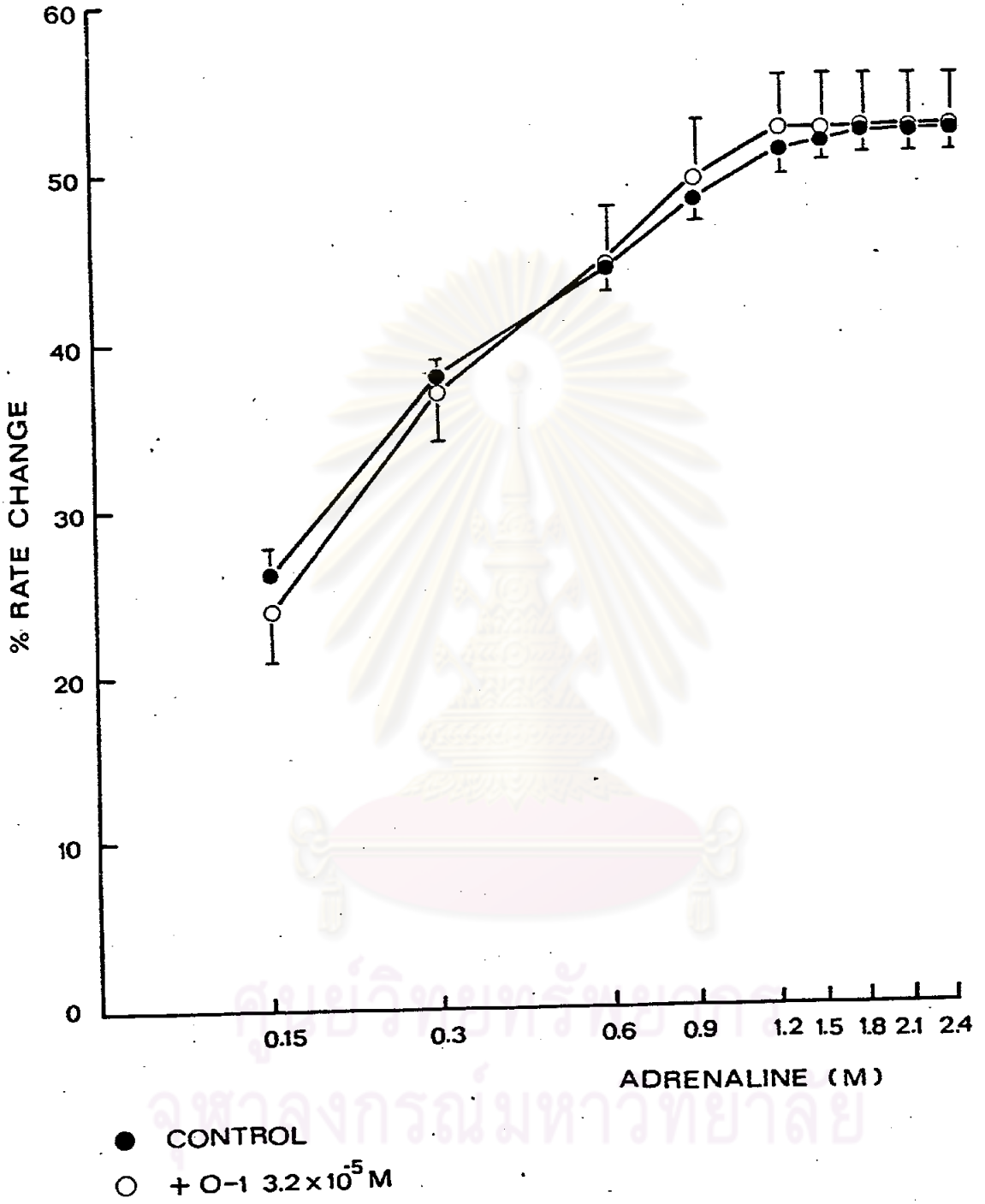

Figure 18. Log concentration-response curves for the inhibition of positive chronotropic effect of adrenaline by O-1 in the rat right atrial strips. Each point is the mean of at least 4 responses. None of the points in this figure is significantly different. ($P > 0.4$)

