CHAPTER 4

RESULTS

The results were divided into two main sections as follows:

Section I: Effects of chronic cocaine treatment on the responsiveness of guinea-pig isolated atria and trachea to β-adrenoceptor agonists (epinephrine and salbutamol).

Cumulative concentration-response curves for the positive inotropic, positive chronotropic and relaxing effects of β-adrenoceptor agonists (epinephrine, and salbutamol) in the absence and presence of β-adrenoceptor antagonist (propranolol) were examined in isolated preparations taken from cocaine- and saline-treated guinea-pigs.

1.1 The effects of chronic cocaine treatment on the responsiveness of guinea-pig isolated atria to β-adrenoceptor agonists.

Both epinephrine and salbutamol produced the same general pattern of effects on the isolated atria of cocaine-and saline-treated guinea-pigs, the representative tracings were shown in the figure 17 and 18. But quantitatively, the atria of all cocaine-treated groups were more sensitive to both agonists than those of saline-treated groups, as summarized in figure 19 and 20, cocaine (2.5 mg/kg) significantly caused parallel shift to the left of the log

concentration-response curve of the positive inotropic and positive chronotropic effects, respectively, of both agonists.

Although, salbutamol had the general pattern of effects on atria similar to that of epinephrine, its maximum effects of both cocaine-and saline-treated groups were significantly lower than those of epinephrine. Thus, salbutamol exhibited partial agonistic activity on the atria (Figure 19 A-20 A). The maximum inotropic effects but not chronotropic effects of epinephrine on isolated atria of cocaine-treated guinea-pigs were significantly higher than those of saline-treated animals, in contrast the maximum chronotropic effects but not inotropic effects of salbutamol on isolated atria of cocaine-treated guinea-pigs were significantly higher than those of saline-treated animals (Figure 19 A-20 A).

Table 9 showed the $[D]_{max50}$ (concentration of agonist that produced 50 % its own maximum response) and the pD_2 values (negative log $[D]_{max50}$) for both positive inotropic and chronotropic effects of the two drugs on the cocaine-and saline-treated groups. The pD_2 values of both agonists of cocaine-treated groups were significantly higher than those of saline-treated groups.

1.2 The effects of chronic cocaine treatment on the responsiveness of guinea-pig isolated tracheas to β-adrenoceptor agonists.

The representative tracing of the relaxing effects of epinephrine and salbutamol on carbachol-induced contraction of isolated trachea of cocaine-and saline-treated guinea-pigs were illustrated in figure 21 and 22, respectively. Cocaine significantly induced leftward shift of the concentration-response curves of both agonists (Fig. 23). The increase in the responsiveness of the cocaine treated-groups were demonstrated by the significant higher

pD_2 values compared to the control groups (Table 9). In contrast to the effect on isolated atria, the maximum relaxing effects on trachea produced by epinephrine and salbutamol in both cocaine- and saline-treated groups were not significantly different (Fig. 23).

1.3 Effects of β-adrenoceptor antagonist, propranolol on the responsiveness of chronic cocaine-treated guinea-pig isolated atria and tracheas to epinephrine.

The representative tracing of the positive inotropic, positive chronotropic and relaxing effects of epinephrine on carbachol-induced contraction of isolated atria and trachea of cocaine-and saline-treated guinea-pigs were illustrated in figure 24 and 25, respectively.

In the absence of propranolol, the isolated guinea-pig atria and tracheas of all cocaine-treated groups were more sensitive to epinephrine than those of saline-treated groups (Figure 19, 20 and 23). Propranolol ($1x10^{-8}$-$3x10^{-6}$ M), significantly caused the concentration-dependent parallel shift to the right of the concentration-response curves of epinephrine on both positive inotropic and chronotropic effects of atria and relaxing effect on carbachol precontracted trachea of both cocaine- and saline-treated groups (Fig. 26-31). However, the tissue from cocaine-treated animals still demonstrated the higher responsiveness than those of saline-treated animals (Fig. 24-31).

The Schild plot (Fig. 32, 33, and 34) of the data (from the experiment shown in Fig. 27, 29, and 31, respectively) displayed the pA_2 values of propranolol on cocaine- and saline-treated groups. All plots exhibited the linear regressions with slope of 0.94-1.15 which were not significantly different from unity. The pA_2 values of propranolol on the antagonism of all effects (positive iontropic, positive chronotropic in atria and relaxing effects in

trachea) of epinephrine of chronic-cocaine treated groups were slightly but significant higher than those of corresponding control groups (Fig 32-34 and Table 10).

Section II: Analysis of cocaine concentrations in plasma, cardiac tissues and tracheal smooth muscle of guinea- pigs.

2.1: Determination of cocaine concentration in plasma, atrial, ventricular, and tracheal smooth muscle of guinea-pigs at 24 hr after the cocaine cessation

The representative chromatograms of cocaine in various tissues were exhibited in figure 35. The concentrations of cocaine in plasma, atrial and ventricular tissues, as well as smooth muscle of trachea taken from guinea-pigs at 24 hr after cessation of cocaine were shown in table 11. It could be detected in plasma and smooth muscle of trachea, but could not be detectable in atria and ventricular muscle.

2.2: Correlation of the $[D]_{max50}$ ratio of the positive chronotropic, positive inotropic and the tracheal relaxing effects of epinephrine and salbutamol with the cocaine concentration in plasma, atrial, ventricular, and tracheal smooth muscle of guinea-pigs at 24 hr after the cocaine cessation

The $[D]_{max50}$ ratio values indicated the degree of supersensitivity of atria and trachea to epinephrine and salbutamol. Table 11 demonstrated that the supersensitivities to both agonists were observed in atria and tracheas of cocaine-treated groups. In addition, it seems likely that tracheal smooth muscle of cocaine-

treated groups were more sensitive (D_{max50} ratios of trachea were highly than those of atria) to both agonists than those of control groups. These were correlated with the higher level of cocaine observed in tracheas than atria and ventricle which were not detectable.

