CHAPTER III

RESULTS

The effect of capsaicin on right atrial rate and left atrial isometric tension is recorded in Fig. 1. Three doses of capsaicin, 0.2, 2, and 10 µg/ml, were tested in these experiments. It is seen that capsaicin initially produced both positive chronotropic and inotropic effects. The positive chronotropic effect showed inverse relationship with doses. The increment in rate was largest with 0.2 µg/ml capsaicin; and the rate did not return to controls even 30 min following capsaicin addition although it declined after the peak effect had been reached. Capsaicin at 10 µg/ml produced a small initial increase in rate which was rapidly followed by depression. On the other hand, three doses of capsaicin showed similar initial positive inotropy followed by depression in which strongest reduction in contractile force was observed with 10 µg/ml capsaicin. The acute stimulatory action on the rate and isometric tension induced by capsaicin was not significantly modified by reserpine pretreatment or by prior exposure to propranolol (Fig. 2). Likewise, additions of propranolol and methysergide 5 min before capsaicin did not affect the capsaicin-evoked positive chronotropy and inotropy (Fig. 3). It is noteworthy that propranolol appeared to enhance the rate and force depression mediated by capsaicin; however, this was not significantly different from that induced by capsaicin alone (Fig. 2).

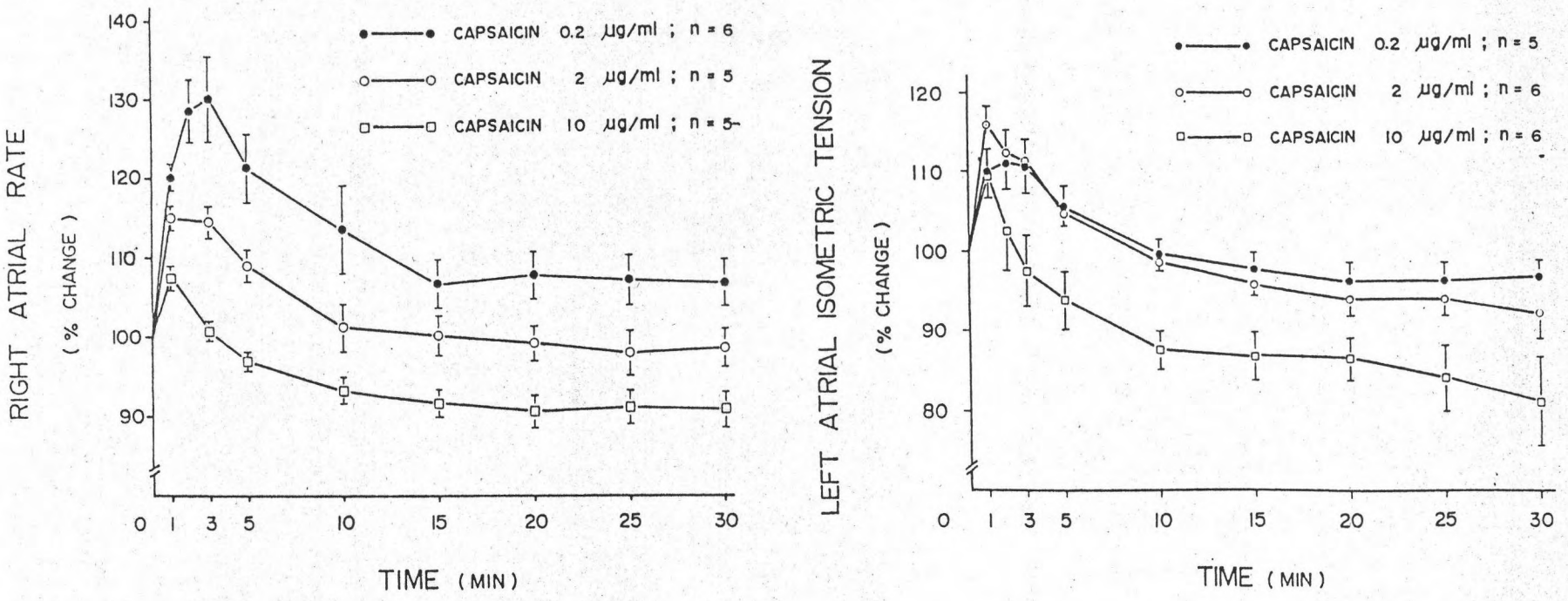

Figure 1 Effect of different doses of capsaicin on the rate and isometric force of isolated rat atria

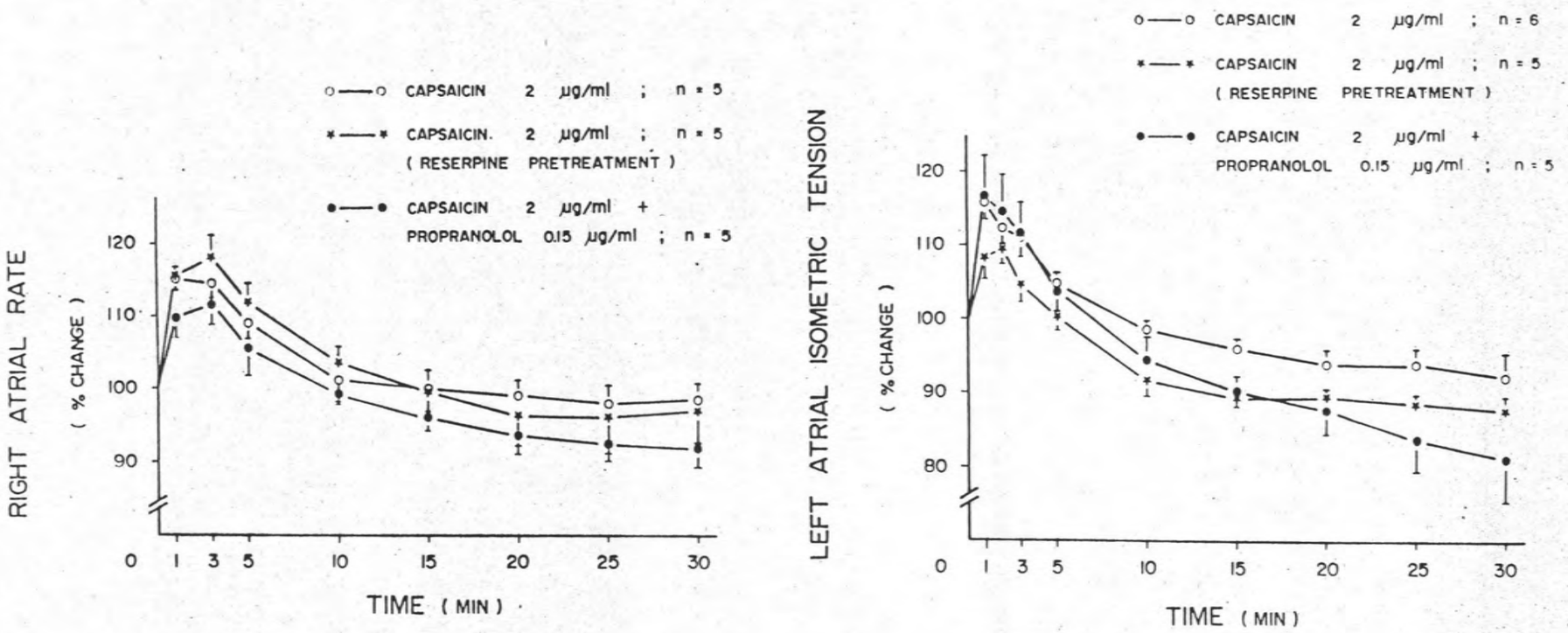

Figure 2 Effect of propranolol and reserpine pretreatment on the response of isolated rat atria to capsaicin.

