CHAPTER IV

RESULTS

Acute Effects of DOX Treatment

Acute Effect of DOX on Rate and Force of Atria.

The effects of DOX on heart rate and developed tension were examined in right and left atrial muscle preparations stimulated at 250 bpm, respectively. Although 100 μM DOX decreased heart rate which was spontaneous contraction gradually and continuously over 3 hours of experiment, heart rate of control group was steady and increased slightly after 120 minutes. The significant difference between DOX and control groups was observed since 10 minutes after the drug was added in the organ bath. At the end of experiment, heart rate of DOX and control groups were -27.65±1.89% and 4.44±2.06%, respectively (Fig. 3A).

Developed tension of right atrial muscle preprarations of both DOX and control groups were decreased gradually with time. The decrease in right atrial force of control was greater than DOX, but there were not different between both groups (Fig. 3B). There was a reciprocal relationship between the right atrial force and heart rate in control group.

Ten minutes after 100 μM DOX was added to the incubation buffer, inhibition of developed tension of left atrial muscle preparations occurred. The decrease in left atrial force progressed through the end of experiment, and the percentage of change was -60.47±4.33 at 180 minutes (Fig. 3C). Left atrial force observed in the control preparations also decreased with time and were -34.50±6.84% at the end of experiment; however, developed tension in the presence of DOX declined more rapidly

and showed significant difference from corresponding control values 20 minutes after the drug addition (Fig. 3C).

Acute Effect of DOX on Post-rest Contraction

During the 3-hour observation period, percentage of change in PRC which was observed after a 30-second quiescent period decreased in DOX group after 120 minutes, but that of control group increased since 60 minutes. There was statistically significant difference starting 40 minutes after the drug addition between both groups (Fig. 4 - 5).

Acute Effect of DOX on Response to Isoproterenol

The effects of DOX on the positive chronotropic and inotropic response to isoproterenol after 30-minute period incubation with DOX or NSS were illustrated in Fig. 6 - 8. In control group, addition of isoproterenol in cumulative doses from 10^{-9} - 10^{-5} M increased all of heart rate, right atrial force and left atrial force. There was an observation that right and left atrial force decreased at the higher doses of isoproterenol because of post-receptor downregulation. Meanwhile, with 100 μM DOX, all of the positive effects and maximal responses (Pmax) of isoproterenol were depressed. The difference of heart rate betweeen DOX and control group was found significantly at lower dose (10^{-9} - 10^{-7} M) and that of left atrial force was found significantly at higher dose (10^{-7} - 10^{-5} M). Post-receptor downregulation also occurred at high dose of isoproterenol. Further observation about the inhibitory effect of propranolol, which was competitive beta-adrenergic receptor antagonist, was performed with the cumulative concentration response curve of isoproterenol 5 minutes after adding 10^{-6} M propranolol in the chamber. The results were shown in Fig. 9, ABC. Positive chronotropic and inotropic responses of isoproterenol at cumulative doses of 10^{-9} - 10^{-7} M were completely blocked with propranolol. In this study, Pmax of heart rate and left atrial force did not reach with maximum dose of isoproterenol. However, DOX caused a decrease in positive responses to isoproterenol, but did not show a blockade of these responses as propranolol.

Acute Effect of DOX on Response to Acetylcholine

The cumulative dose response curve for acetylcholine was generated after 30-minute incubation in the presence of 100 μM DOX or NSS. All of cumulative doses of acetylcholine (10^{-7} – 10^{-3} M) decreased heart rate, right atrial force and left atrial force in buffer which was incubated with NSS. DOX reduced the response of acetylcholine and caused a right-ward shift of the cumulative dose response curves (Fig. 10 - 12). The significant differences between DOX and NSS were shown at lower concentration of acetylcholine. Additionally, DOX did not modify the maximal negative inotropic and chronotropic effects at high concentrations of acetylcholine.

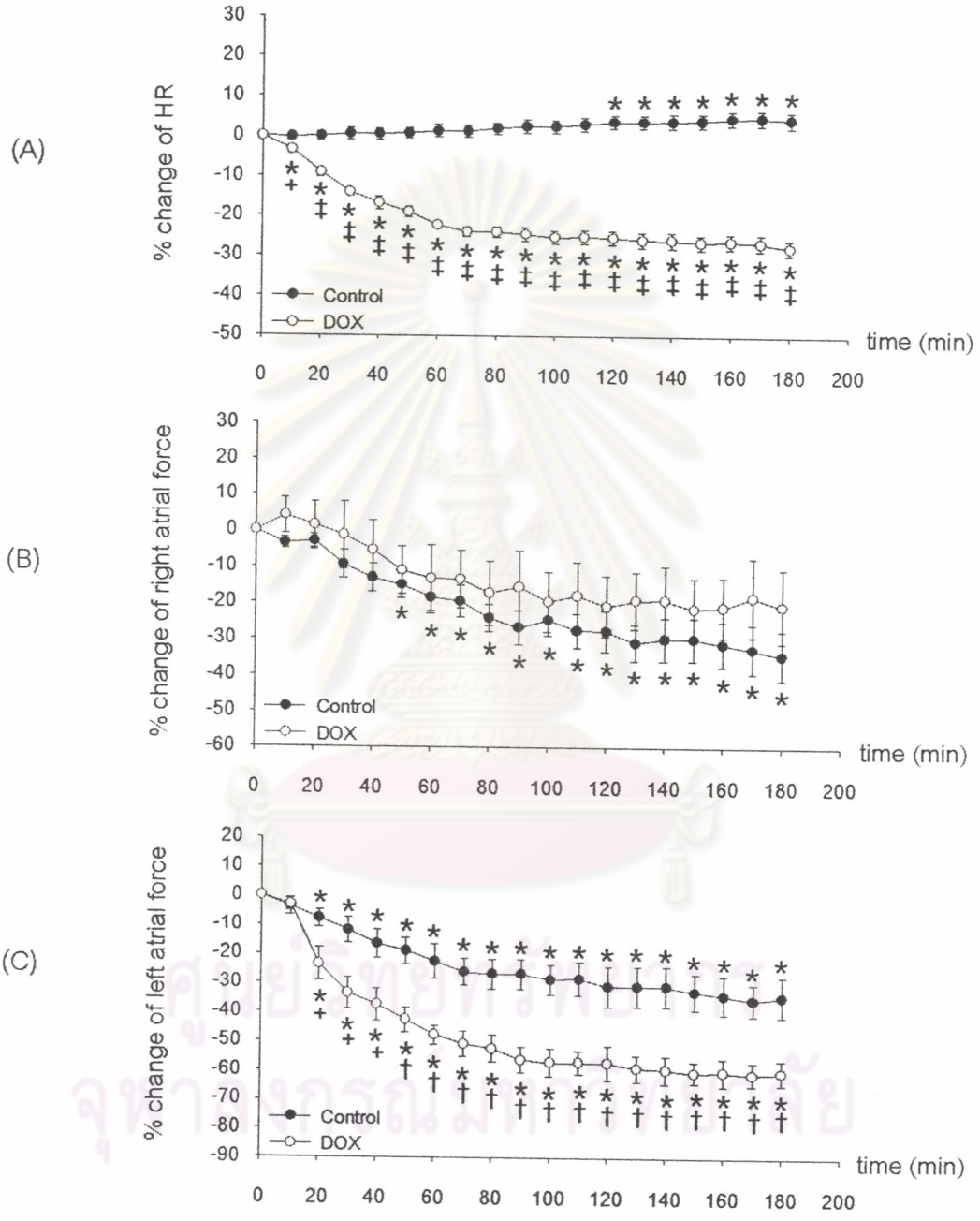

Fig. 3. Acute effects of DOX on heart rate, right atrial force and left atrial force. Each point represents the mean of 8 experiments. Vertical lines indicate the S.E. *, $p \leq 0.05$ versus 0 min; +, $p \leq 0.05$ versus control; †, $p \leq 0.01$; ‡, $p \leq 0.001$.

(A)

(B)

Fig. 4. Changes in post-rest contraction of the isolated left atrium from control (A) and acute DOX-treated (B) animals.