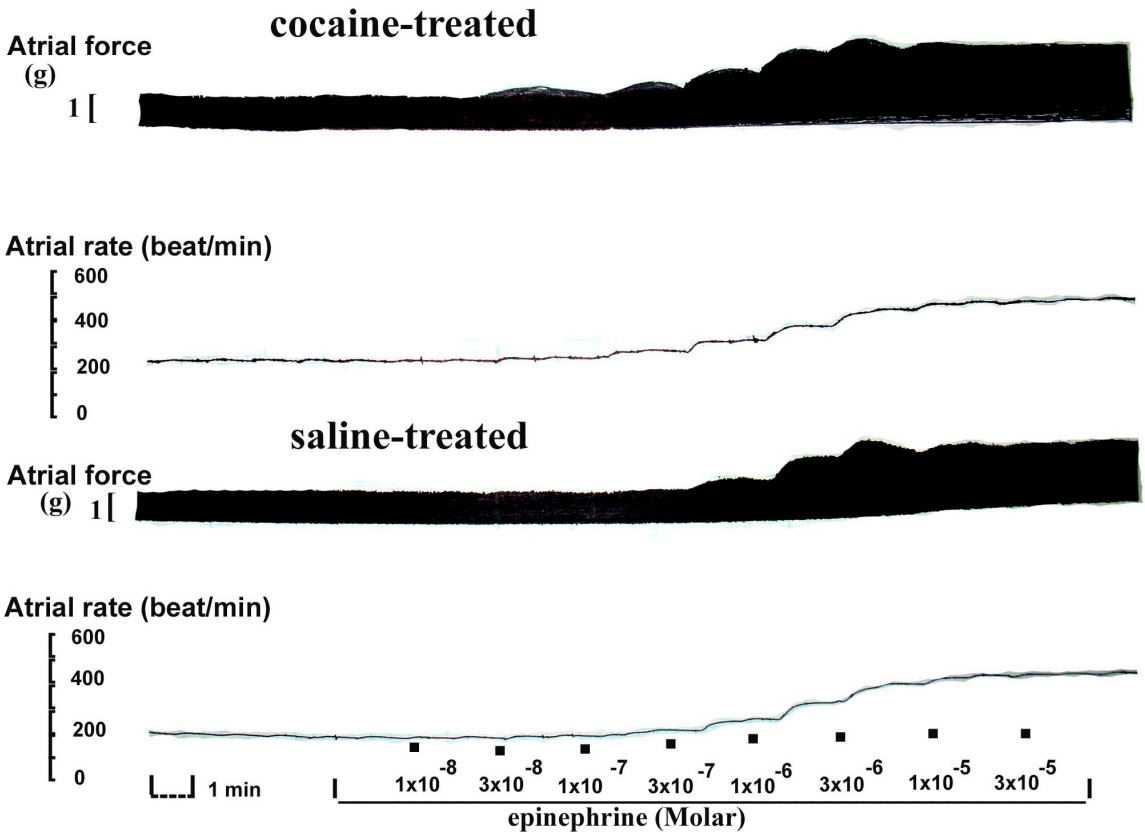


Figure 17 The representative tracing of the *positive inotropic and positive chronotropic* effects of the cumulative increase in concentrations of *epinephrine* in isolated atria of cocaine -and saline -treated guinea-pigs.

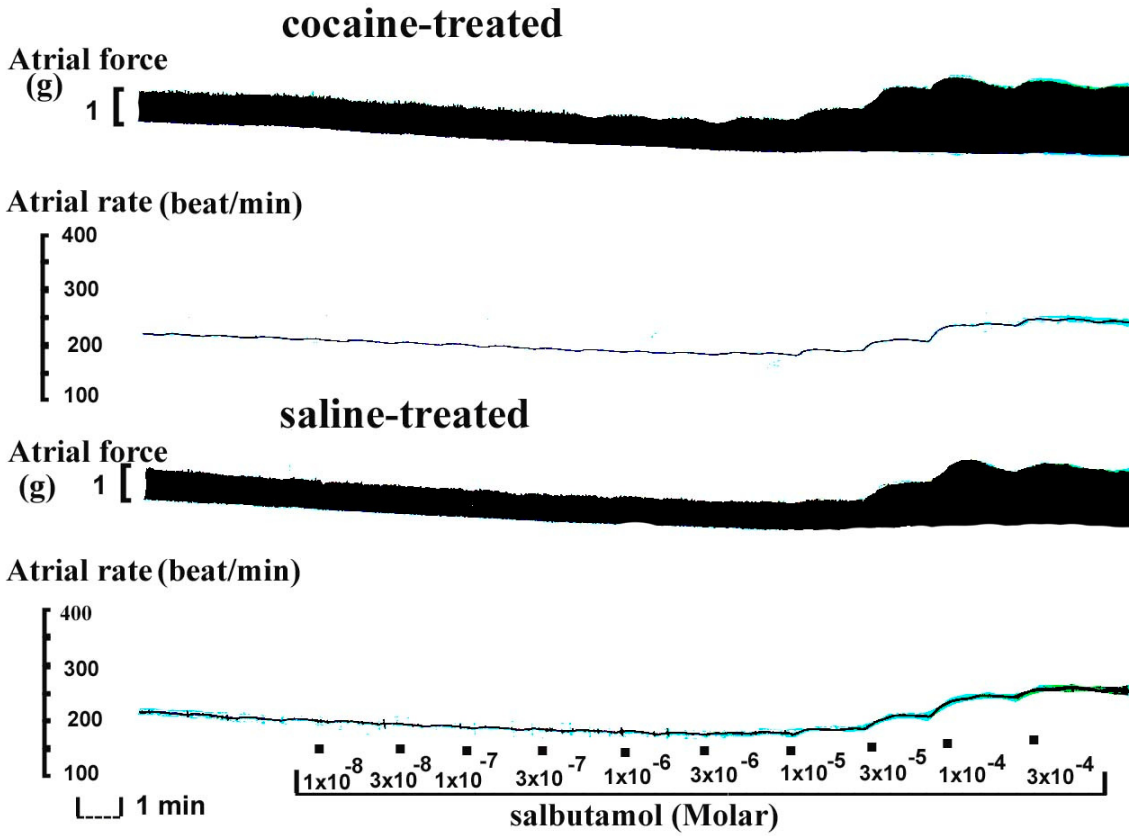


Figure 18 The representative tracing of the *positive inotropic and positive chronotropic* effects of the cumulative increase in concentrations of *salbutamol* in isolated atria of cocaine-and saline-treated guinea-pigs.