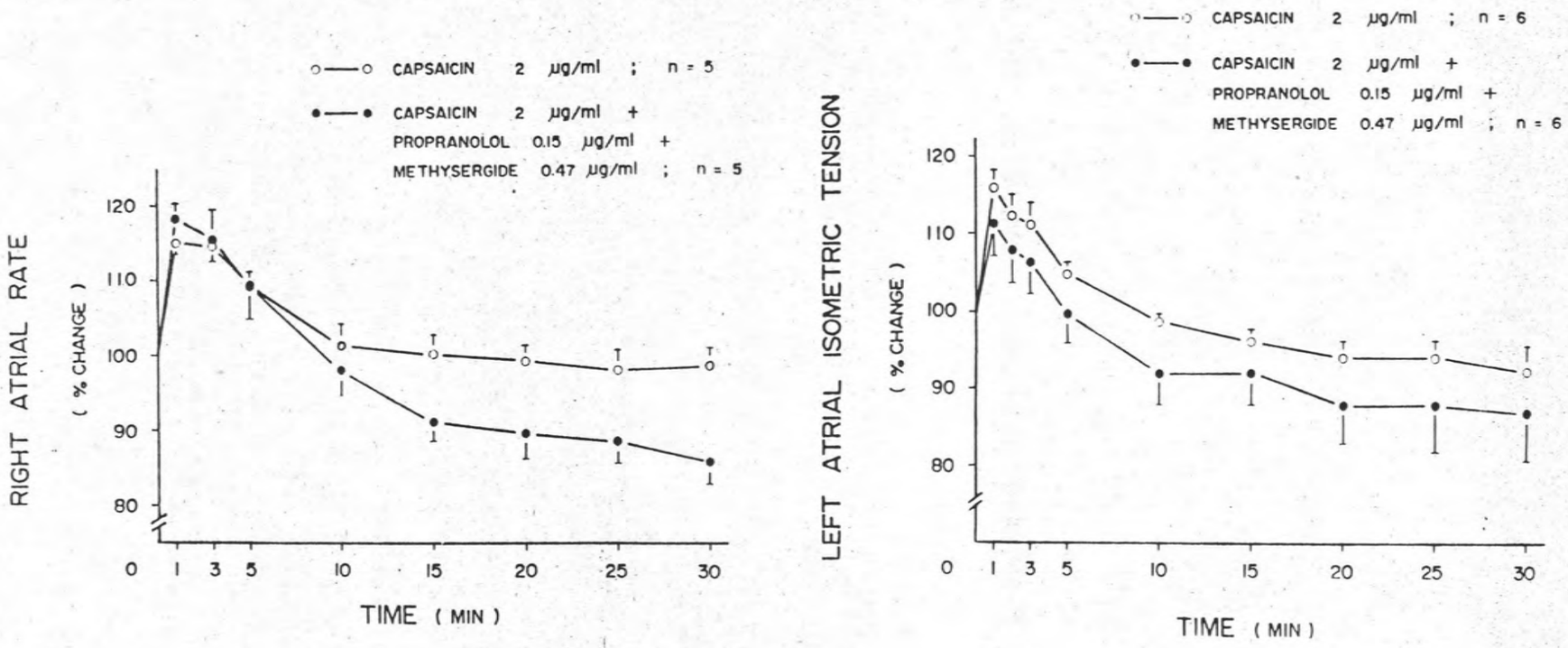

Figure 3 Effect of propranolol plus methysergide on the response of isolated rat atria to capsaicin.

Fig. 4 shows the effects of verapamil (0.05 µg/ml), capsaicin (0.2 µg/ml), and verapamil plus capsaicin on the rate and contractile force. Verapamil produced the well-known negative chronotropic and inotropic effects. The effect on the force was evidently more pronounced than on the rate. Thirty minutes after verapamil was added, the rate and isometric tension were reduced by approximately 25 and 45 % respectively. When verapamil and capsaicin were administered simultaneously, the initial stimulatory effect of capsaicin on the rate and force was attenuated. Most interestingly, however, was the observation that there was a significant diminution of verapamil action on both the rate and isometric force. These findings indicated the antagonism between these two drugs. Thus, while verapamil mitigated the initial stimulation evoked by capsaicin on the rate and force, the verapamil-mediated negative chronotropy and inotropy were diminished by capsaicin. It can also be seen that capsaicin apparently antagonized verapamil effect on the rate more effectively than on the force. More striking results were obtained when capsaicin concentrations were raised to 2 and 10 µg/ml (Fig. 5 and Fig. 6 respectively). Capsaicin at 10 µg/ml caused complete inhibition of the verapamil - induced negative chronotropy since the curve depicted percentage changes in atrial rate in the presence of verapamil plus capsaicin was practically identical to that caused by capsaicin alone. The verapamil-evoked negative inotropy, on the other hand, was somewhat more resistant to inhibition by the same dose of capsaicin (Fig. 6). Thus, these results confirmed earlier observation that capsaicin was more efficacious in reversing the effect of verapamil on the rate than on the contractile force. It should also be pointed out that