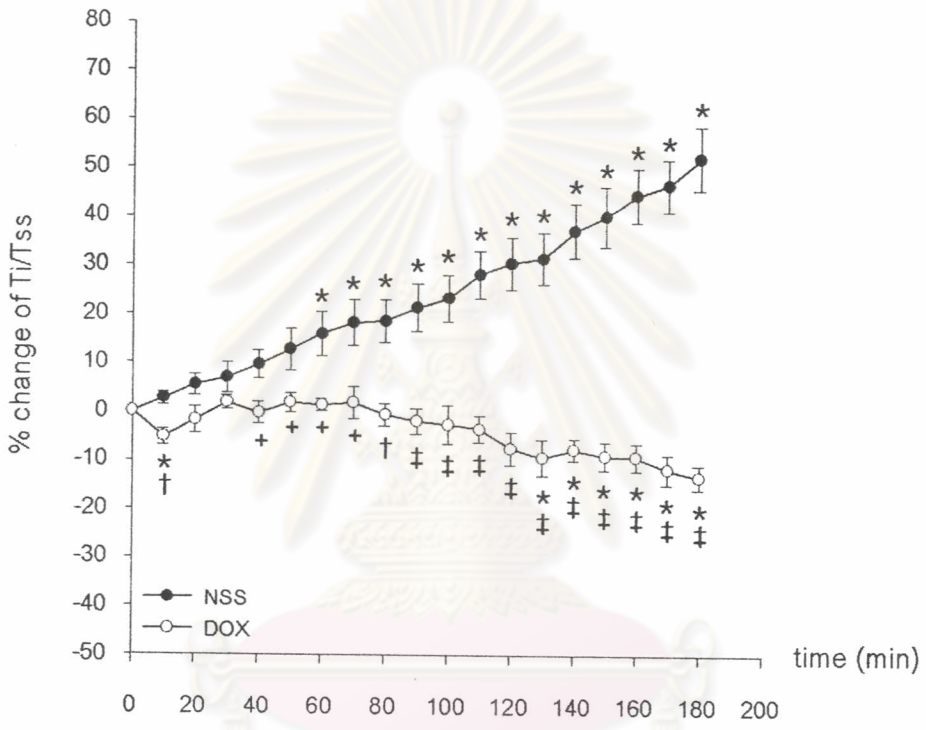

Fig. 5. Acute effect of DOX on post-rest contraction. Each point represents the mean of 8 experiments. Vertical lines indicate the S.E. *, $p \leq 0.05$ versus 0 min; +, $p \leq 0.05$ versus control; †, $p \leq 0.01$; ‡, $p \leq 0.001$. Ti and Tss indicate an initial tension after resting period and steady-state tension, respectively.

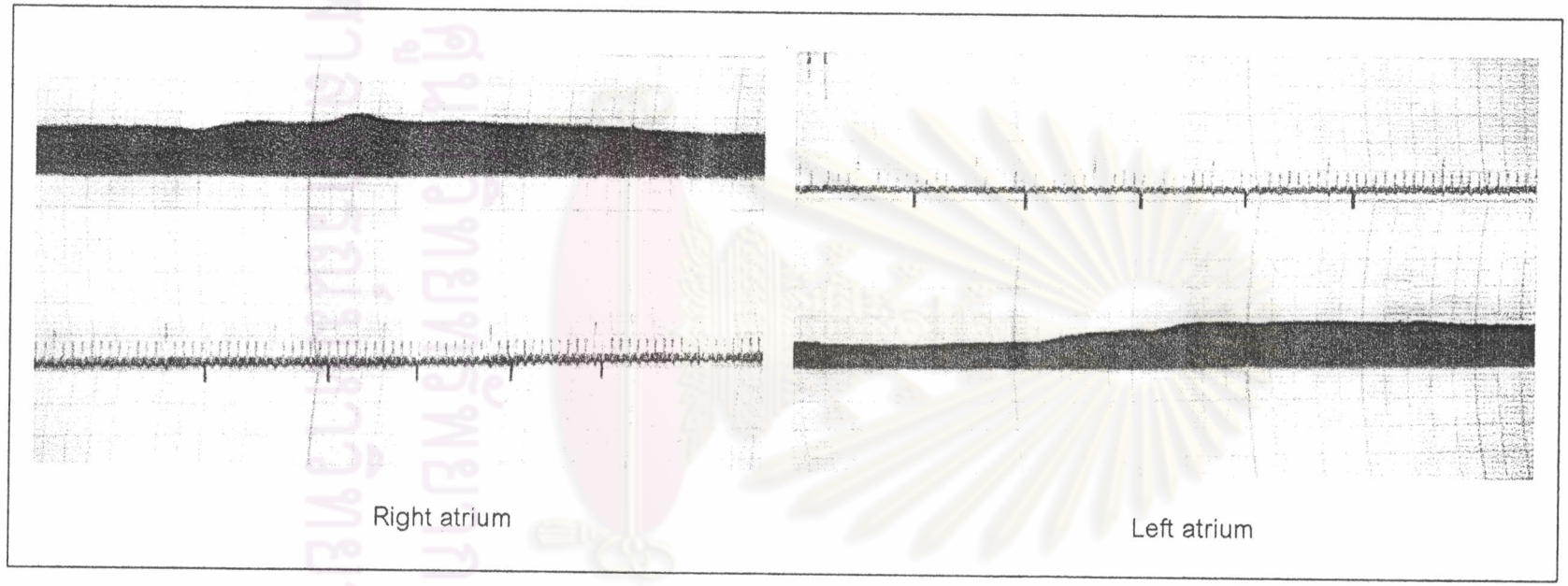

Fig. 6. Changes in cumulative dose-response of isoproterenol of right and left atria from acute control animal.

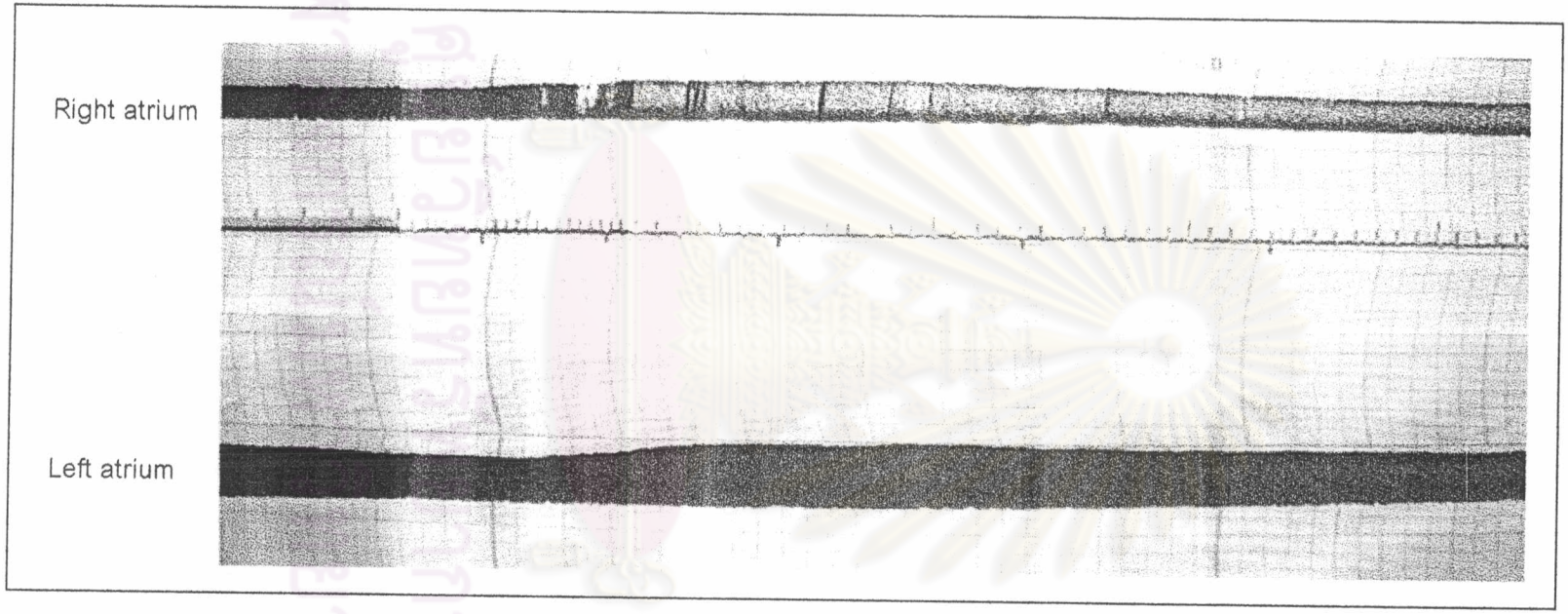

Fig. 7. Changes in cumulative dose-response of isoproterenol of right and left atria from acute DOX-treated animal.