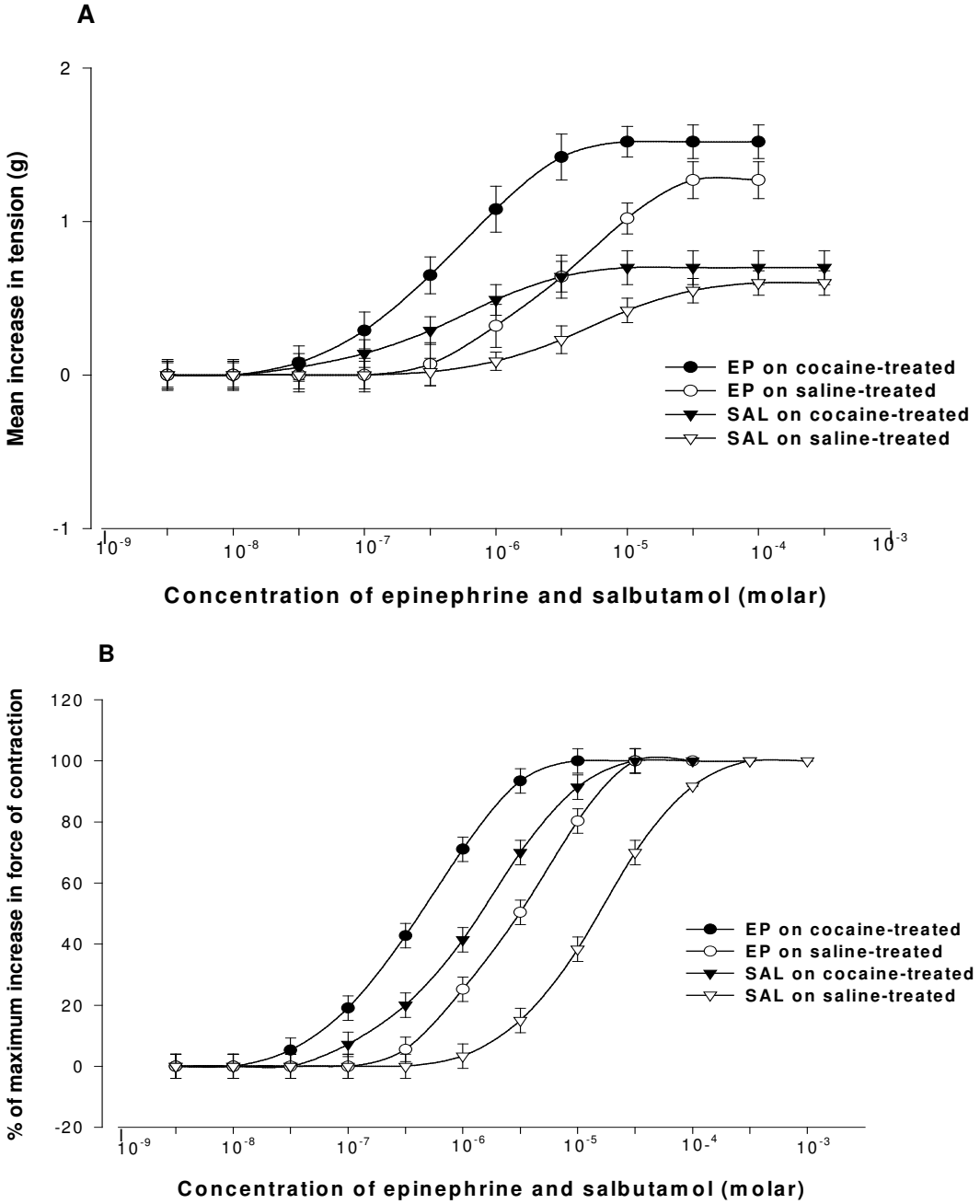


Figure 19 Cumulative concentration-effect curves of epinephrine (EP) and salbutamol (SAL) on *force of contraction* of isolated atria of cocaine- and saline-treated guinea-pigs. Each point represents mean ± S.E.M. of 8 experiments. A:

expressed as gram tension B: expressed as percentage of their maximum responses.