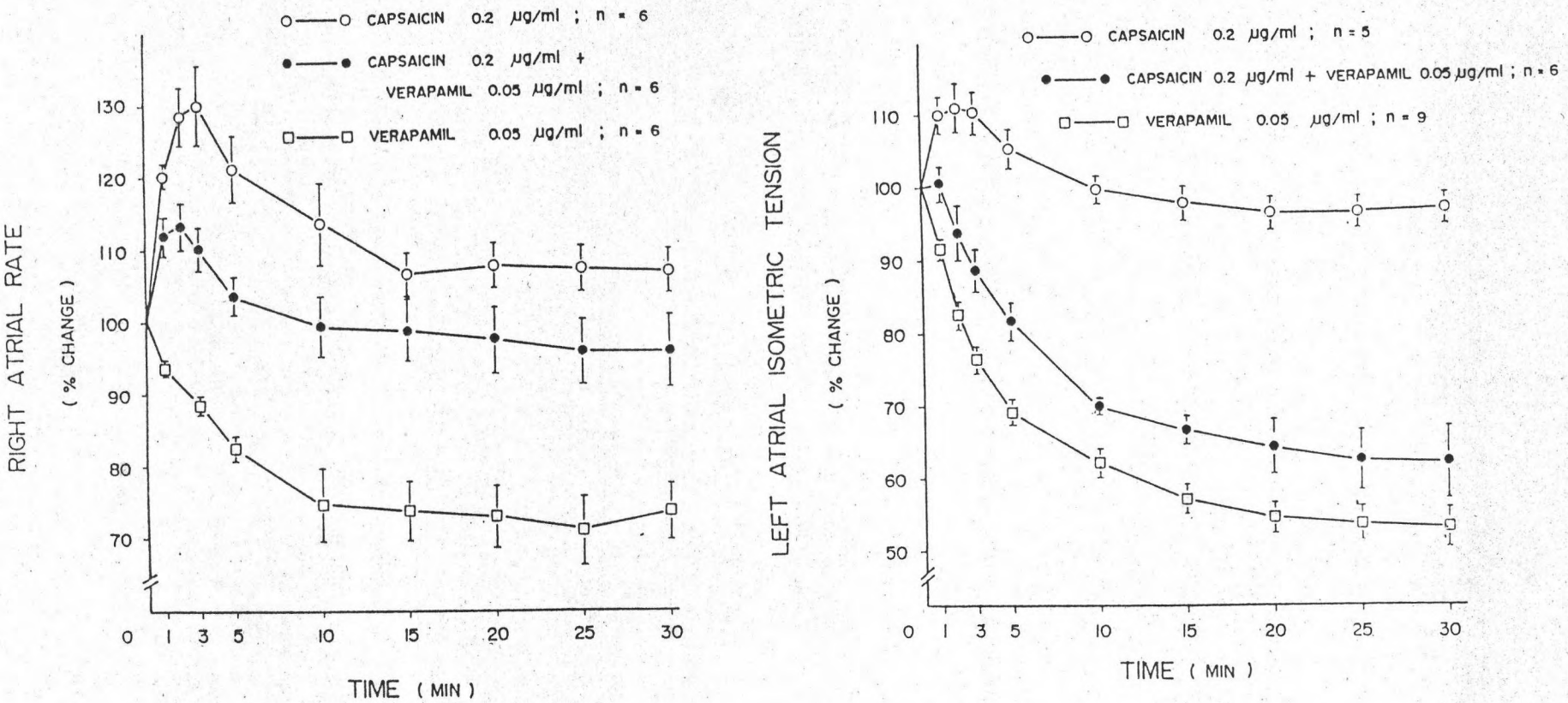

Figure 4 Antagonism of the verapamil-induced negative chronotropy and inotropy by capsaicin at the concentration of 0.2 µg/ml.

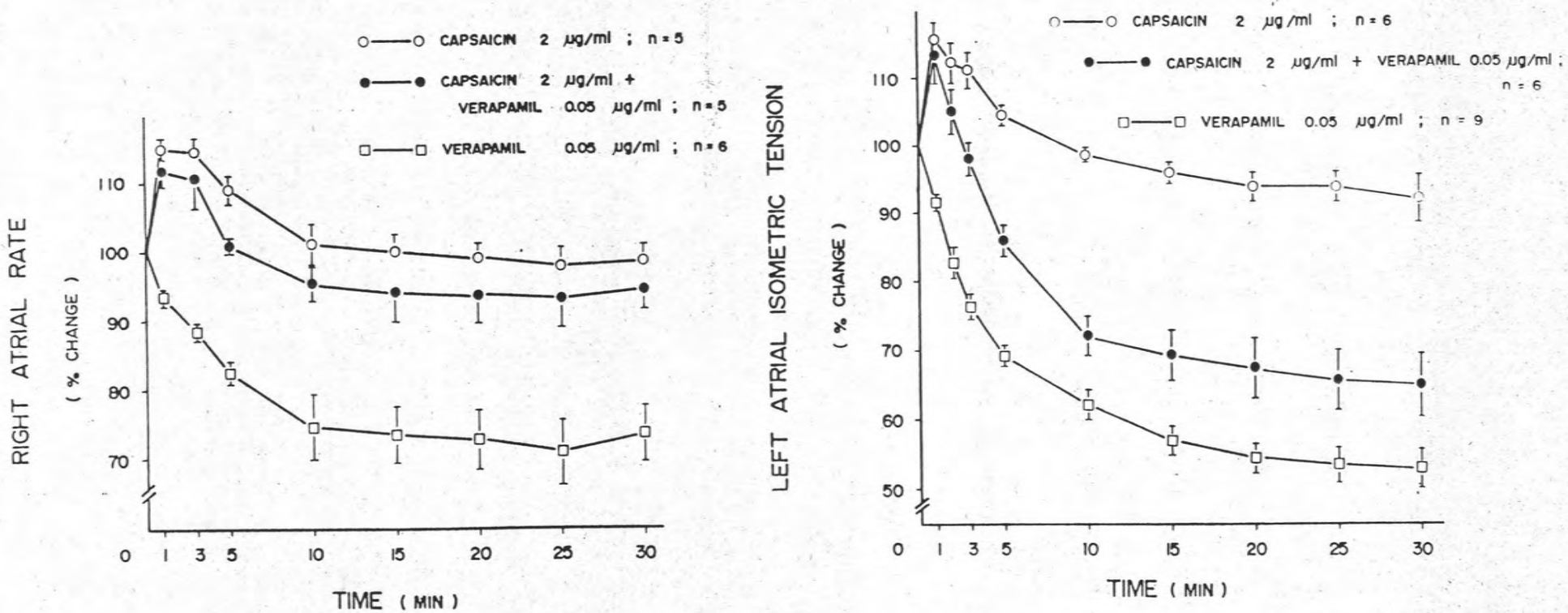

Figure 5 Antagonism of the verapamil-induced negative chronotropy and inotropy by capsaicin at the concentration of 2 µg/ml.

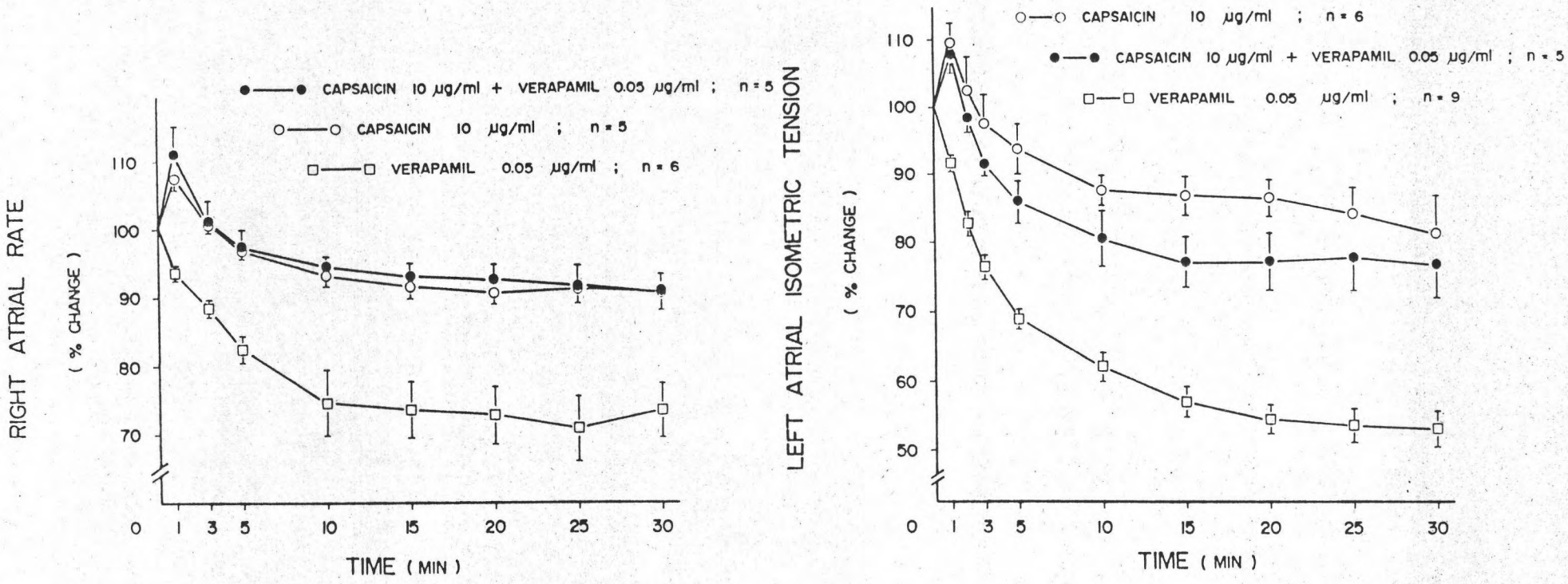

Figure 6 Antagonism of the verapamil-induced negative chronotropy and inotropy by capsaicin at the concentration of 10 µg/ml.

verapamil could not significantly reduce the intial stimulation of the rate and contractile force when capsaicin was present in high doses (Fig. 5 and Fig. 6) whereas it could with low dose of capsaicin (Fig. 4). Reserpine pretreatment did not abolish this antagonizing activity of capsaicin indicating that capsaicin did not reverse verapamil effect by releasing endogenous catecholamines (Fig. 7).