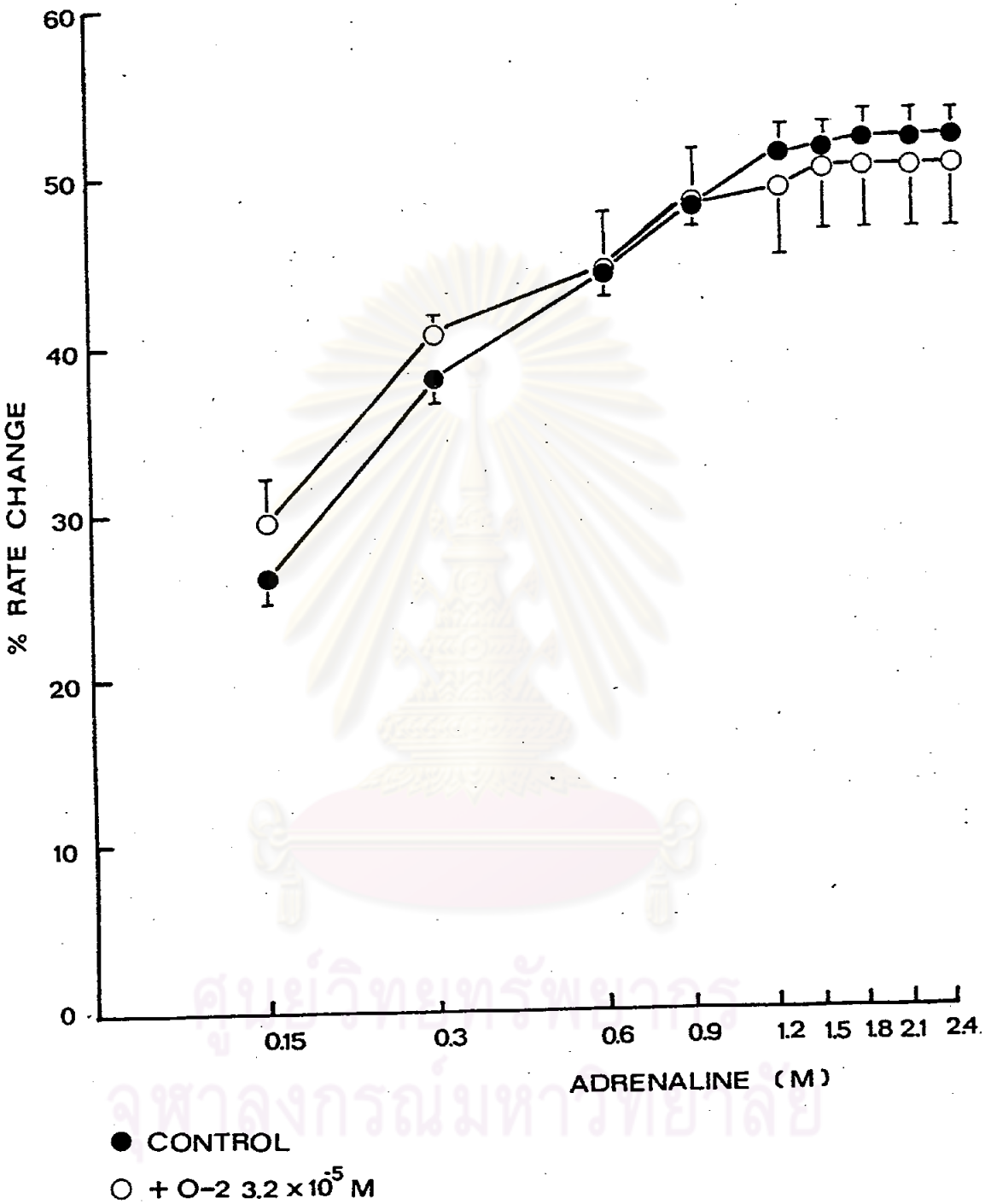

Figure 19. Log concentration-response curves for the inhibition of positive chronotropic effect of adrenaline by O-2 in the rat right atrial strips. Each point is the mean of at least 4 responses. None of the points in this figure is significantly different. ($P > 0.5$)