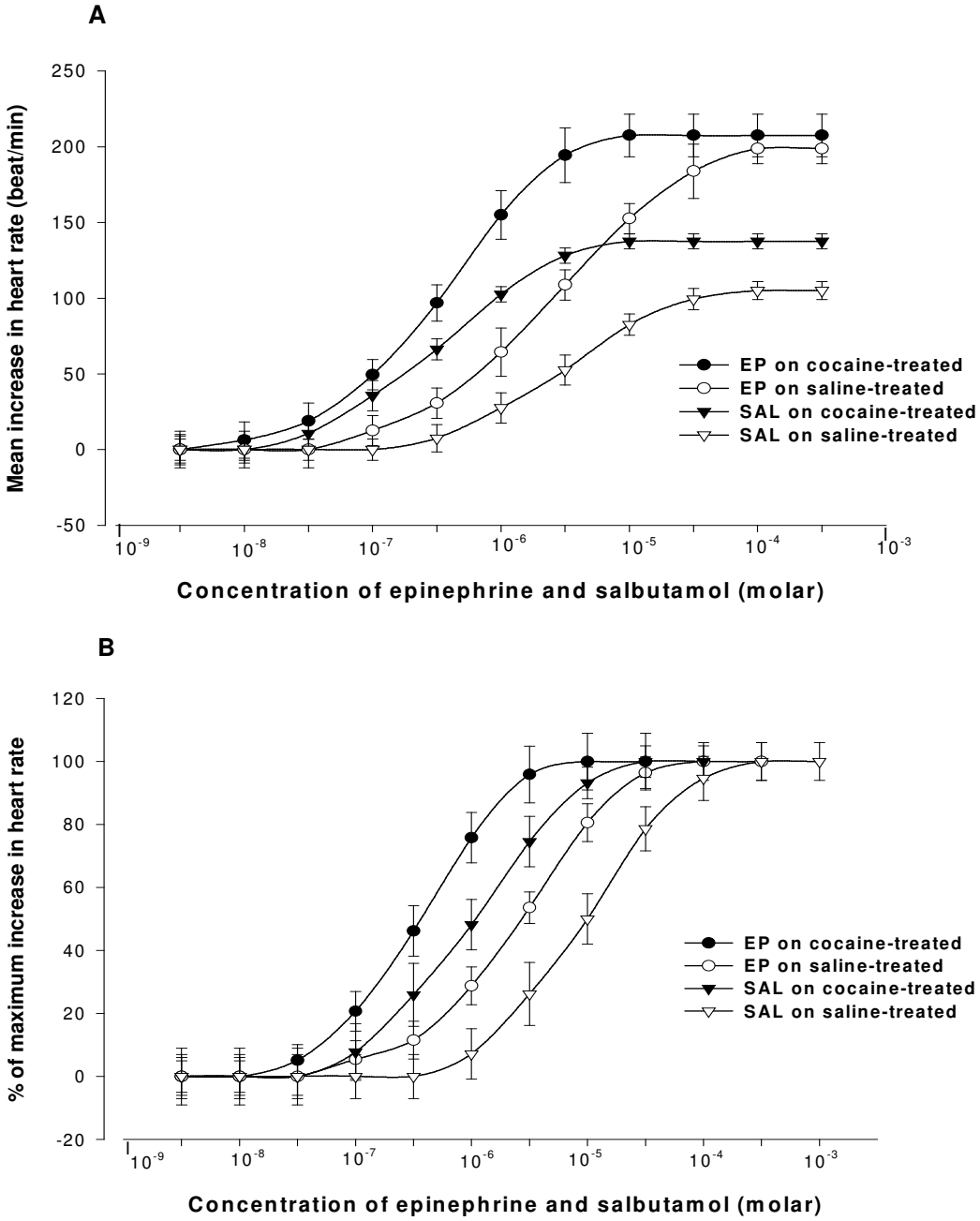


Figure 20 Cumulative concentration-effect curves of epinephrine (EP) and salbutamol (SAL) on *heart rate* of isolated atria of cocaine- and saline-treated guinea-pigs. Each

point represents mean ± S.E.M. of 8 experiments. A: expressed as atrial rate (beat/min) B: expressed as percentage of their maximum responses.

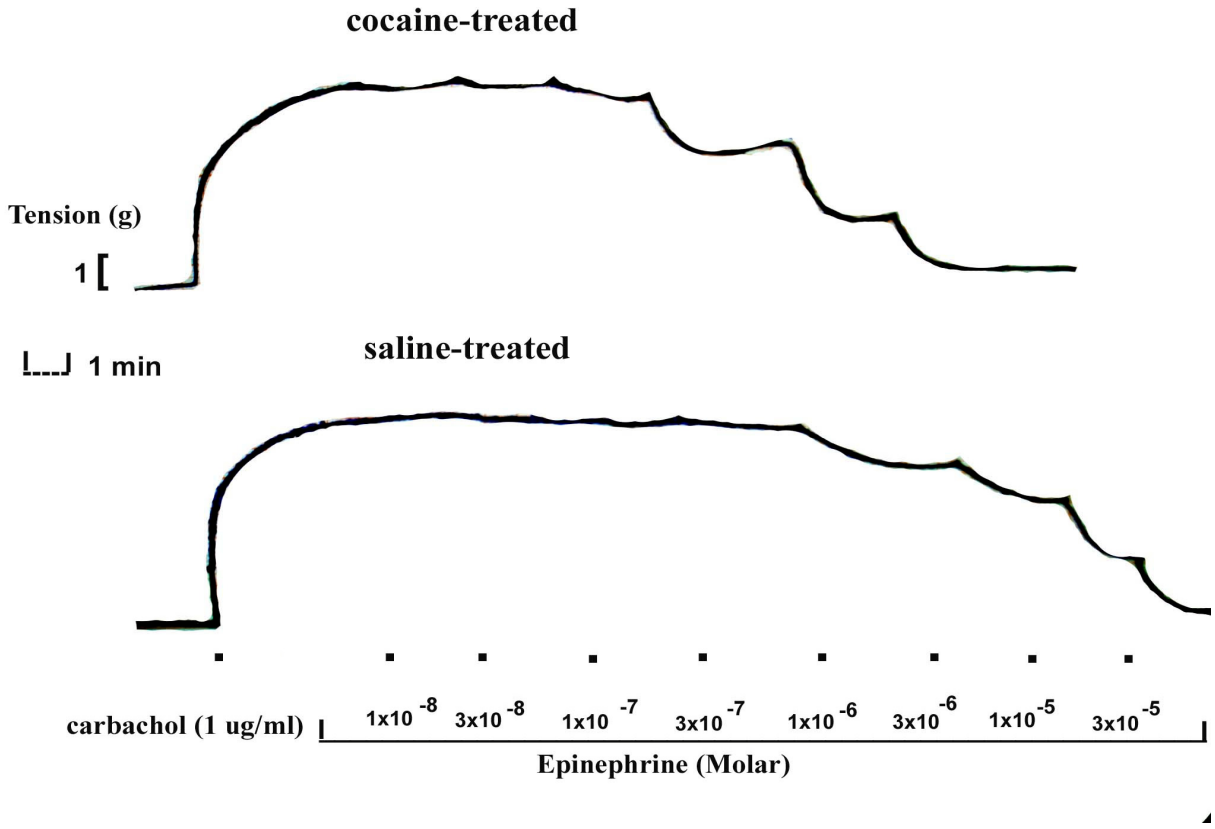


Figure 21 The representative tracing of *relaxing effect* of the cumulative increase in concentrations of *epinephrine* on

carbachol-induced contraction of cocaine- and saline-treated guinea-pig tracheas.