In order to determine whether the initial stimulatory action of capsaicin was involved in the capacity of this compound to alleviate the negative chronotropy and inotropy mediated by verapamil, experiments were performed in which verapamil was added 15 min after the atria had been exposed to capsaicin. The results of these experiments are reported in Fig. 8, 9 and 10. The initial capsaicin-evoked cardiac stimulation usually subsided 15 min after capsaicin was added. Qualitative inspection of the curves suggested that capsaicin still retained the antagonizing activity even though verapamil was added at the time when most of the capsaicin-induced cardiac stimulation had declined. This can be most clearly seen from Fig. 10 in which verapamil added 15 min after 10 µg/ml capsaicin caused only a small reduction in atrial rate and isometric tension. Note also that prior to verapamil addition, not only the acute stimulatory effect of 10 µg/ml capsaicin had completely ceased but both the rate and contractile force had already been depressed below control values. Thus, to make the correct quantitative comparison, the percentage changes in rate and tension after verapamil in Fig. 8, 9 and 10 were recalculated using the values at 15 min as controls (i.e., the rate and tension in the presence of capsaicin at 15 min just before verapamil addition were taken as

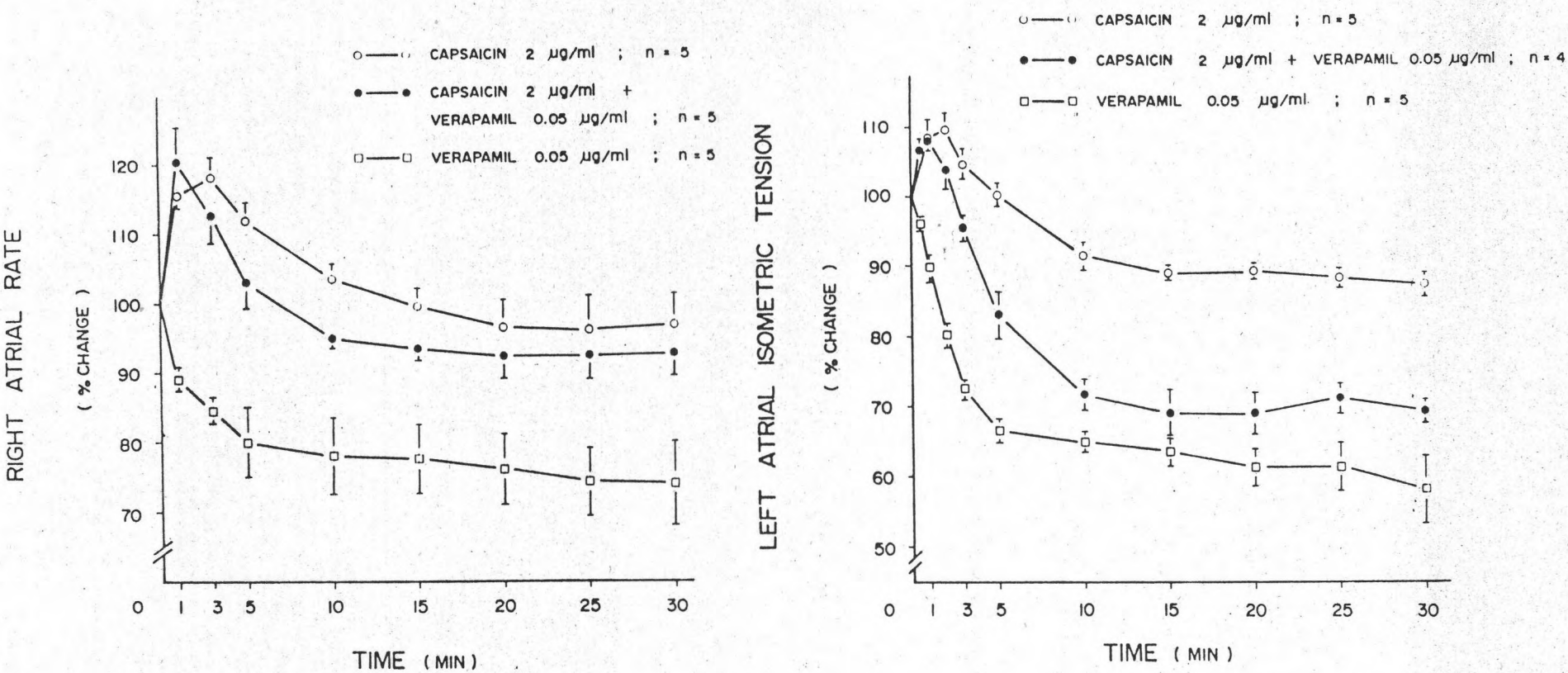

Figure 7 Effect of reserpine pretreatment on the capsaicin-induced reversal of verapamil action on the rate and isometric force.