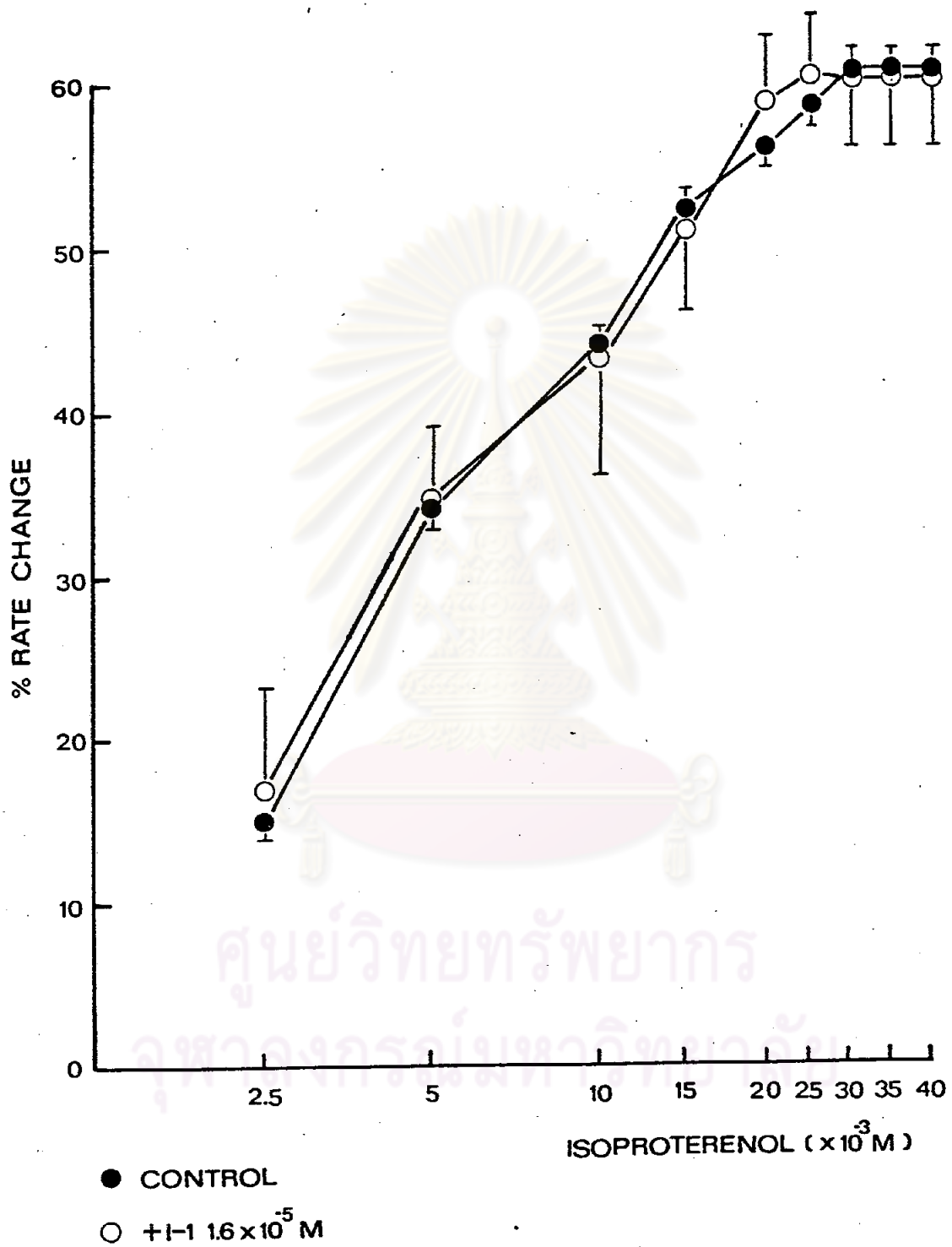

Figure 20. Log concentration-response curves for the inhibition of positive chronotropic effect of isoproterenol by I-1 in the guinea-pigs right atrial strips. Each point is the mean of at least 4 responses. None of the points in this figure is significantly different. ($P > 0.5$)

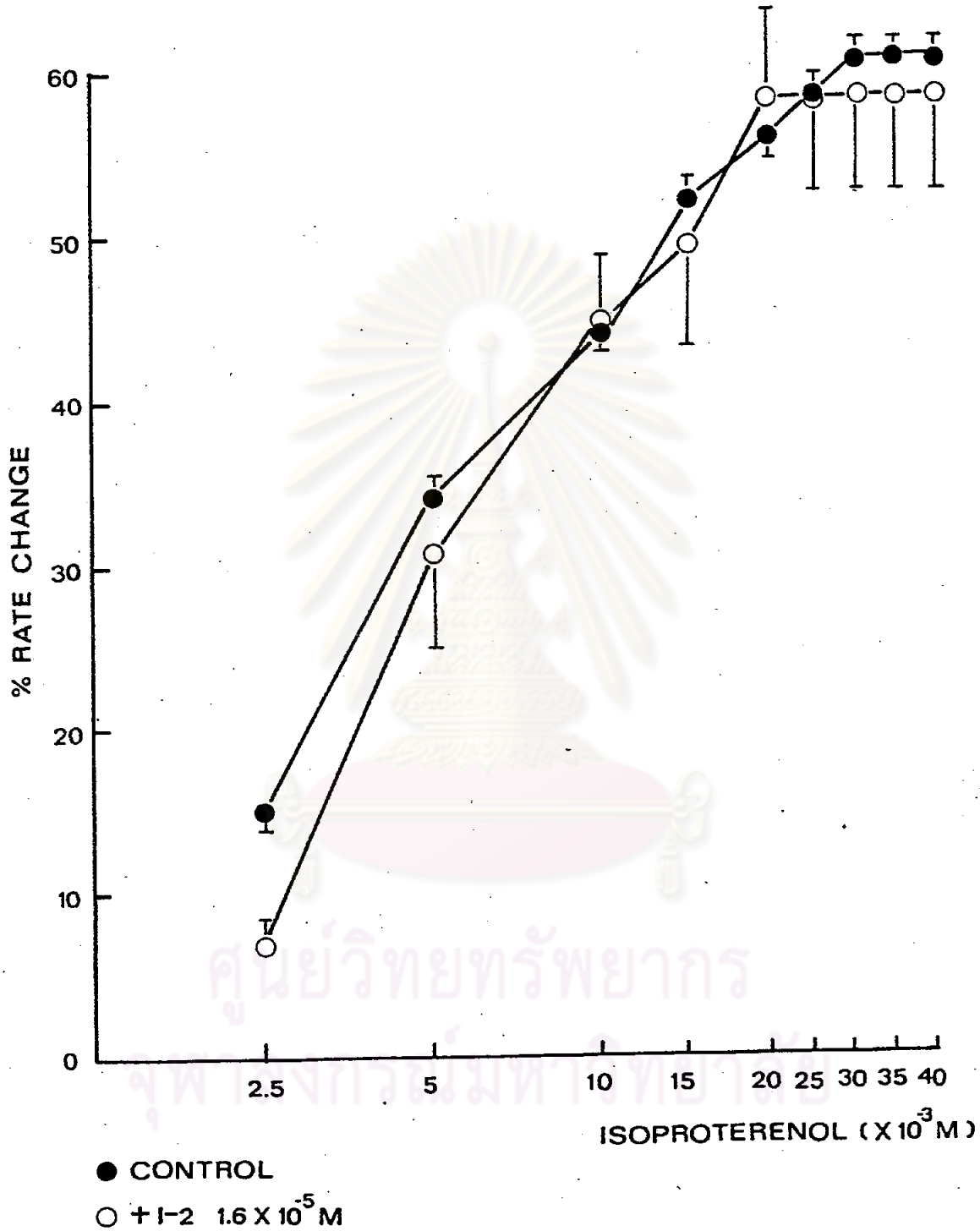

Figure 21. Log concentration-response curves for the inhibition of positive chronotropic effect of isoproterenol by I-2 in the guinea-pigs right atrial strips. Each point is the mean of at least 4 responses. None of the points in this figure is significantly different. ($P > 0.5$)