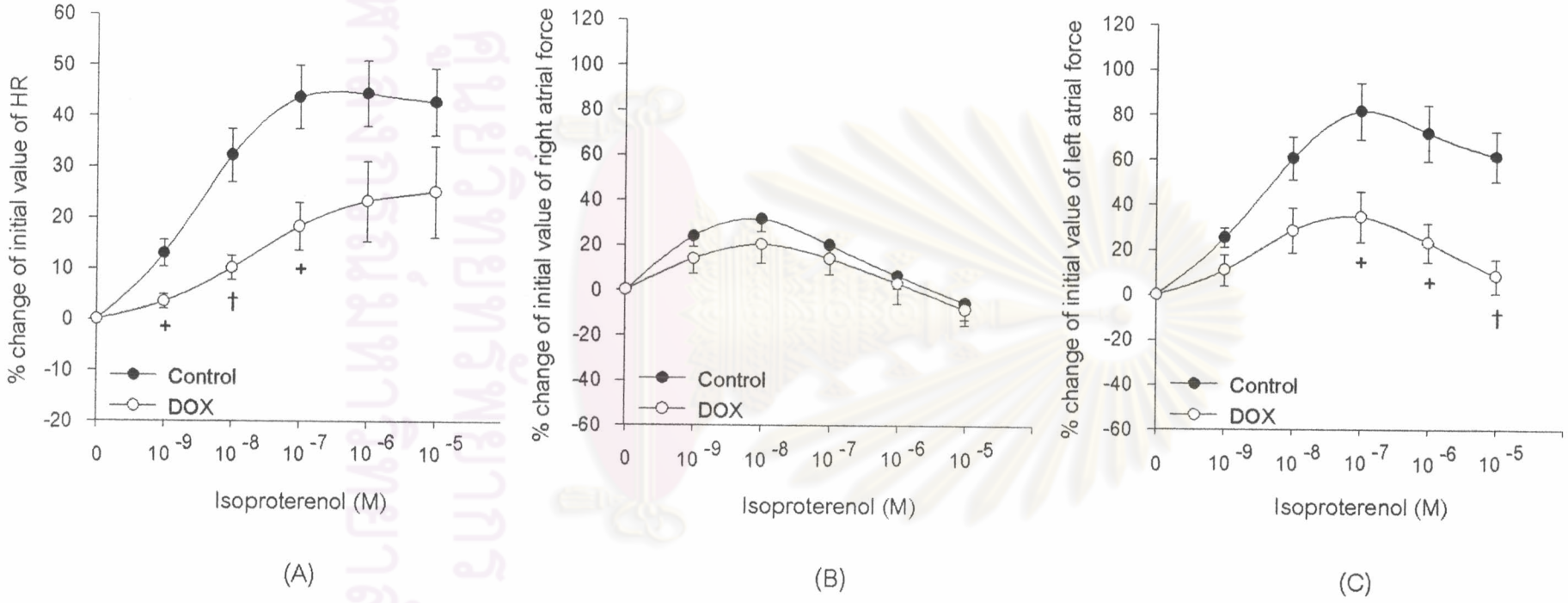

Fig. 8. Acute effects of DOX on positive chronotropic and inotropic effects of isoproterenol in isolated rat right and left atria, respectively. Each point represents the mean of 8 experiments. Vertical lines indicate the S.E. *, $p \leq 0.05$ versus 0 min; +, $p \leq 0.05$ versus control; †, $p \leq 0.01$.

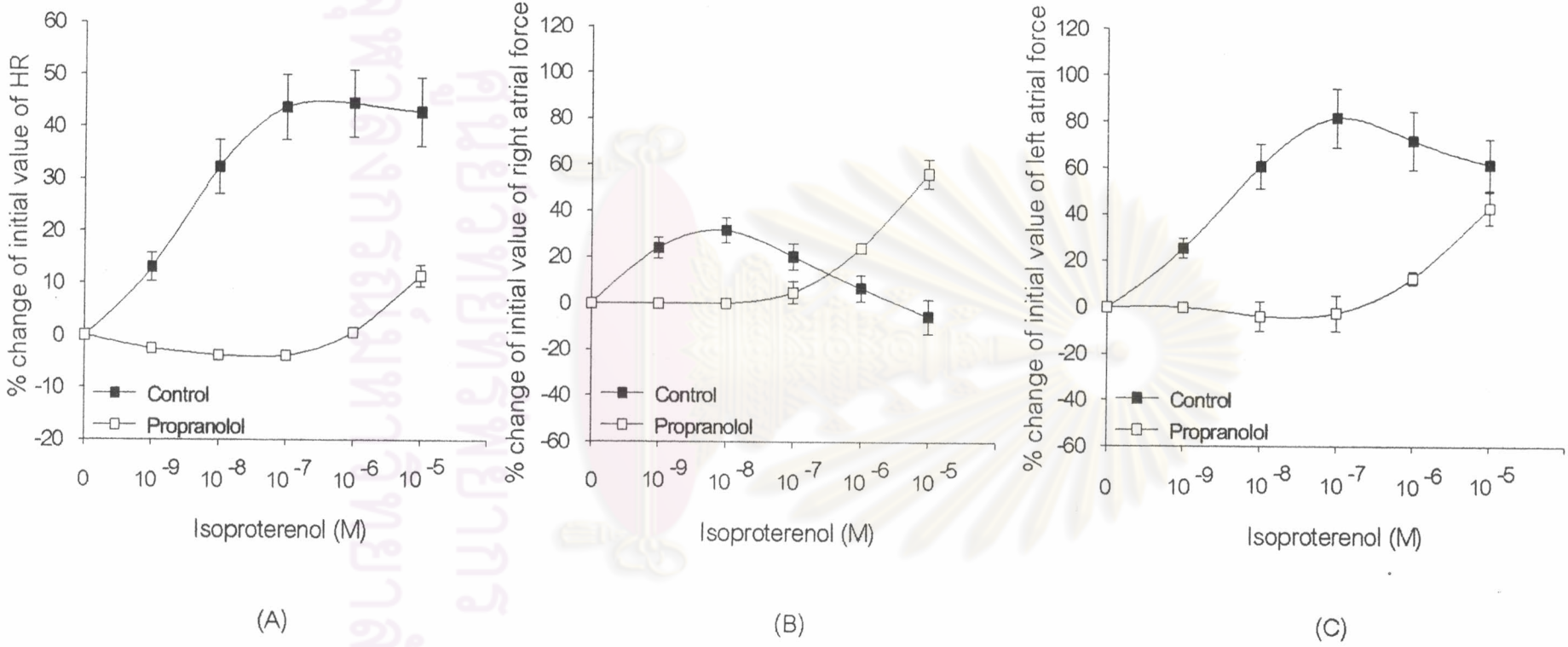

Fig. 9. Effects of propranolol on positive chronotropic and inotropic effects of isoproterenol in isolated rat right and left atria, respectively. Each point of propranolol group represents the mean of 2 experiments. Vertical lines indicate the S.E.