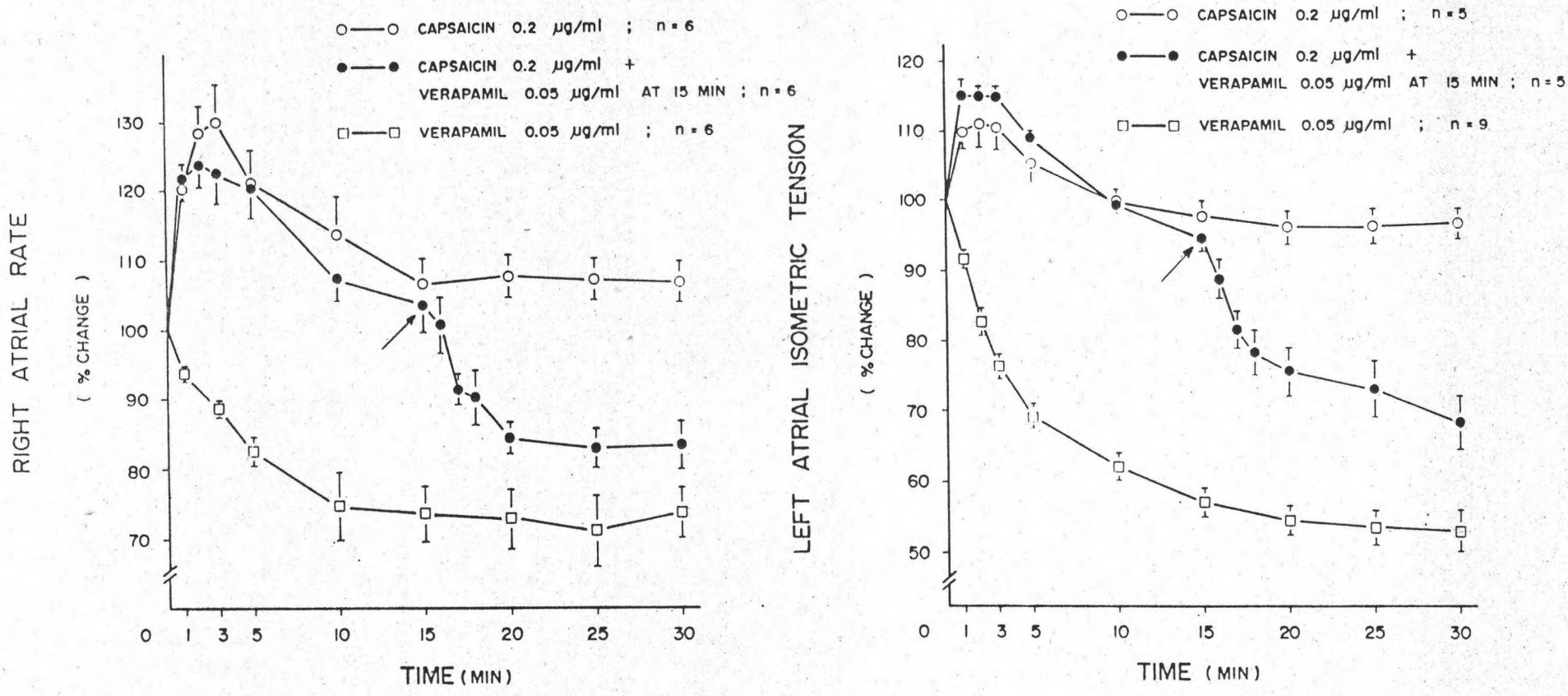

Figure 8 Modification of the verapamil-induced negative chronotropy and inotropy by prior addition of 0.2 µg/ml capsaicin.

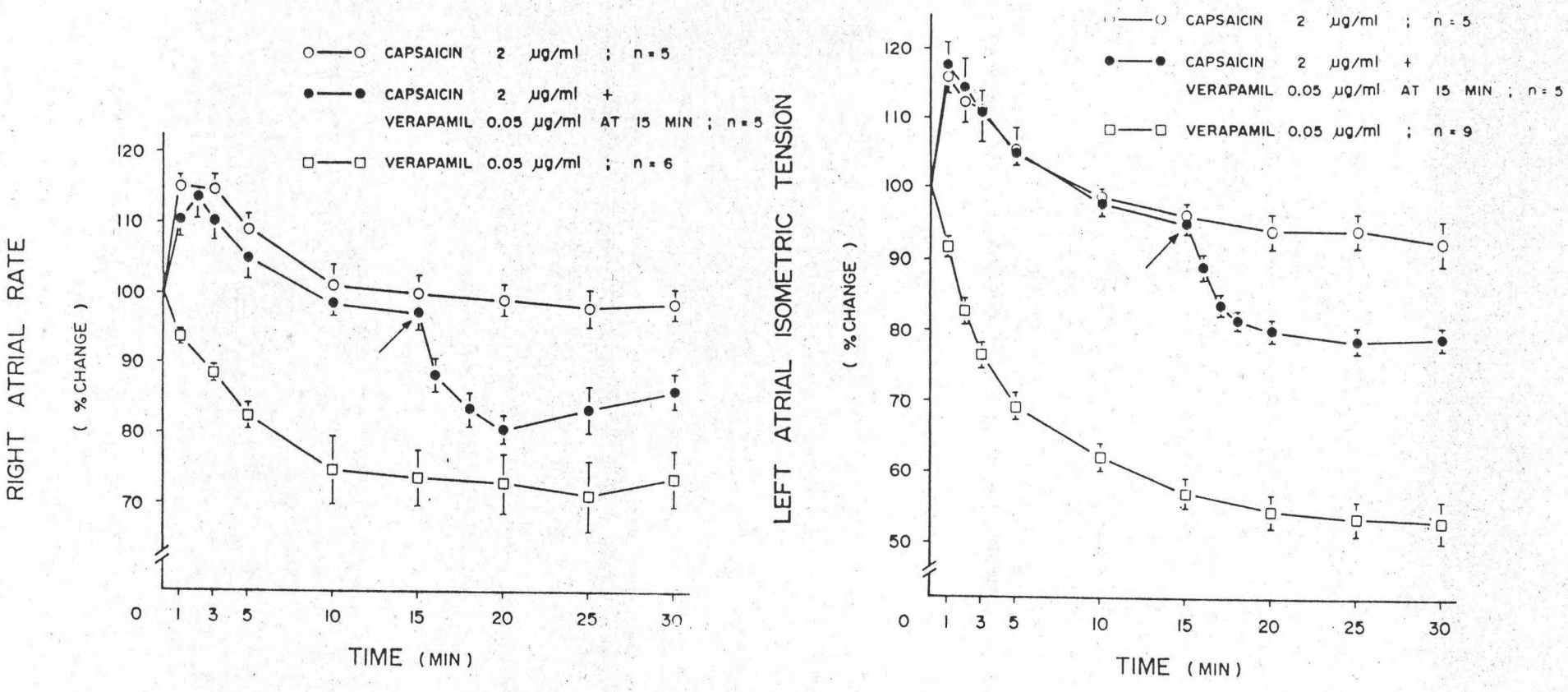

Figure 9 Modification of the verapamil-induced negative chronotropy and inotropy by prior addition of 2 µg/ml capsaicin.

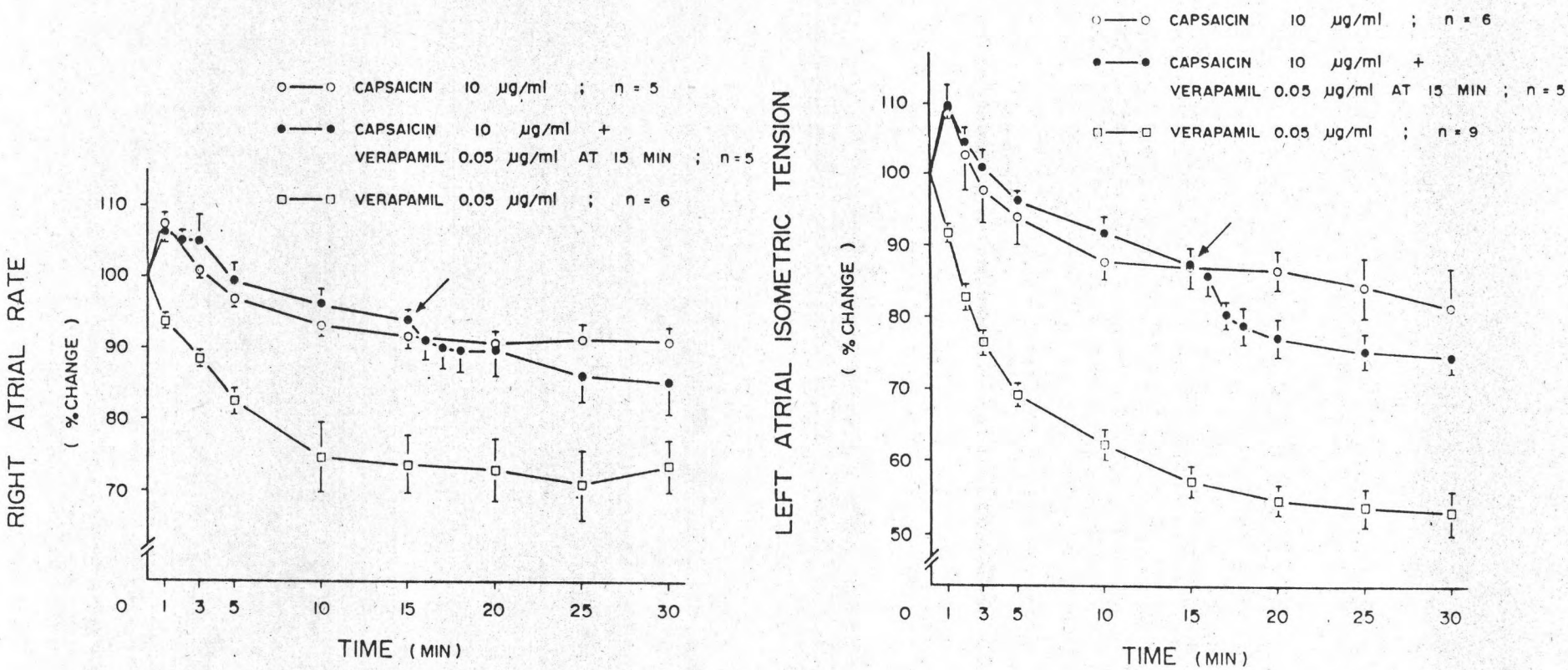

Figure 10 Modification of the verapamil-induced negative chronotropy and inotropy by prior addition of 10 µg/ml capsaicin.