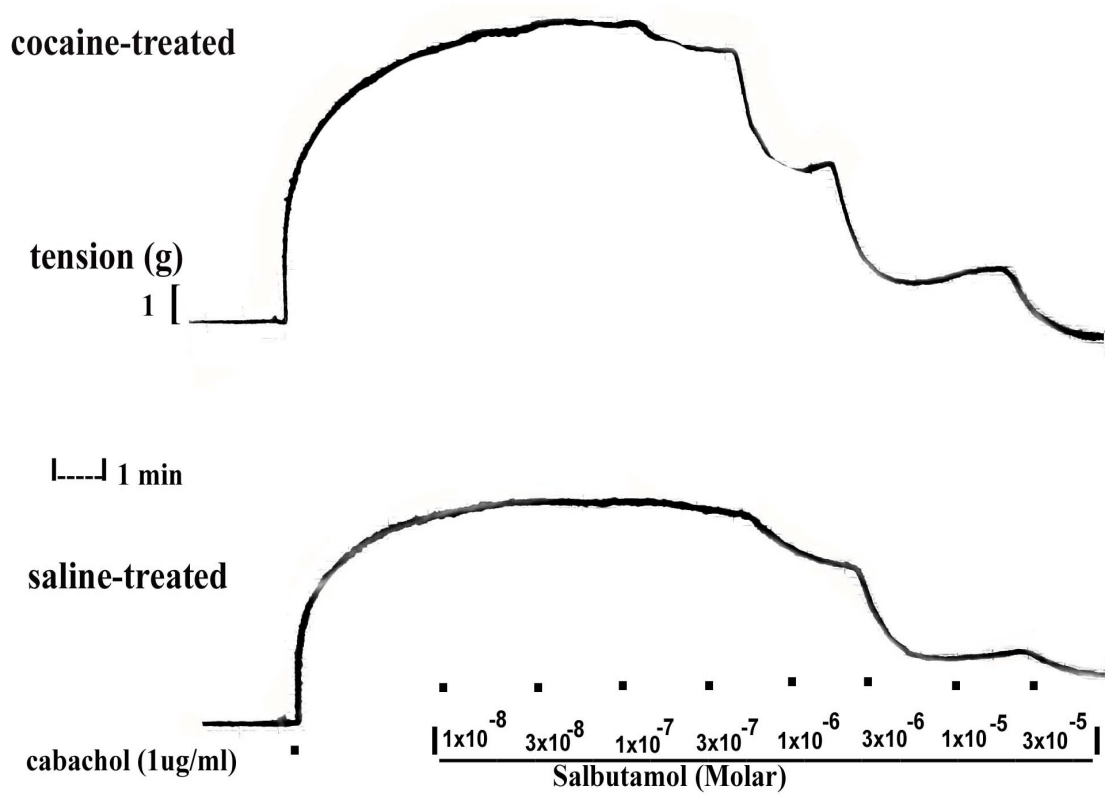


Figure 22 The representative tracing of *relaxing effect* of the cumulative increase in concentrations of *salbutamol* on

carbachol-induced contraction of cocaine- and saline-treated guinea-pig tracheas.