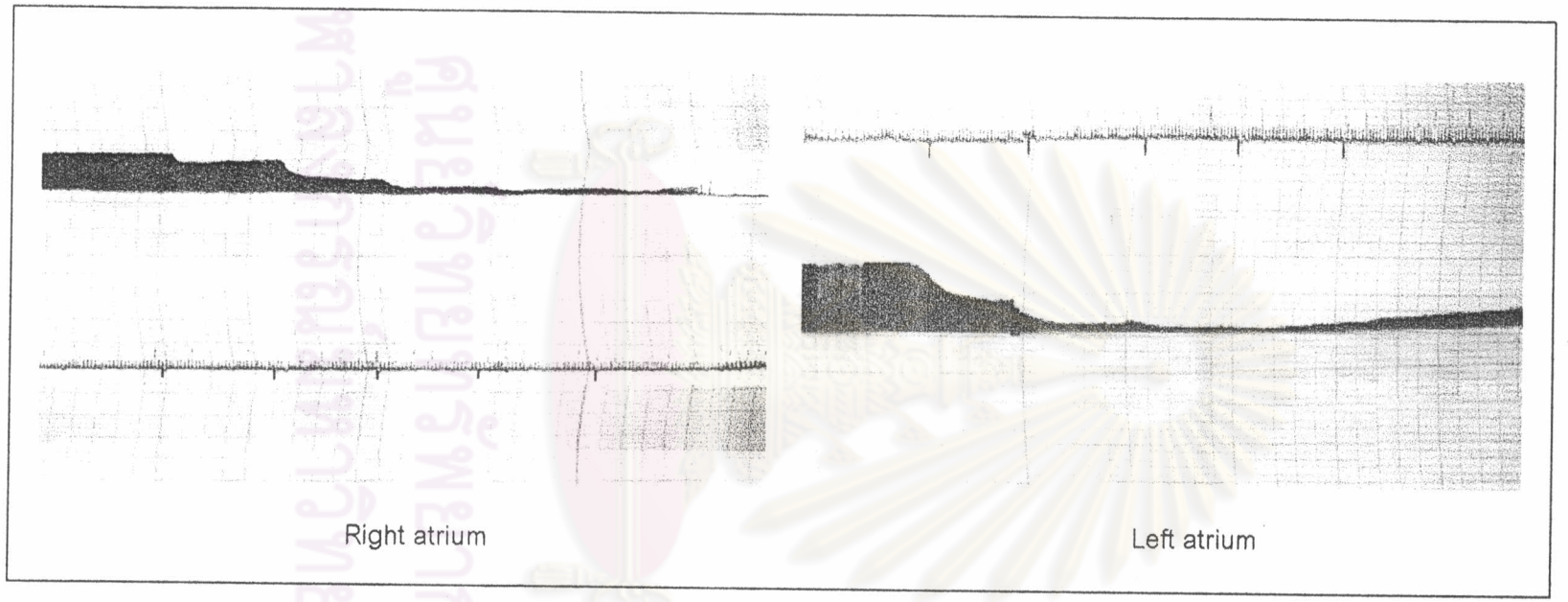

Fig. 10. Changes in cumulative dose-response of acetylcholine of right and left atria from acute control animal.

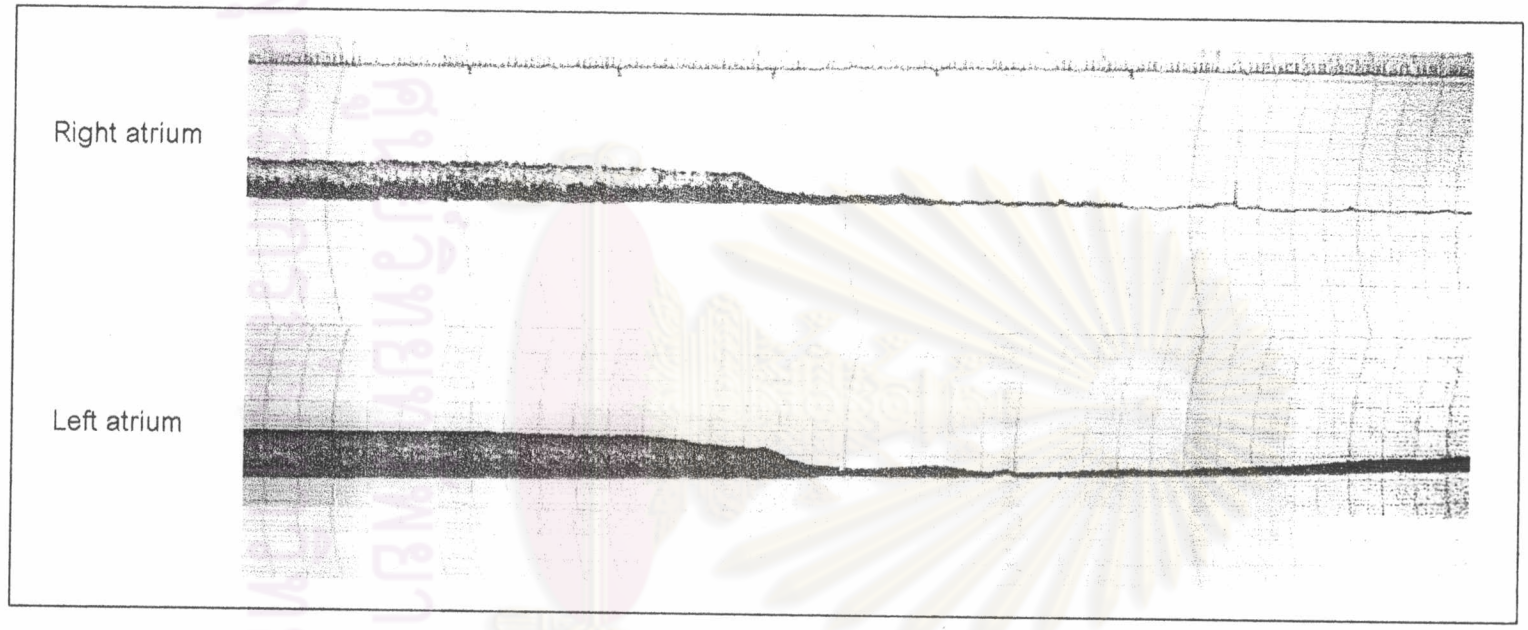

Fig. 11. Changes in cumulative dose-response of acetylcholine of right and left atria from acute DOX-treated animal.