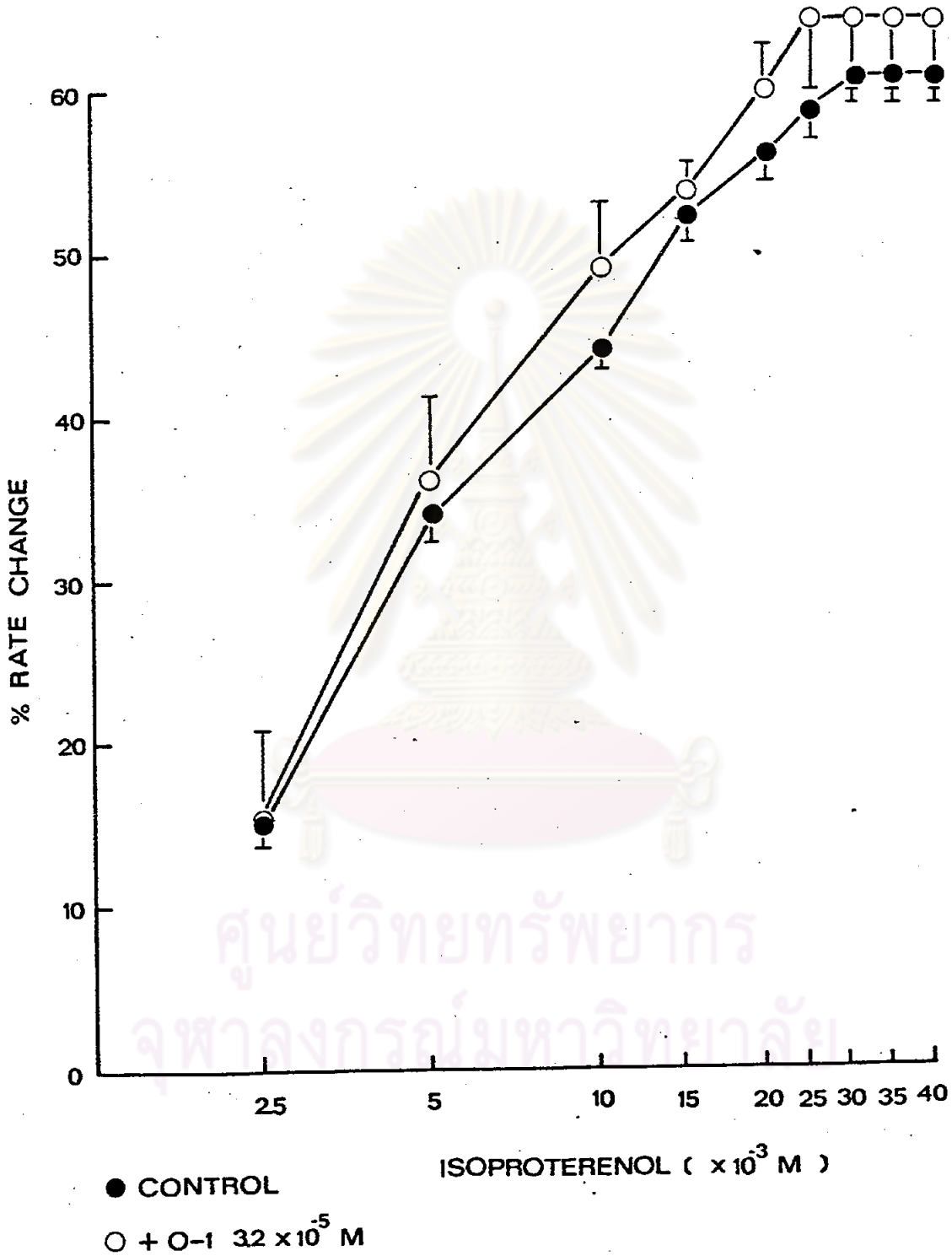

Figure 22. Log concentration-response curves for the inhibition of positive chronotropic effect of isoproterenol by O-1 in the guinea-pigs right atrial strips. Each point is the mean of at least 5 responses. None of the points in this figure is significantly different. ($P > 0.2$)