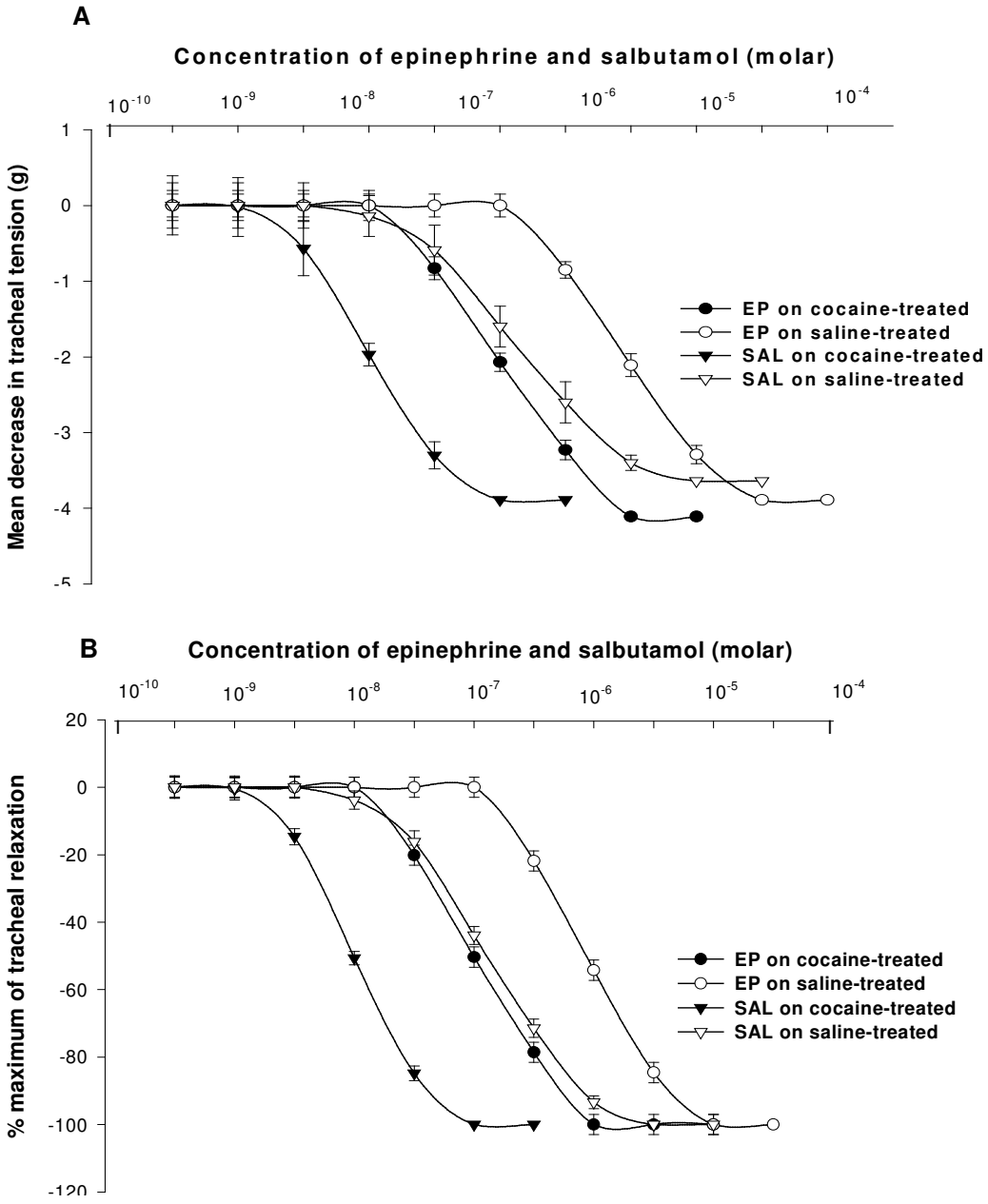


Figure 23 Cumulative concentration-effect curves of epinephrine (EP) and salbutamol (SAL) on *carbachol-induced contraction* of isolated trachea of cocaine- and saline-treated guinea-pigs. Each point represents mean ± S.E.M. of 8 experiments. A: expressed as decrease in tension (g) B: expressed as percentage of their maximum responses.

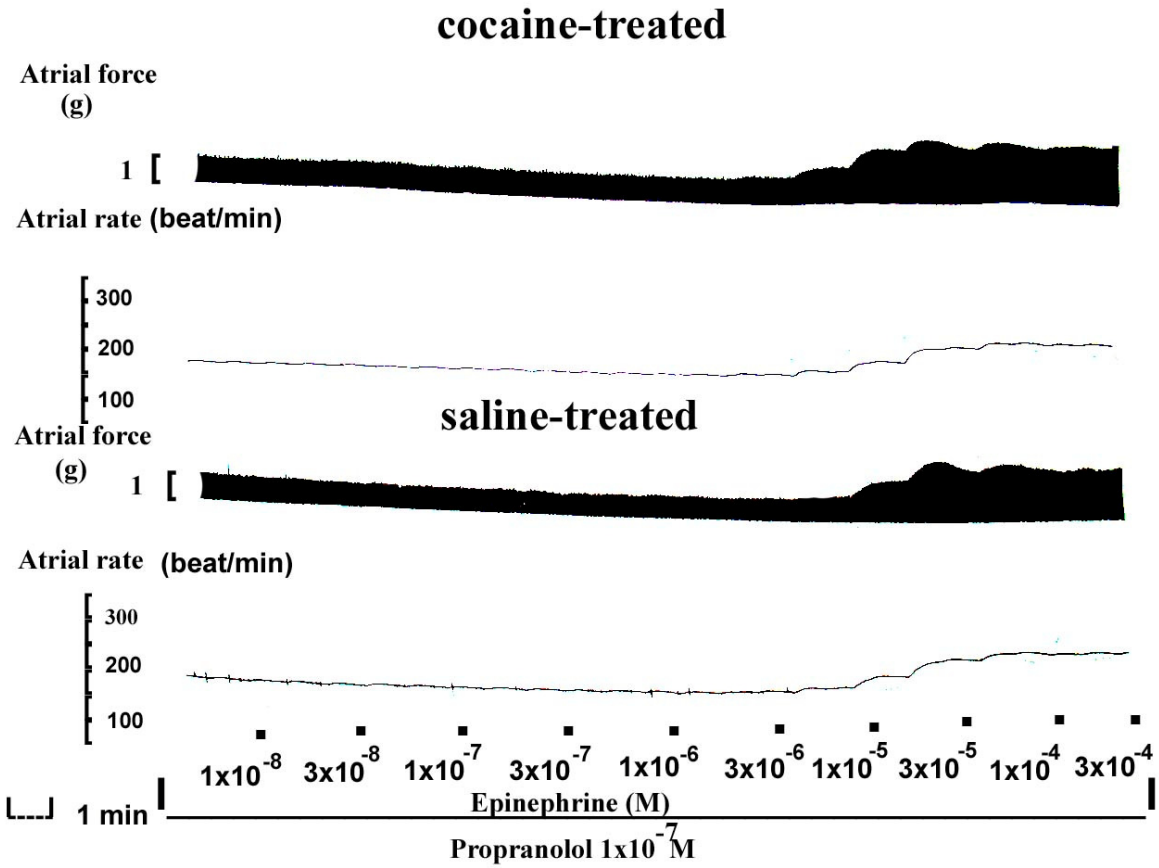


Figure 24 The representative tracing of *positive inotropic* and *positive chronotropic* effects of the cumulative increase in concentrations of *epinephrine* in the presence of *propranolol* of cocaine-and saline-treated guinea-pig atria.