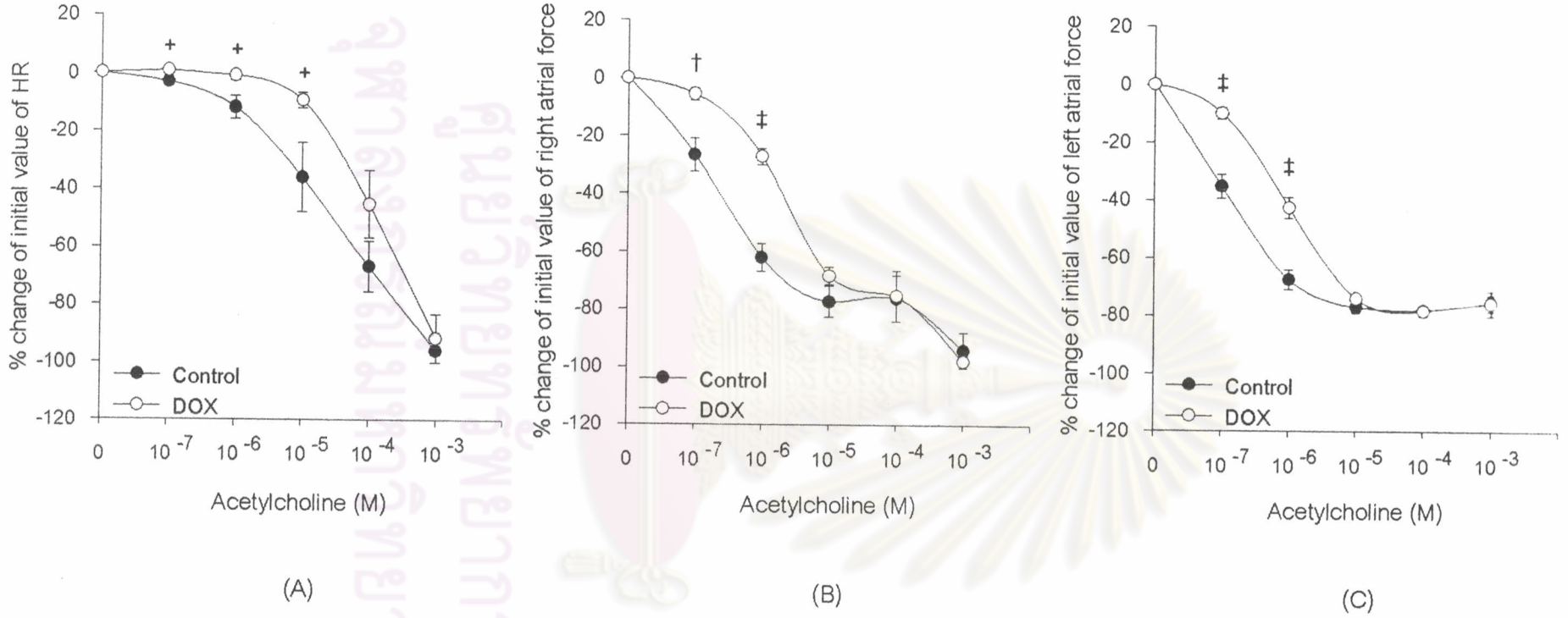

Fig. 12. Acute effects of DOX on negative chronotropic and inotropic effects of acetylcholine in isolated rat right and left atria, respectively. Each point represents the mean of 8 experiments. Vertical lines indicate the S.E. *, $p \leq 0.05$ versus 0 min; +, $p \leq 0.05$ versus control; †, $p \leq 0.01$; ‡, $p \leq 0.001$.

Subacute Effects of DOX treatment

Subacute Effect of DOX on PRC

Clinical cardiotoxicity of DOX developed only after a subacute or chronic exposure to the drug. To examine the delayed DOX cardiotoxicity, subacute treatment of rats with DOX was performed. After 2 injections with total doses 5 mg/kg of DOX in rats, which lasted 10 days, atrial muscle preparations isolated from the drug-treated animals were used for study the contractile performance. Beginning with the experiment of PRC which was observed after a 30-second quiescent period, percentage of changes in PRC increased slightly in DOX group throughout the experiment, while that of control group increased obviously through the end of the experiment. There was statistically significant difference 70 minutes after the drug addition between both groups (Fig. 13 - 14). Because Ca^{2+} accumulated in the SR is a time-dependent manner during the resting period, thereby augmenting the first contraction evoked after the rest period was represented by PRC.

Subacute Effects of DOX on Response to Isoproterenol and Acetylcholine

Determining the subacute effects of DOX on adrenergic and cholinergic function found that changes in positive response of isoproterenol and negative response of acetylcholine in atrial muscle preparations of rats treated with DOX did not differ from those of control group (Fig. 15 - 20).

(A)

(B)

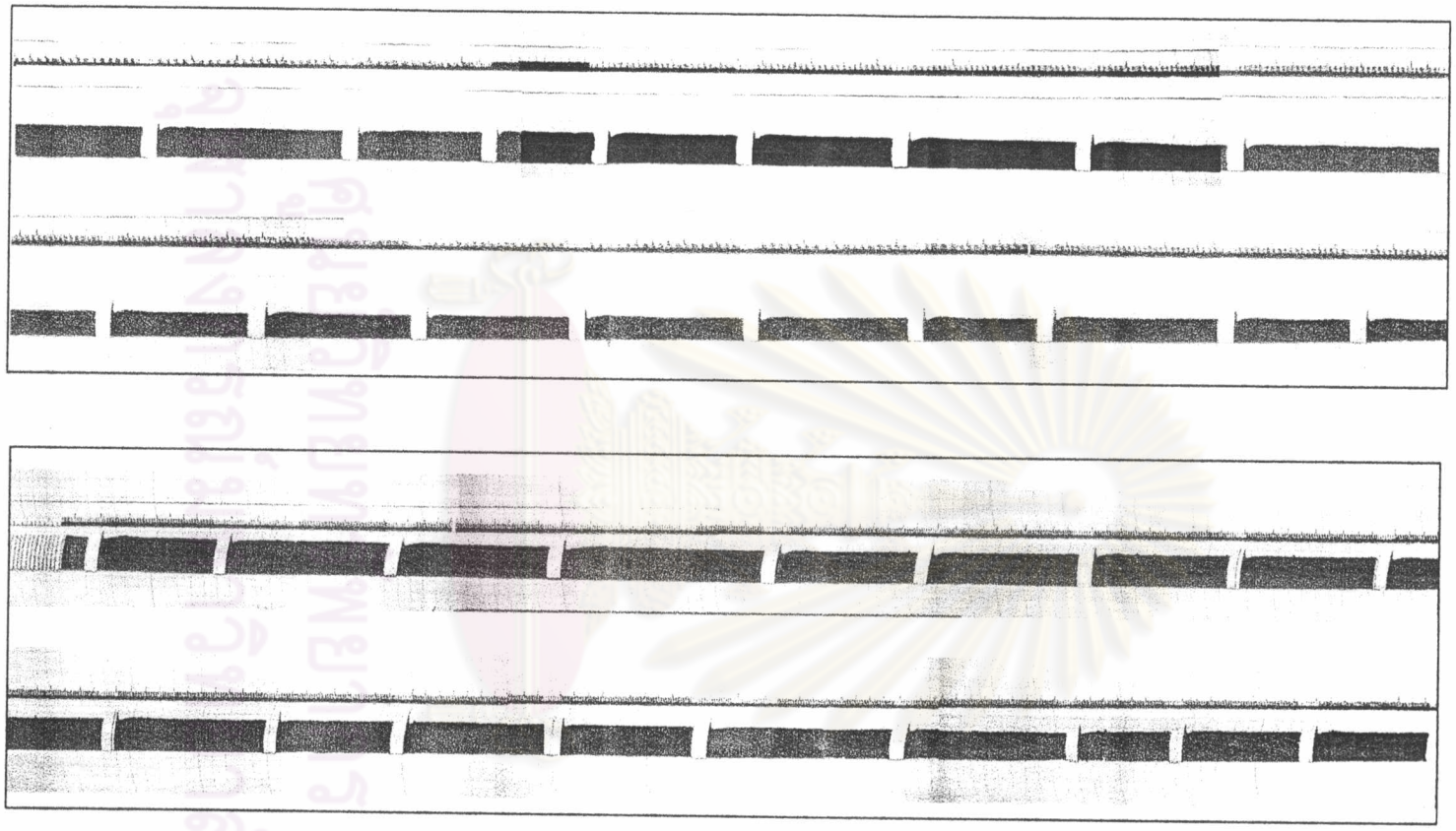

Fig. 13. Changes in post-rest contraction of the isolated left atrium from control (A) and subacute DOX-treated (B) animals

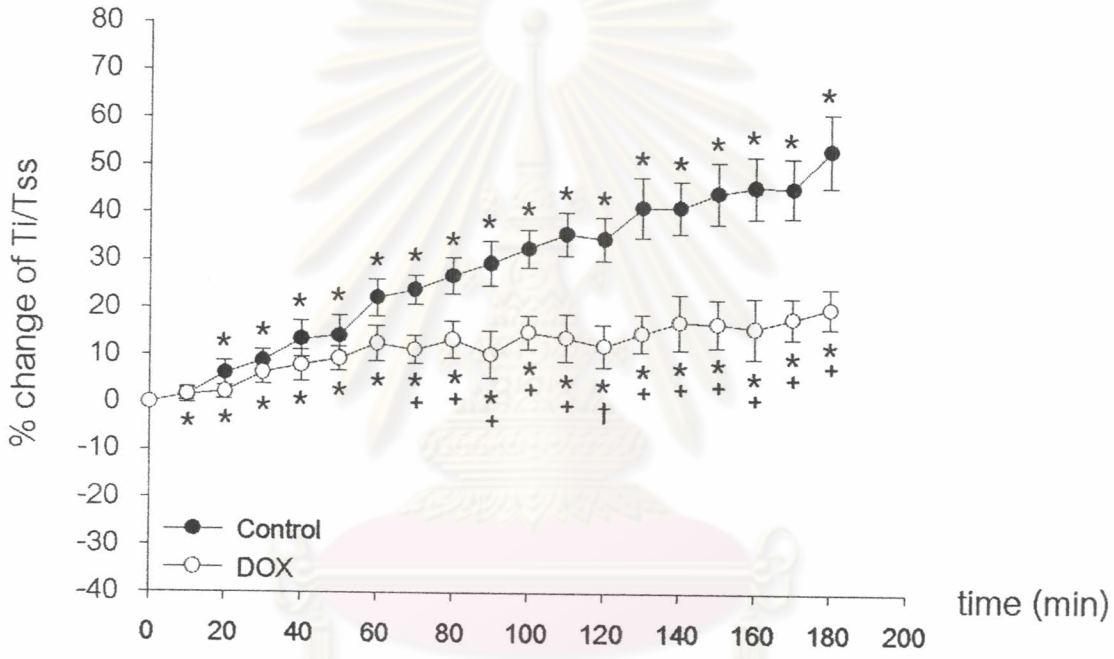

Fig. 14. Subacute effect of DOX on post rest contraction. Each point represents the mean of 8 experiments. Vertical lines indicate the S.E. *, $p \leq 0.05$ versus 0 min; +, $p \leq 0.05$ versus control; †, $p \leq 0.01$.