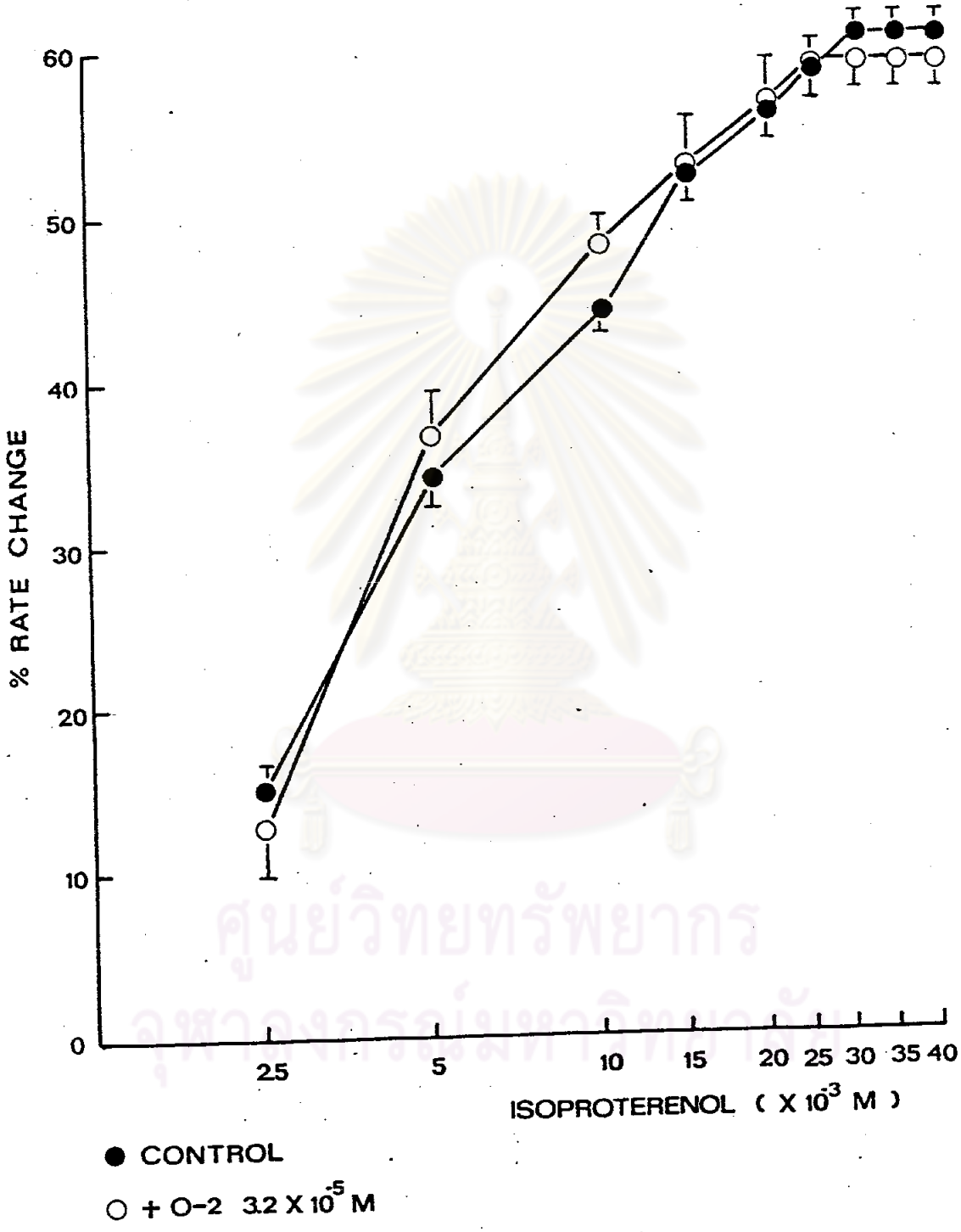

Figure 23. Log concentration-response curves for the inhibition of positive chronotropic effect of isoproterenol by O-2 in the guinea-pigs right atrial strips. Each point is the mean of at least 5 responses. None of the points in this figure is significantly different. ($P > 0.4$)

2.7 Effects of Indole Alkaloids on Positive Chronotropic Action of 5-Hydroxytryptamine (5-HT)

Administration of 5-HT in the cumulative dose regimen (4×10^{-6}-3.2×10^{-5} M) to the bath fluid always caused an increase in the rate of contraction of the right atrial strips of the rats. Four indole alkaloids could mitigate positive chronotropic effect induced by 5-HT. From the Fig. 24 and 25, the ability of low concentrations of I-1 and I-2 (1.2×10^{-5} and 1.6×10^{-5} M) caused a rightward shift of the curves to 5-HT ($P < 0.001$), together with depression of the maximum response. In contrast, O-1 and O-2 in the lower concentration (1.6×10^{-5} M) could not alleviate the positive chronotropic effect of 5-HT (Fig. 26 and 27). In order to reduce the effect of 5-HT, the concentrations of O-1 and O-2 were 2 times more than I-1 and I-2 (3.2×10^{-5} M). Fig. 26 and 27 illustrated log concentration - response curves in the presence of O-1 and O-2, curves were shifted to the right and there were significant changes in maximal responses ($P < 0.001$) and $P < 0.025$) respectively).

The affinity of a non-competitive antagonist for its receptor, pD_2, is the negative logarithm of the molar concentration of the antagonist that produces 50% reduction of the maximum response obtained with an agonist (Broucke and Lemli, 1982). The calculated mean pD_2 values obtained for four indole alkaloids against 5-HT are summarized in Table 4. It can be seen from Table 4 that the affinity of I-1 was stronger than I-2, O-1 and O-2 respectively.