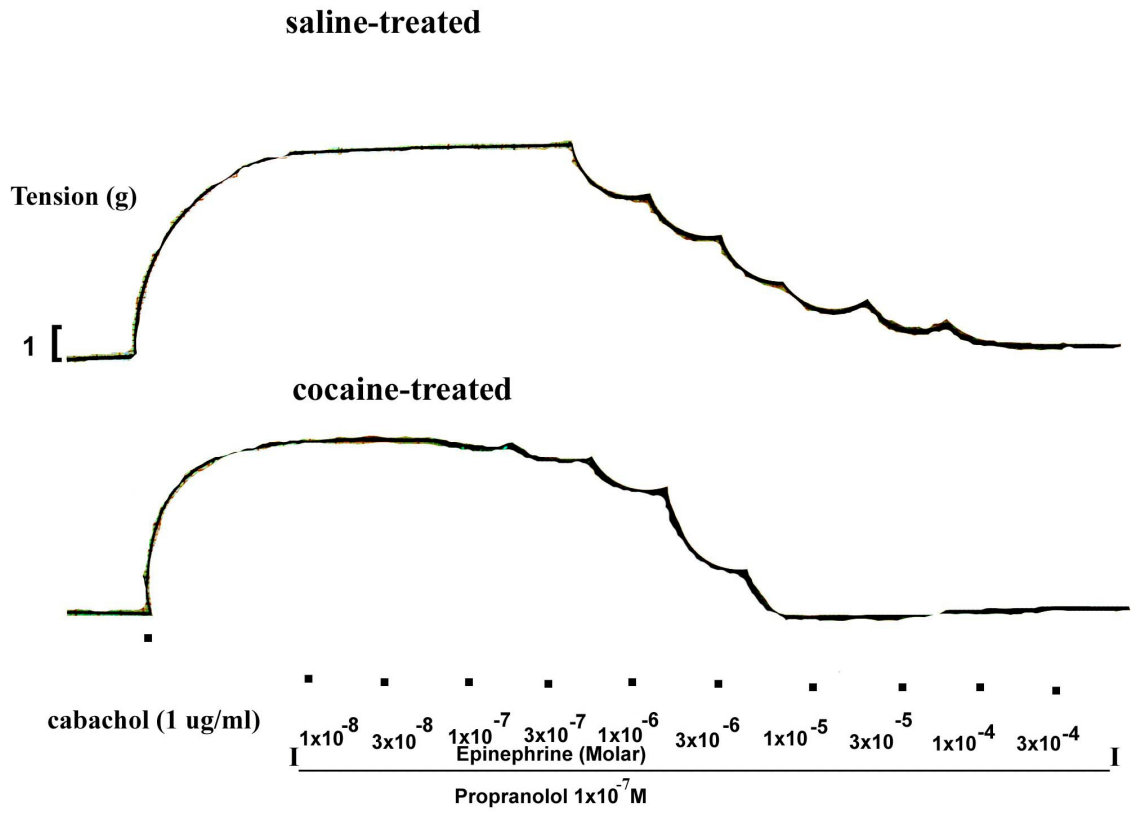


Figure 25 The representative tracing of the cumulative increase in concentrations of *epinephrine* in the presence of *propranolol* on *carbacol-induced tracheal contraction* of cocaine-and saline-treated guinea-pig tracheas.

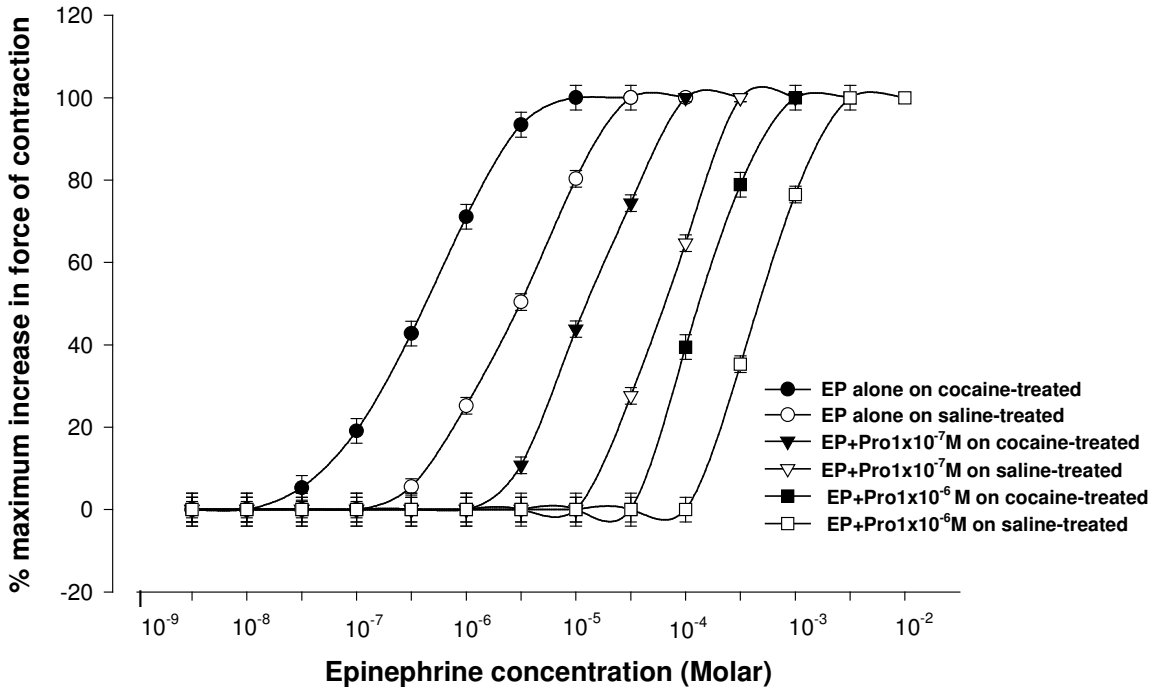
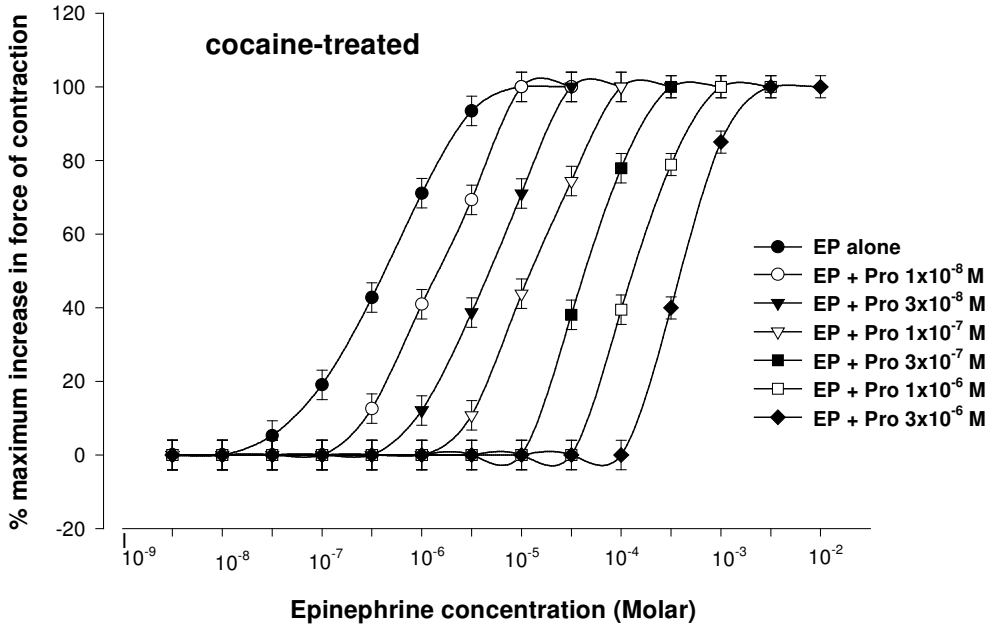


Figure 26 Effects of some concentrations (10^{-7}-10^{-6} M) of propranolol on the cumulative-concentration stimulation curves of epinephrine *on force of contraction* of isolated atria of cocaine-treated group compared to those of saline-treated group. Symbols represent means and vertical bars represent standard error of means (n = 8).