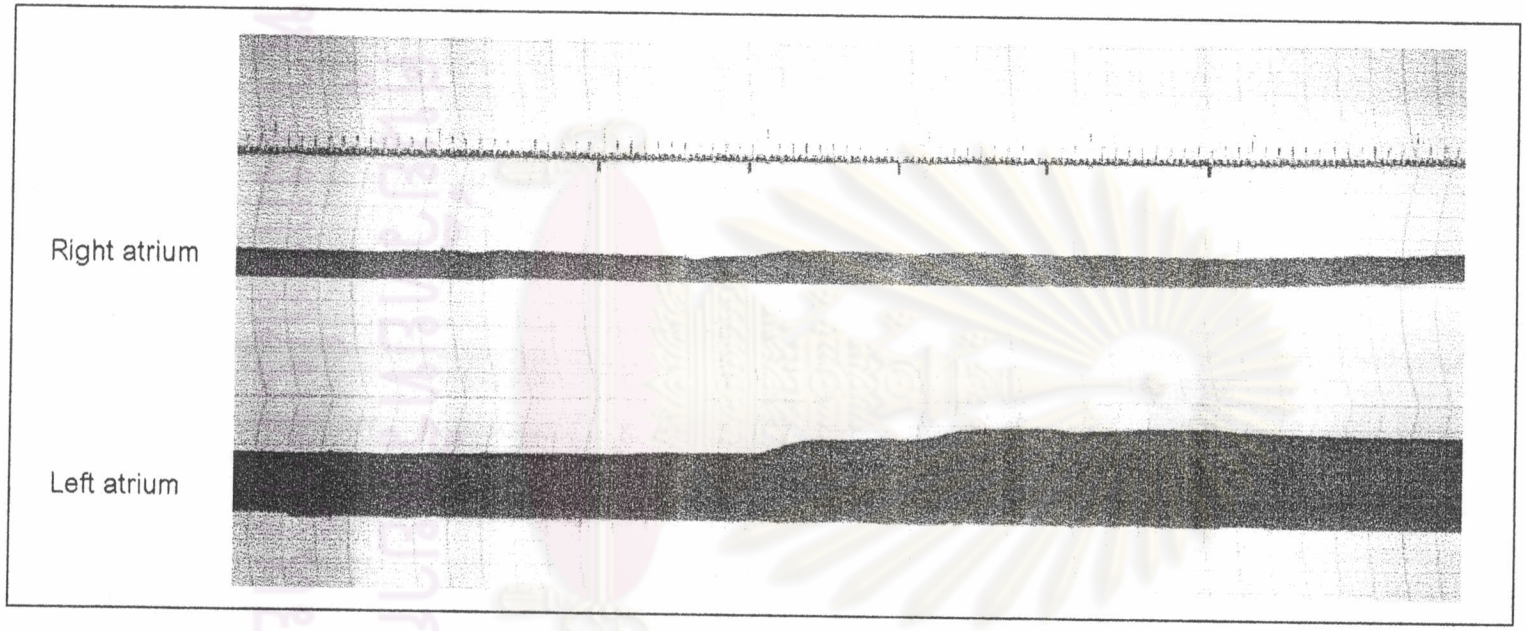

Fig. 15. Changes in cumulative dose-response of isoproterenol of right and left atria from subacute control animal.

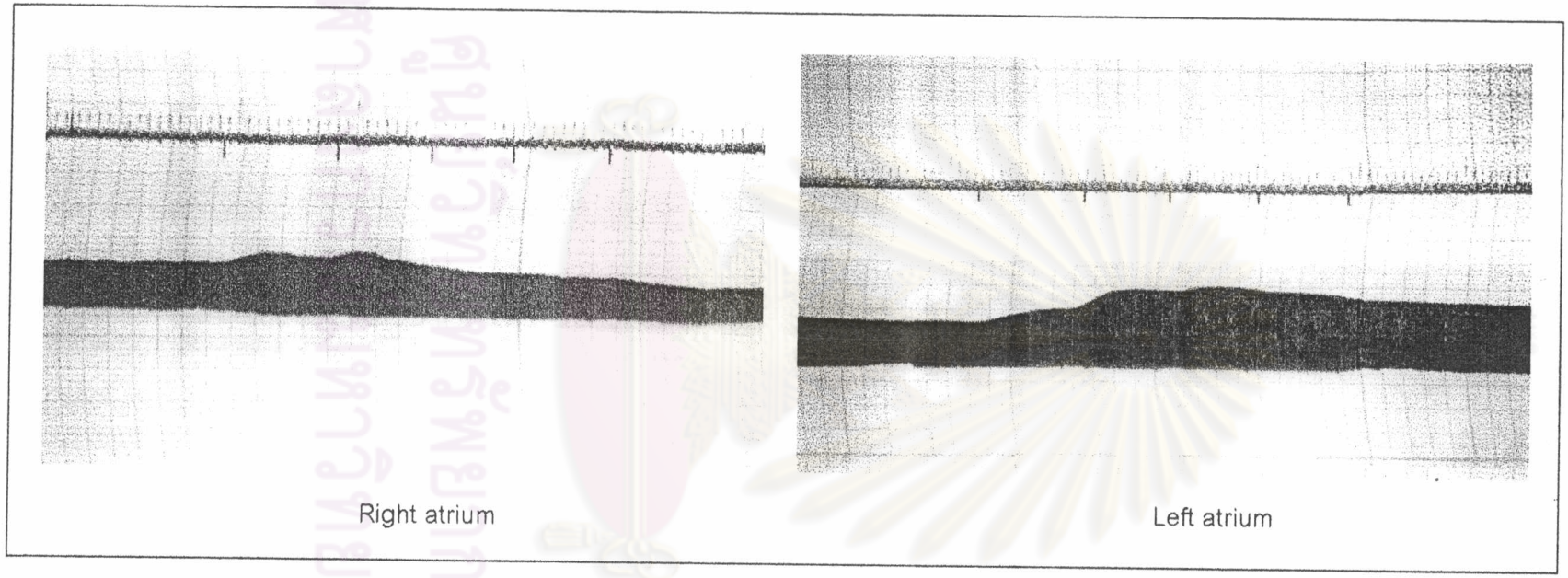

Fig. 16. Changes in cumulative dose-response of isoproterenol of right and left atria from subacute DOX-treated animal.