100%). The recalculated values were tabulated in Tables 1 and 2. Statistical analyses revealed that the decrease in rate following verapamil added 15 min after 0.2 µg/ml capsaicin was not significantly different from that produced by verapamil alone whereas it was with respect to force. With higher doses of capsaicin the antagonism was more evident, particularly regarding the force. Thus capsaicin appeared less efficacious in antagonizing verapamil action on the rate than on the force when the latter was added after the capsaicin-induced cardiac stimulation had declined.

Experiments were also performed to study the antagonistic action of capsaicin when this compound was added after verapamil. The results are shown in Fig. 11 and 12 in which capsaicin (2 µg/ml) was given at 15 and 5 min after verapamil (0.05 µg/ml) respectively. It is seen that capsaicin, added after verapamil, caused the usual initial stimulation of both the rate and isometric force. However, the rate and force remained significantly elevated at 15 min after capsaicin which indicated the antagonism of verapamil action by capsaicin. The antagonism was more apparent in Fig. 12 in which the time course of percentage changes in rate and force was followed for 25 min after capsaicin addition. It should be pointed out that the augmented rate and force mediated by capsaicin (2 µg/ml) alone had returned to control values within 15 min after capsaicin was added.

Fig. 13 shows the effect of procainamide (5 µg/ml), capsaicin (2 µg/ml), and procainamide plus capsaicin on the rate and isometric tension. Procainamide had only slight effect on both the rate and

Table 1 Quantitative analyses of the capsaicin-induced reversal of verapamil action on right atrial rate

	% change of atrial rate					
	0	1	3	5	10	15 min
0.05 µg/ml verapamil (n = 6)	100	93.64 ± 0.97	88.50 ± 1.21	82.39 ± 1.77	74.67 ± 4.98	73.57 ± 4.18
verapamil after 0.2 µg/ml capsaicin (n = 6)	100	97.19 ± 1.90	87.30 ± 2.75	81.82 ± 3.06	80.27 ± 2.07	81.98 ± 2.69
verapamil after 2 µg/ml capsaicin (n = 5)	100	90.88 ± 2.11	85.94 ± 1.15	84.17 ± 2.03	86.08 ± 2.49	87.52 ± 1.79[a]
verapamil after 10 µg/ml capsaicin (n = 5)	100	96.69 ± 1.61	95.17 ± 2.17[a]	95.33 ± 3.24[b]	91.39 ± 3.43[a]	90.55 ± 4.44[a]

When both drugs were present, verapamil was added 15 min after capsaicin.

[a] $P < 0.05$ compared with verapamil alone

[b] $P < 0.01$ compared with verapamil alone

Table 2 Quantitative analyses of the capsaicin-induced reversal of verapamil action on left atrial isometric force

	% change of tension						
	0	1	2	3	5	10	15 min
0.05 µg/ml verapamil (n = 9)	100	91.68 ± 0.79	82.73 ± 1.96	76.32 ± 1.80	69.13 ± 1.59	62.12 ± 2.03	56.99 ± 2.13
verapamil after 0.2 µg/ml capsaicin (n = 5)	100	93.94 ± 1.82	86.17 ± 1.73	82.75 ± 2.67	80.00 ± 3.45 [a]	77.18 ± 4.44 [b]	72.26 ± 4.23 [b]
verapamil after 2 µg/ml capsaicin (n = 5)	100	93.80 ± 1.16	88.20 ± 1.27 [a]	86.11 ± 1.14 [d]	84.44 ± 1.74 [d]	82.94 ± 2.17 [d]	83.37 ± 2.23 [d]
verapamil after 10 µg/ml capsaicin (n = 5)	100	97.95 ± 1.29 [c]	91.86 ± 0.59 [d]	90.04 ± 2.12 [d]	88.03 ± 1.74 [d]	85.92 ± 2.09 [d]	85.09 ± 1.63 [d]

When both drugs were present, verapamil was added 15 min after capsaicin.

[a] $p < 0.05$ compared with verapamil alone

[b] $p < 0.01$ compared with verapamil alone

[c] $p < 0.005$ compared with verapamil alone

[d] $p < 0.001$ compared with verapamil alone

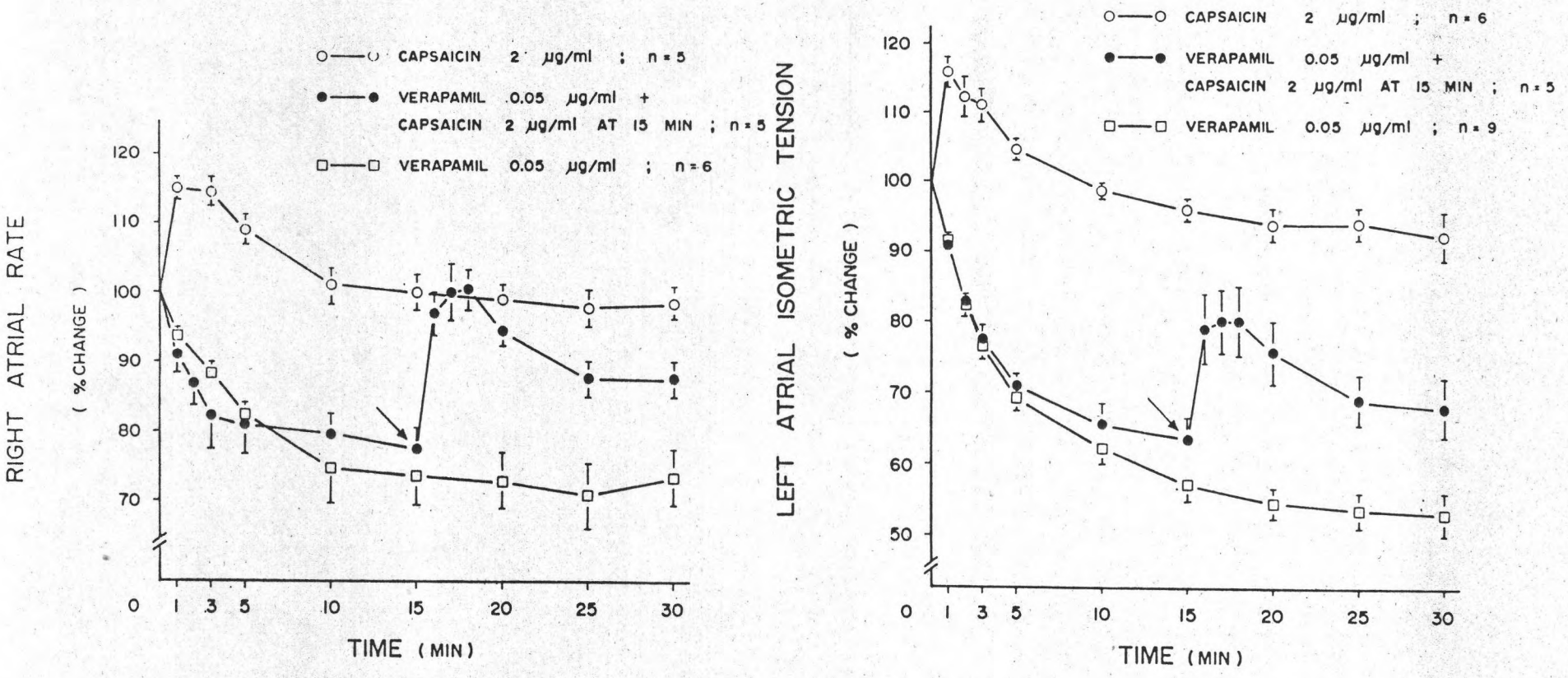

Figure 11 Antagonistic action of capsaicin, added 15 min after verapamil, on the verapamil-mediated negative chronotropy and inotropy.