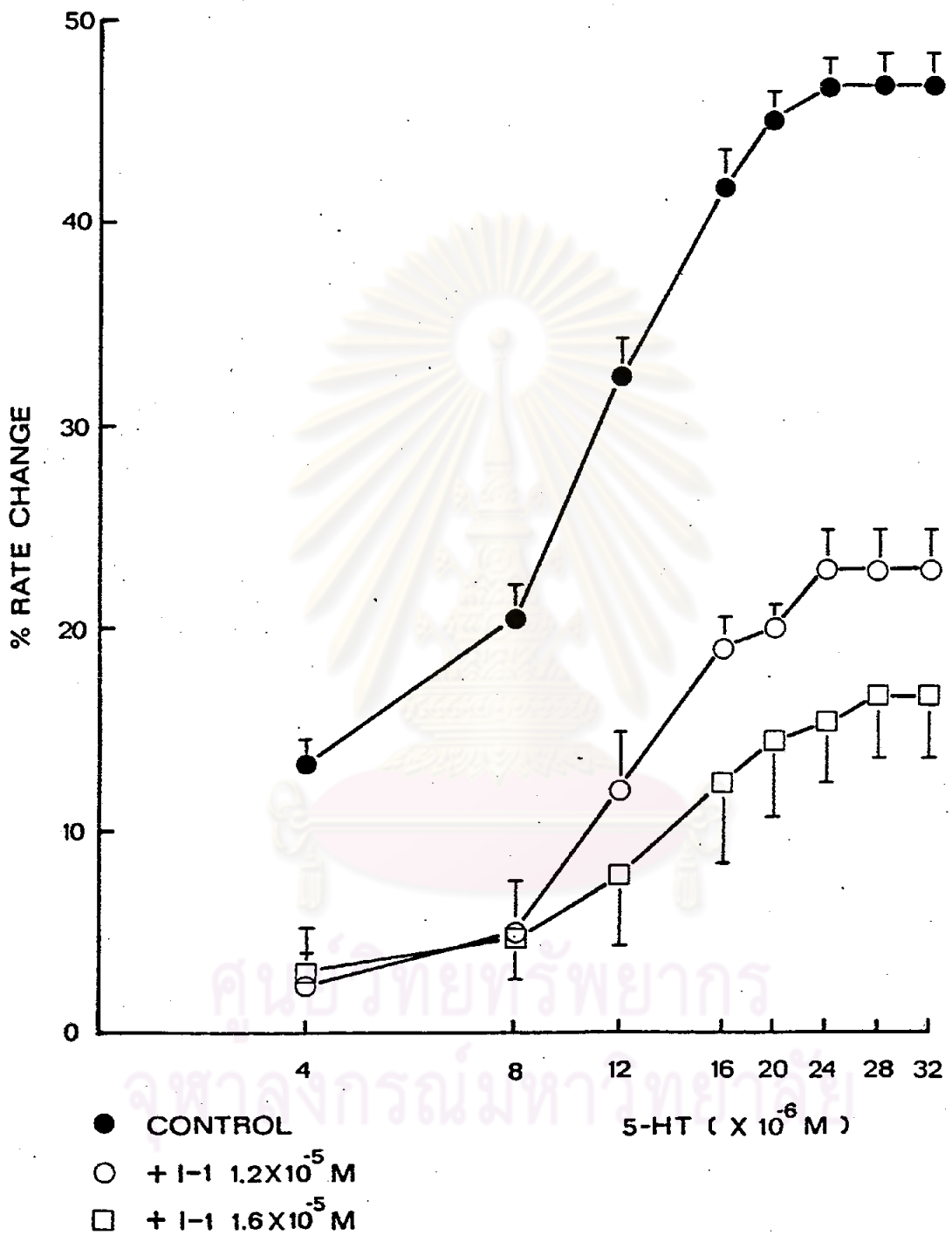

Figure 24. Log concentration-response curves for the inhibition of positive chronotropic effect of 5-hydroxytryptamine (5-HT) by I-1 in the rat right atrial strips. Each point is the mean of at least 5 measurements. All points on the control and both concentrations of I-1 curves are significantly different ($P < 0.001$; Student's t test).