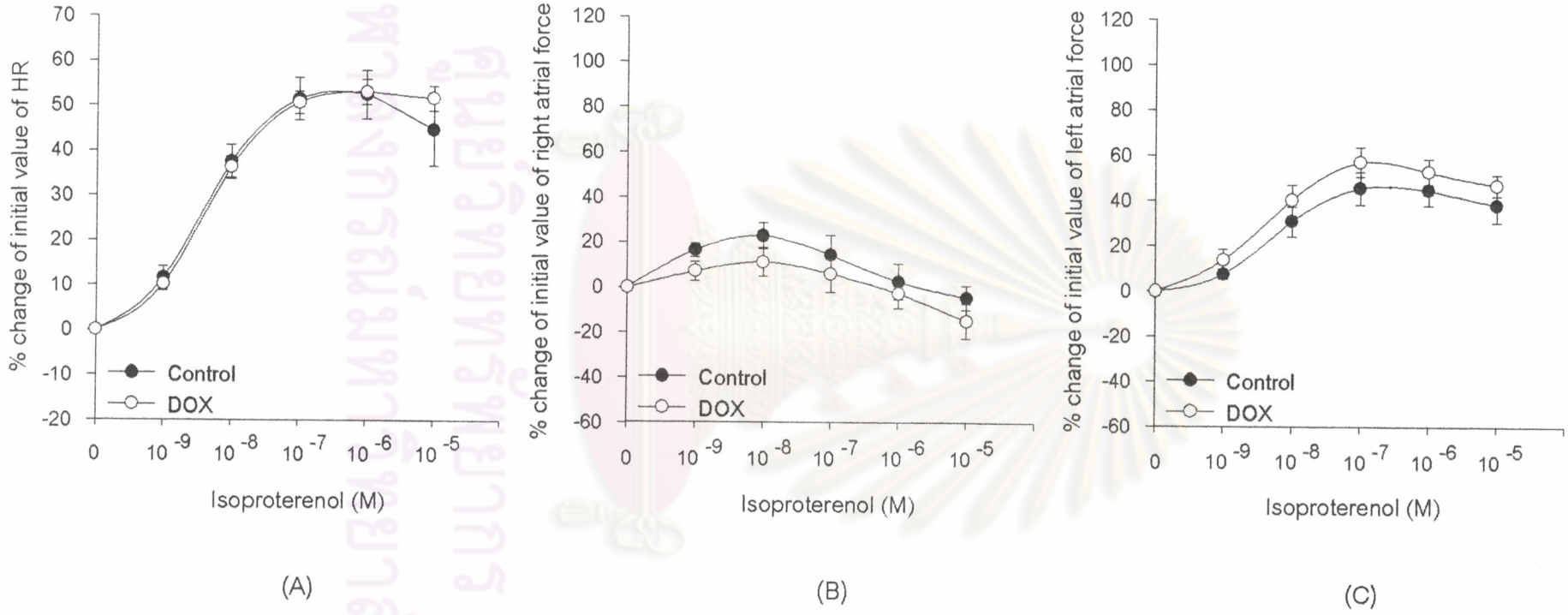

Fig. 17. Subacute effect of DOX on positive chronotropic and inotropic effect of isoproterenol in isolated rat right and left atria. Each point represents the mean of 8 experiments. Vertical lines indicate the S.E.

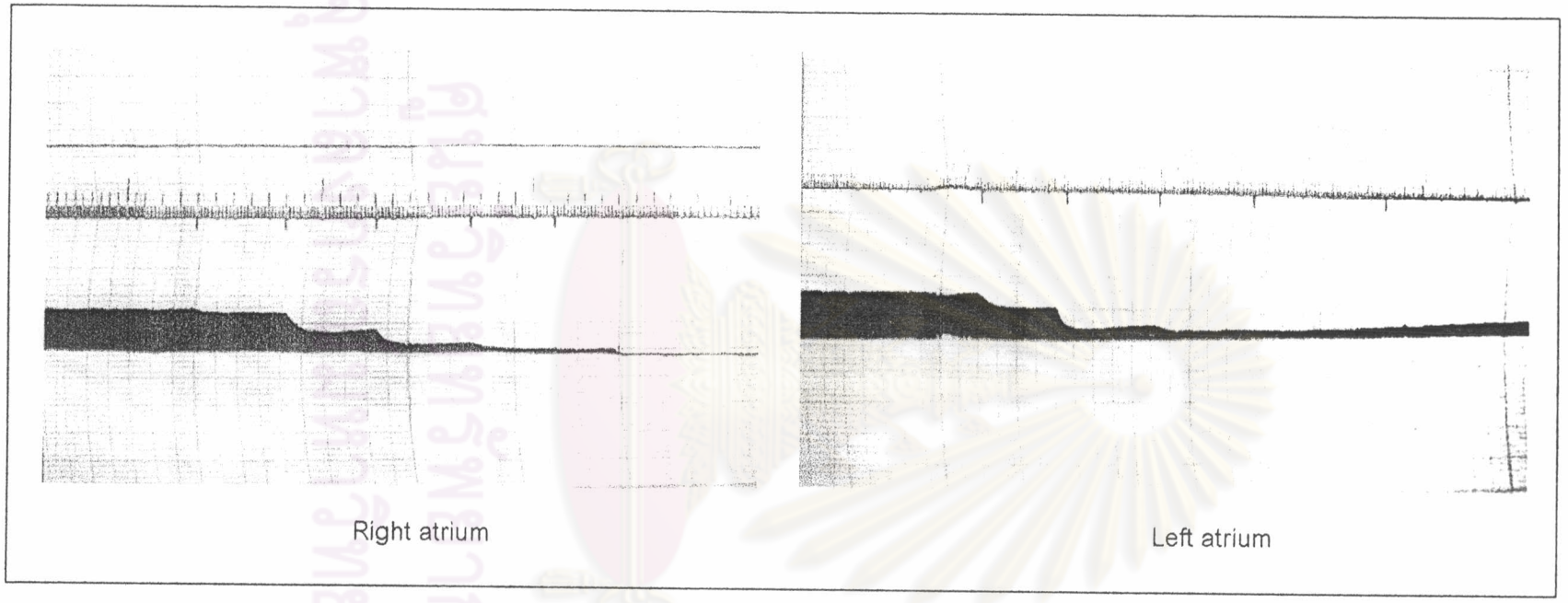

Fig. 18. Changes in cumulative dose-response of acetylcholine of right and left atria from subacute control animal.

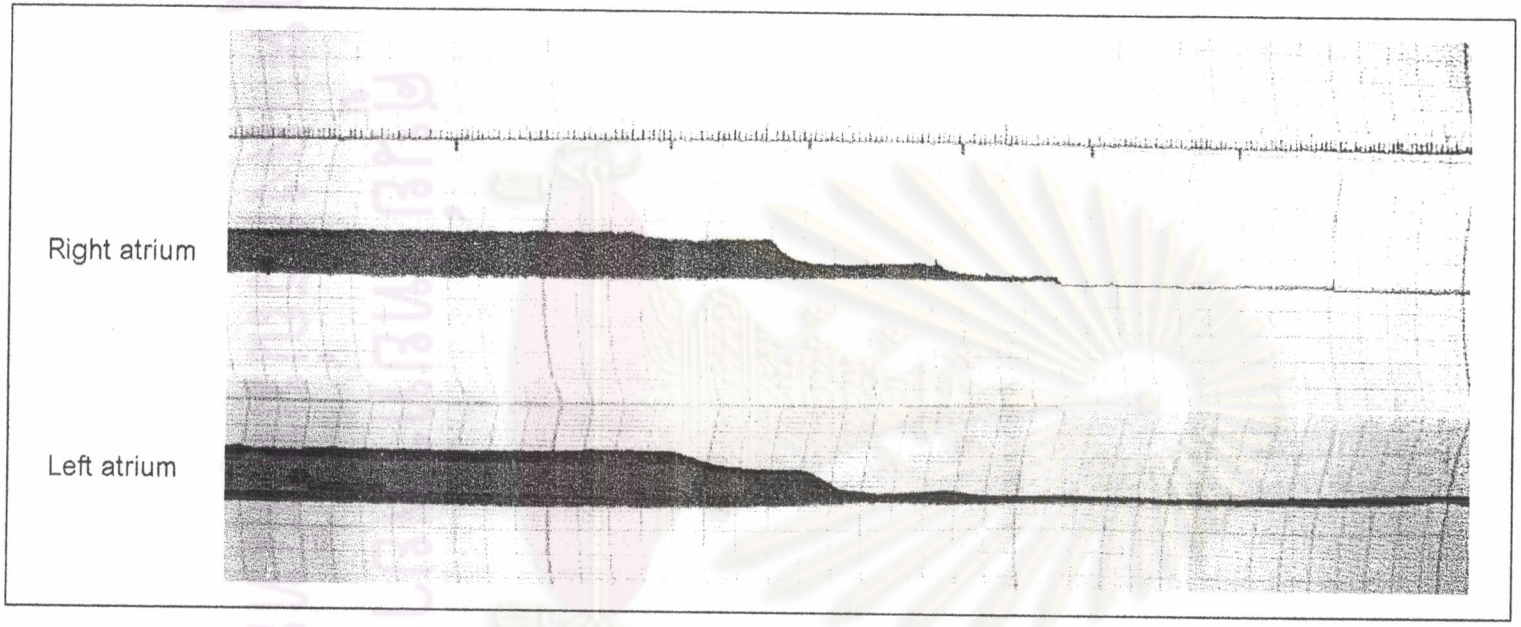

Fig. 19. Changes in cumulative dose-response of acetylcholine of right and left atria from subacute DOX-treated animal.