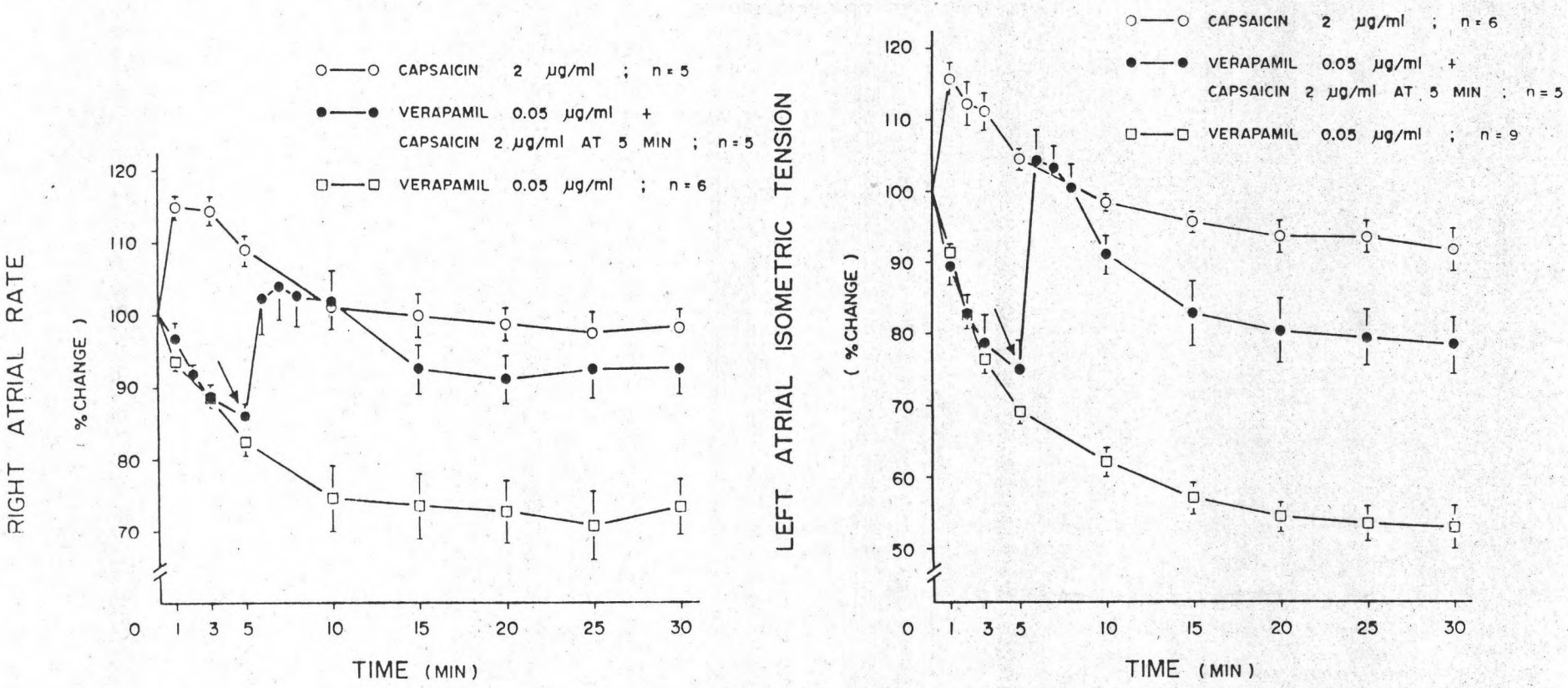

Figure 12 Antagonistic action of capsaicin, added 5 min after verapamil, on the verapamil-mediated negative chronotropy and inotropy.

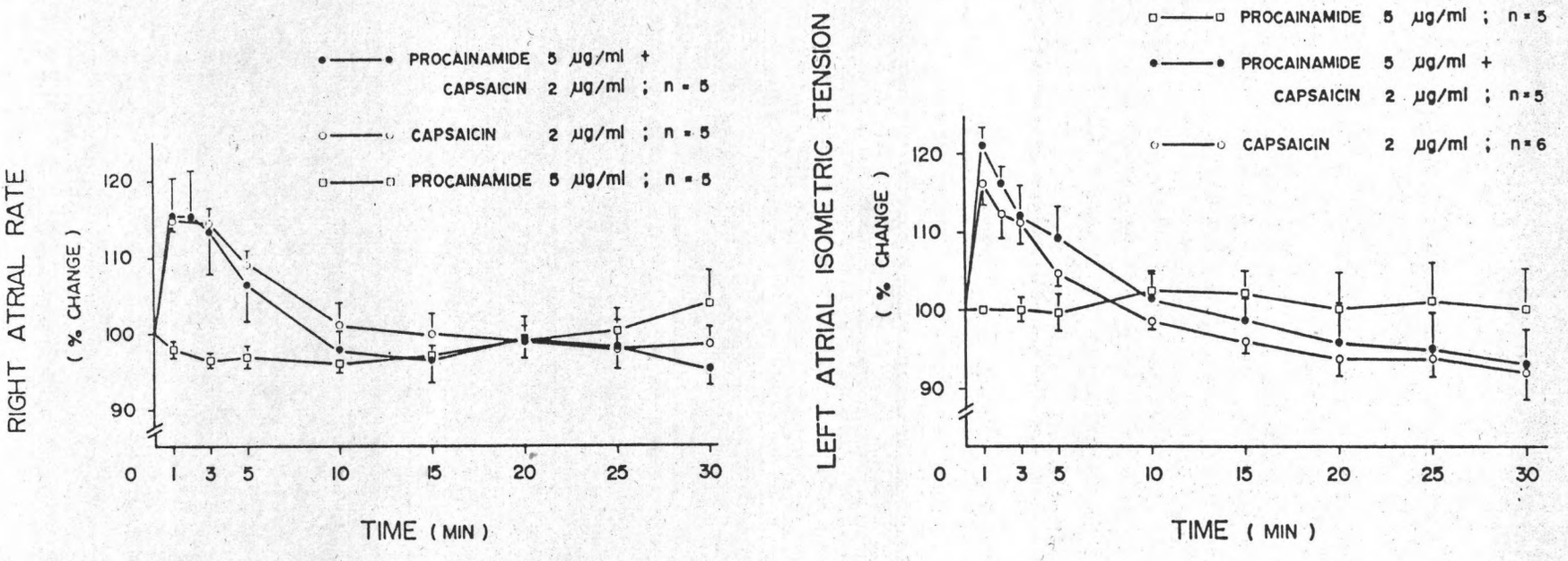

Figure 13 Effect of procainamide with and without capsaicin on the rate and isometric force of isolated rat atria.

force. When both procainamide and capsaicin were present, the changes in rate and contractile force were essentially the same as those caused by capsaicin alone. Thus, there seemed to be no significant interaction between the two compounds.