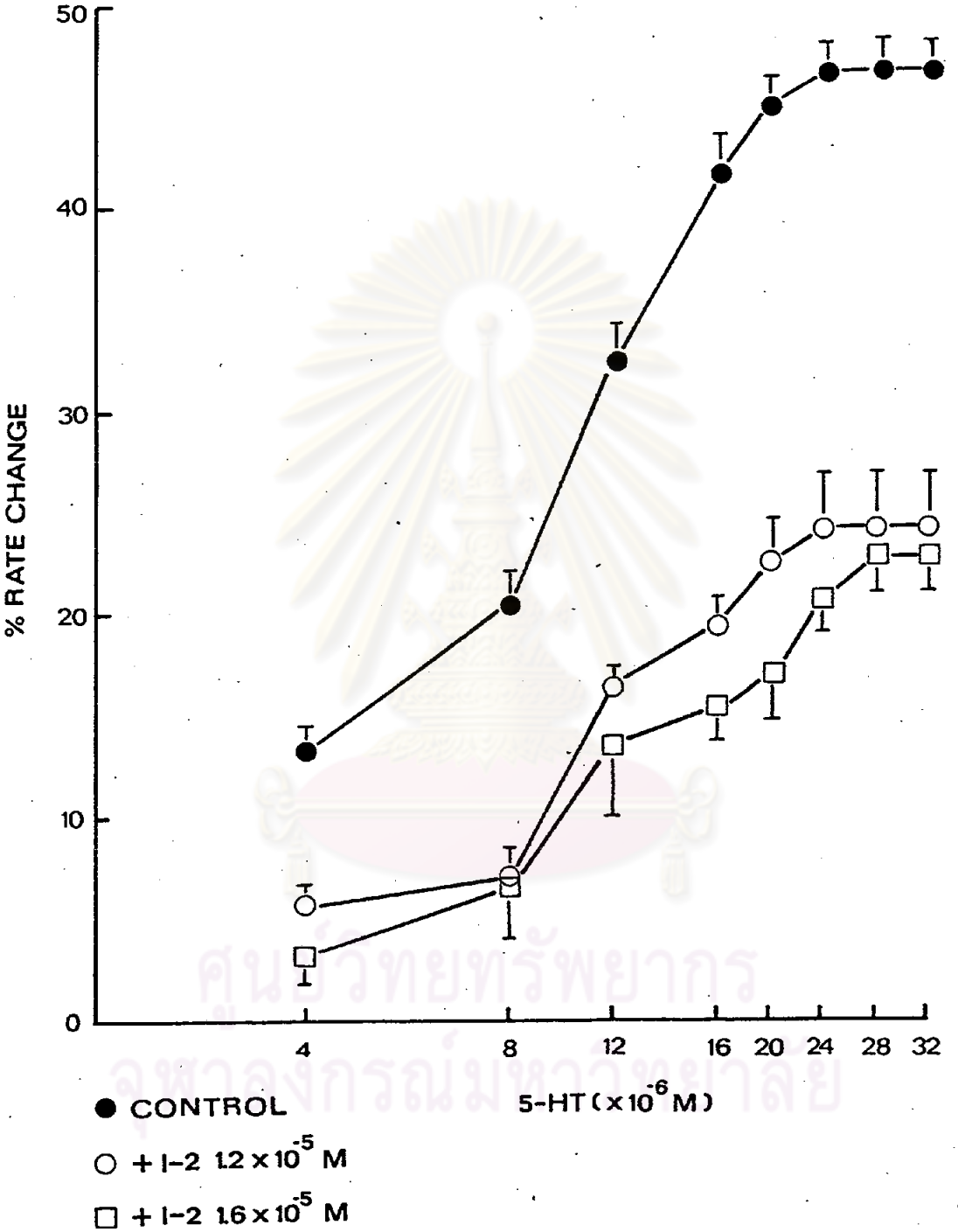

Figure 25. Log concentration-response curves for the inhibition of positive chronotropic effect of 5-hydroxytryptamine (5-HT) by I-2 in the rat right atrial strips. Each point is the mean of at least 4 measurements. All points on the control and both concentrations of I-2 curves are significantly different ($P < 0.001$; Student's t test).

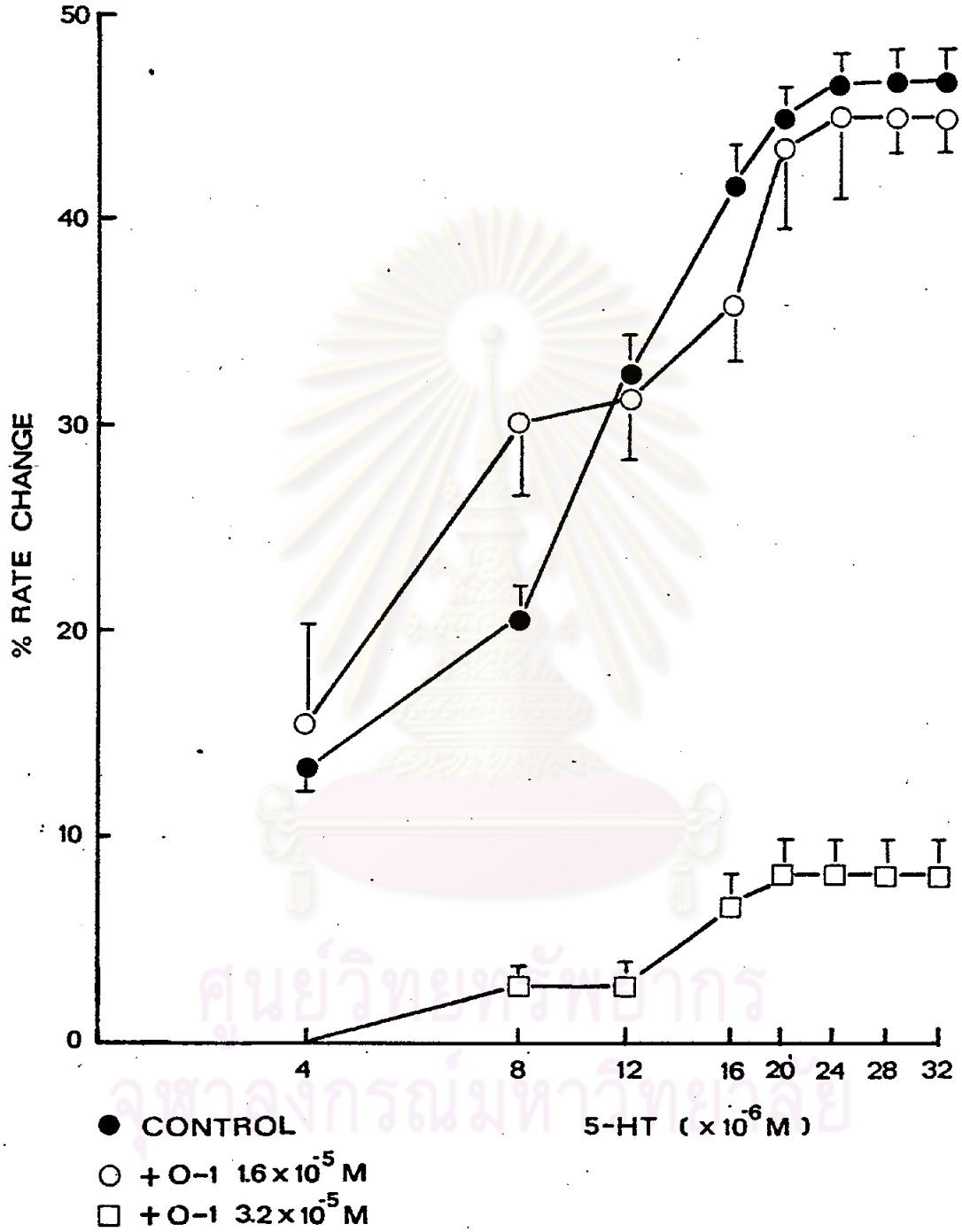

Figure 26. Log concentration-response curves for the inhibition of positive chronotropic effect of 5-hydroxytryptamine (5-HT) by O-1 in the rat right atrial strips. Each point is the mean of at least 4 measurements. None of the points on the control and 1.6×10^{-5} M O-1 curves is significantly different whereas all points in 3.2×10^{-5} M O-1 curves are significantly different ($P < 0.001$; Student's test).