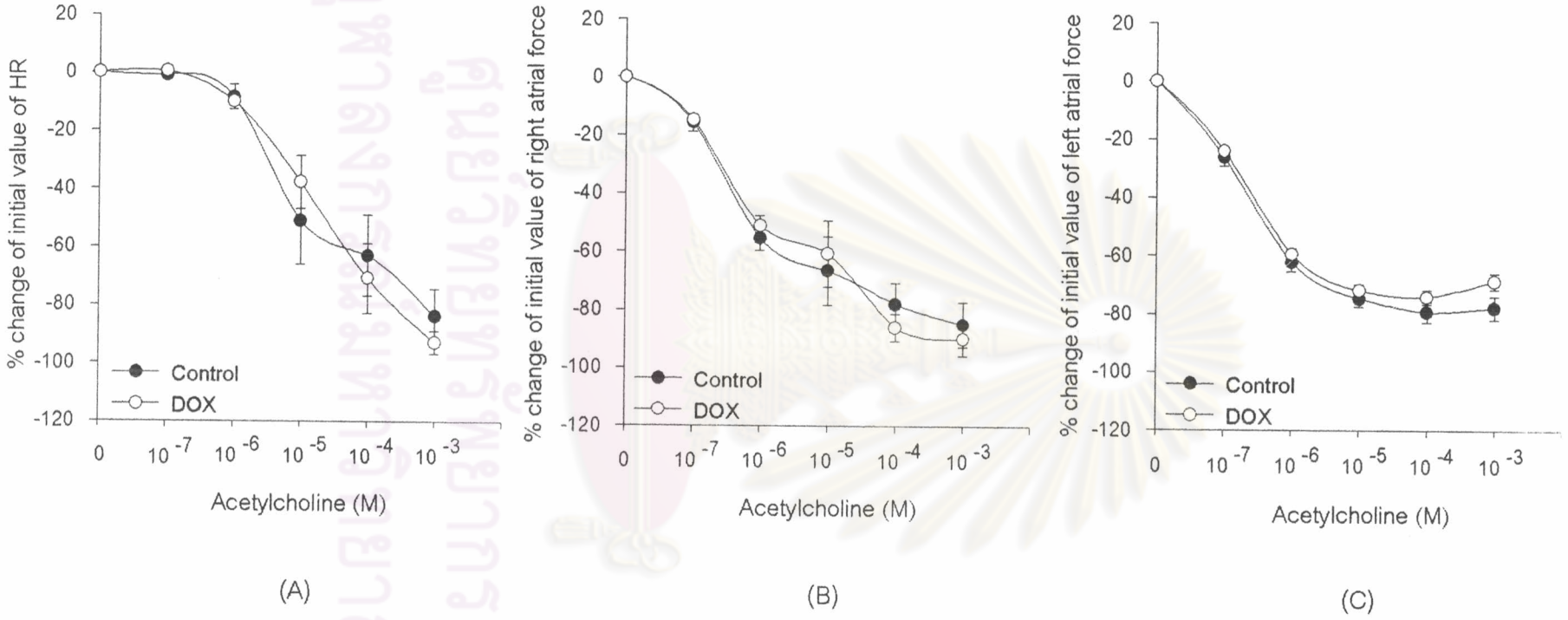

Fig. 20. Subacute effect of DOX on negative chronotropic and inotropic effects of acetylcholine in isolated rat right and left atria. Each point represents the mean of 8 experiments. Vertical lines indicate the S.E.

Subacute Effect of DOX on Cardiac Enzyme Activity

The ventricular muscles of rats treated with total dose 5 mg/kg of DOX were obtained to determine the myosin-ATPase and actomyosin-ATPase activities. The myosin-ATPase activity was 0.255±0.009 and 0.264±0.009 mM P/mg protein in DOX and control group, respectively. The activity of actomyosin ATPase was divided into presence or absence of Ca^{2+}. In this experiment, there was no difference between the activities of actomyosin ATPase in the presence and absence of Ca^{2+}. The actomyosin ATPase activity in the presence of Ca^{2+} was 0.193±0.007 and 0.181±0.011 mM P/mg protein in DOX and control group, respectively and the actomyosin ATPase in the absence of Ca^{2+} was 0.187±0.006 and 0.191±0.013 mM P/mg protein in DOX and control group, respectively. There were no differences in all of these ATPase activities between DOX and control groups (Fig. 21).

CK activity was evaluated using ventricular muscles of rat treated with DOX or NSS. CK activities of DOX and control groups were presented in Table 1. Rats treated with total dose 5 mg/kg of DOX showed the less CK activity than control group, significantly.

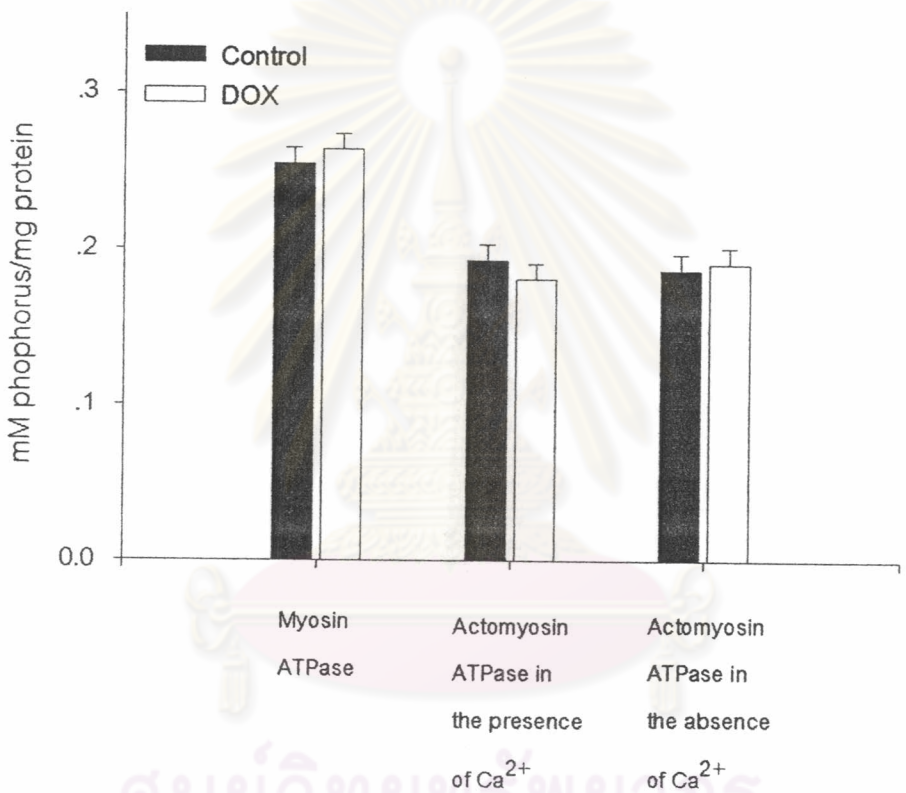

Fig. 21. Subacute effect of DOX on myosin-ATPase and actomyosin-ATPase (presence or absence of Ca^{2+}) activities. Each bar represents the mean of 24 experiments. Vertical lines indicate the S.E.

Table 1. Subacute effect of DOX on CK activity.

Treatment group	CK activity ($x10^{-4}$ U/L)
Control (n=24)	5.92 ± 0.17
DOX (n=24)	5.33 ± 0.21 *

* significant difference from control group, $p \leq 0.05$