Since capsaicin was found to inhibit the negative chronotropic and inotropic effect of verapamil, it was therefore interesting to investigate whether procainamide possesses similar activity. As reported in Fig. 14, 5 µg/ml procainamide was found to reduce the verapamil-induced negative chronotropy and inotropy although the effect on rate was not significantly different from that caused by verapamil alone. Increasing the dose of procainamide to 10 µg/ml clearly antagonized the depressive action of verapamil on both the rate and isometric tension (Fig. 15).

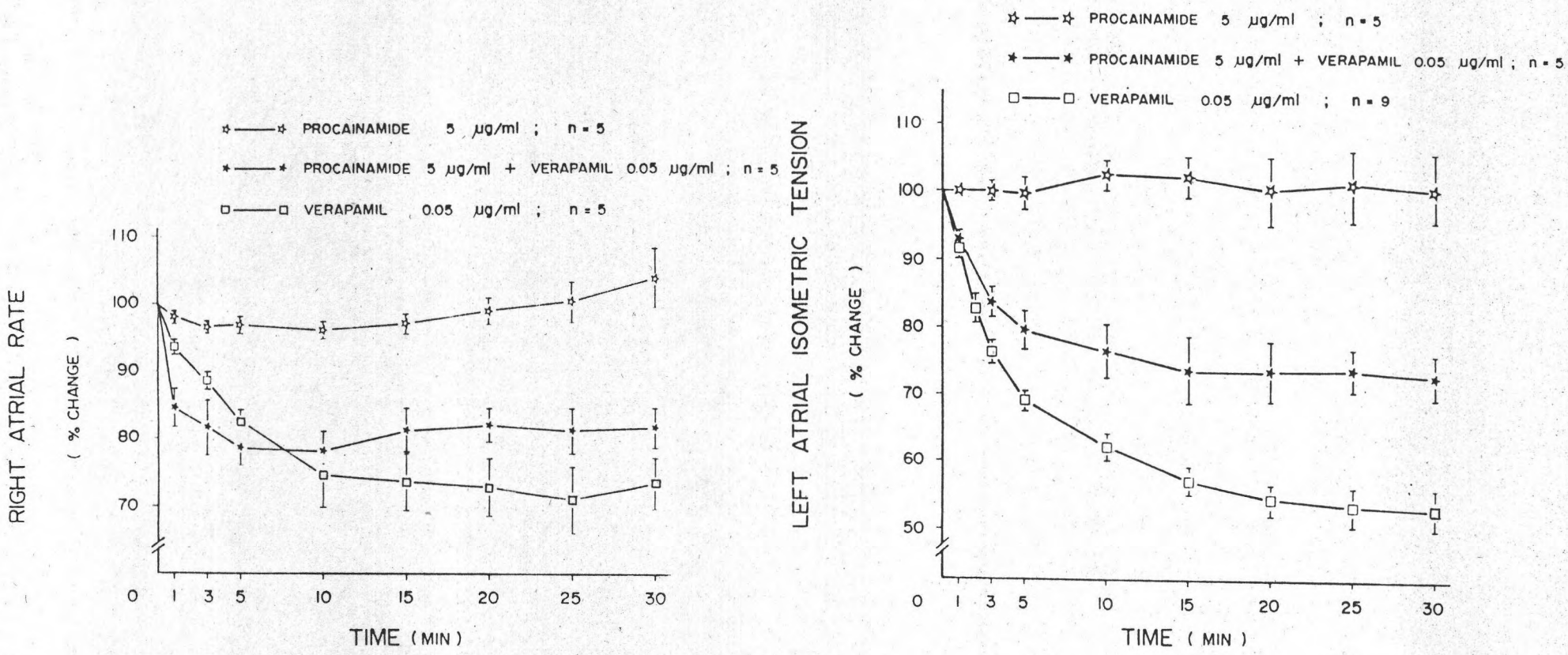

Figure 14 Reduction of verapamil action on the rate and isometric force by 5 µg/ml procainamide

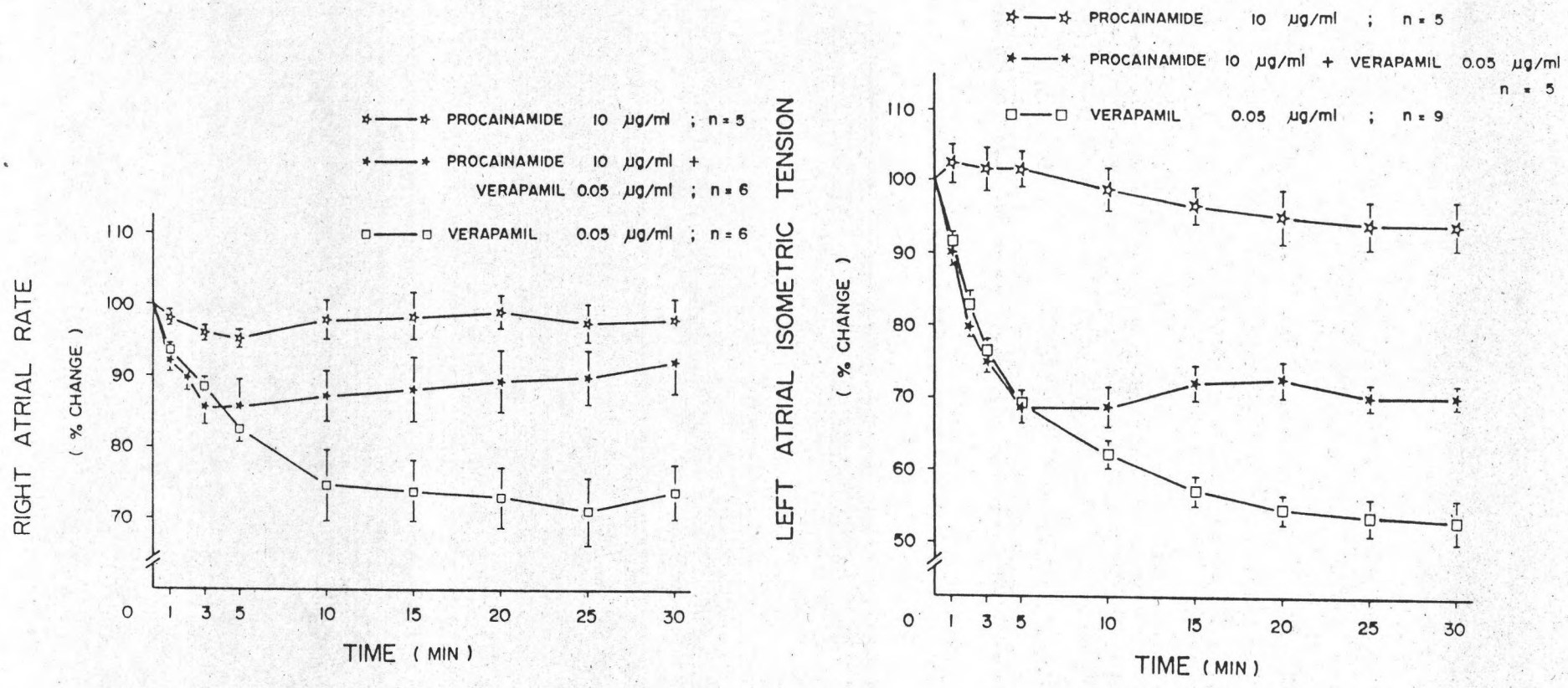

Figure 15 Reduction of verapamil action on the rate and isometric force by 10 µg/ml procainamide.