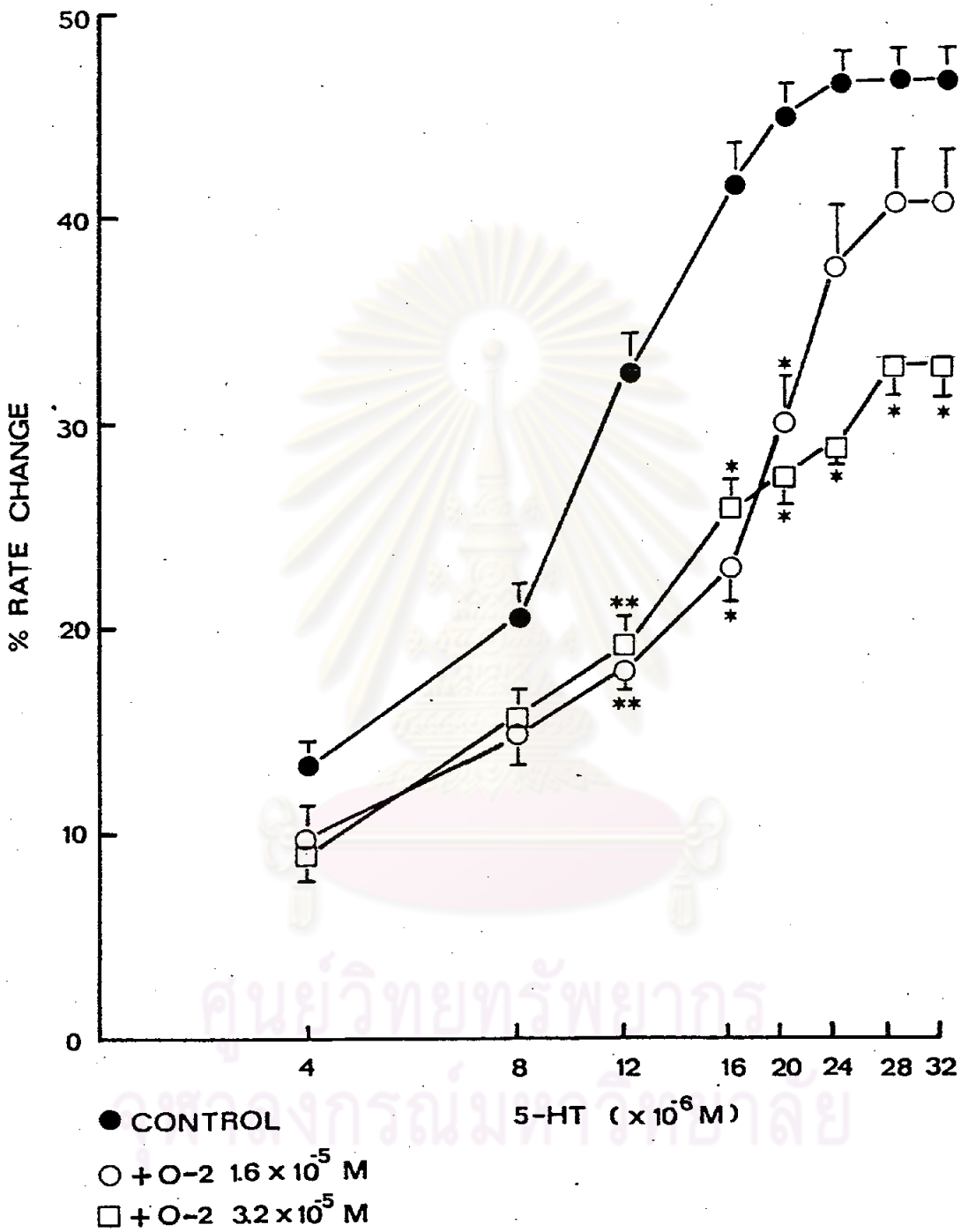

Figure 27. Log concentration-response curves for the inhibition of positive chronotropic effect of (5-HT) by O-2 in the rat right atrial strips. Each point is the mean of at least 4 measurements. None of the points on the control and 1.6×10^{-5} M O-2 curves except the third, fourth and fifth points is significantly different. All points except the two lowest points in 3.2×10^{-5} M O-2 curves are significantly different. * $P < 0.001$ ** $P < 0.025$

AGONIST	ANTAGONIST	pD_2 VALUE
5-HT	1.2×10^{-5} M I-1	4.94 ± 0.04 (n = 5)
	1.6×10^{-5} M I-1	4.95 ± 0.07 (n = 6)
5-HT	1.2×10^{-5} M I-2	4.87 ± 0.1 (n = 4)
	1.6×10^{-5} M I-2	4.81 ± 0.1 (n = 4)
5-HT	1.6×10^{-5} M O-1*	-
	3.2×10^{-5} M O-1	5.27 ± 0.12 (n = 4)
5-HT	1.6×10^{-5} M O-2*	-
	3.2×10^{-5} M O-2	4.1 ± 0.07 (n = 4)

Table 4. pD_2 values of indole alkaloids against 5-hydroxytryptamine (5-HT) on rat isolated right atria. The number of observations (n) is given in parenthesis. The Table shows mean pD_2 values and standard errors of means.

* This concentration cannot block effect of 5-HT.