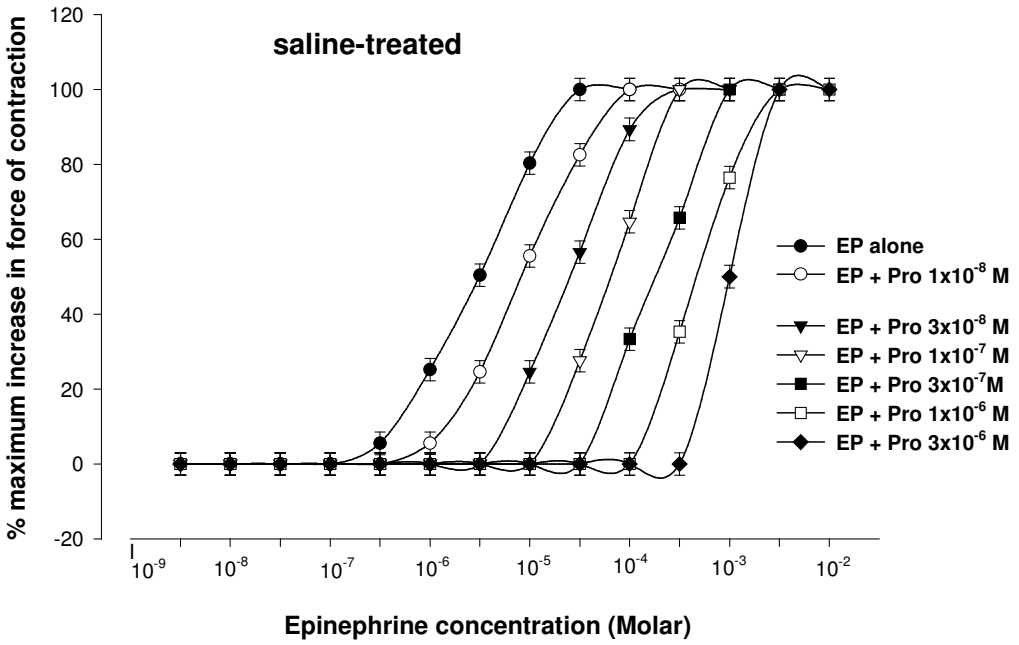


Figure 27 Effects of propranolol (Pro) on the cumulative-concentration response curves of *positive inotropic effects* of epinephrine (EP) on isolated atria of cocaine-treated (above) and saline-treated (below) guinea-pigs. Symbols represent means and vertical bars represent standard error of means (n = 8).

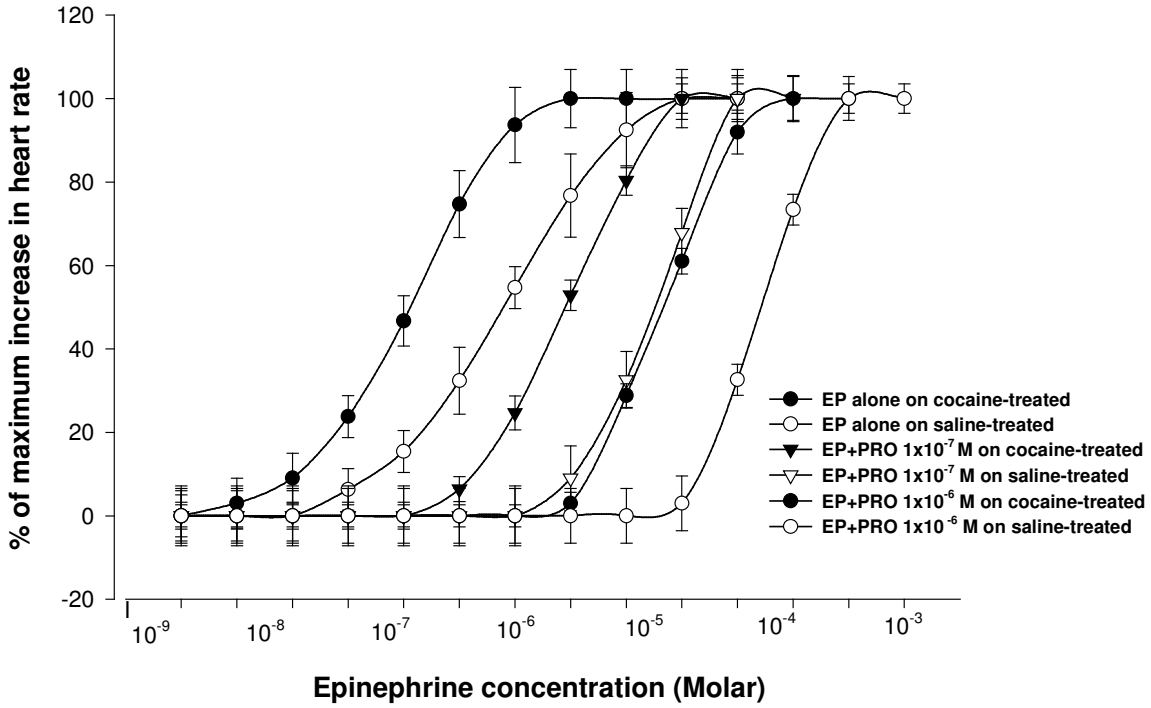


Figure 28 Effects of some concentrations (10^{-7}-10^{-6} M) of propranolol on the cumulative-concentration stimulation curves of epinephrine *on heart rate* of isolated atria of cocaine-treated group compared to those of saline-treated group. Symbols represent means and vertical bars represent standard error of means (n = 8).

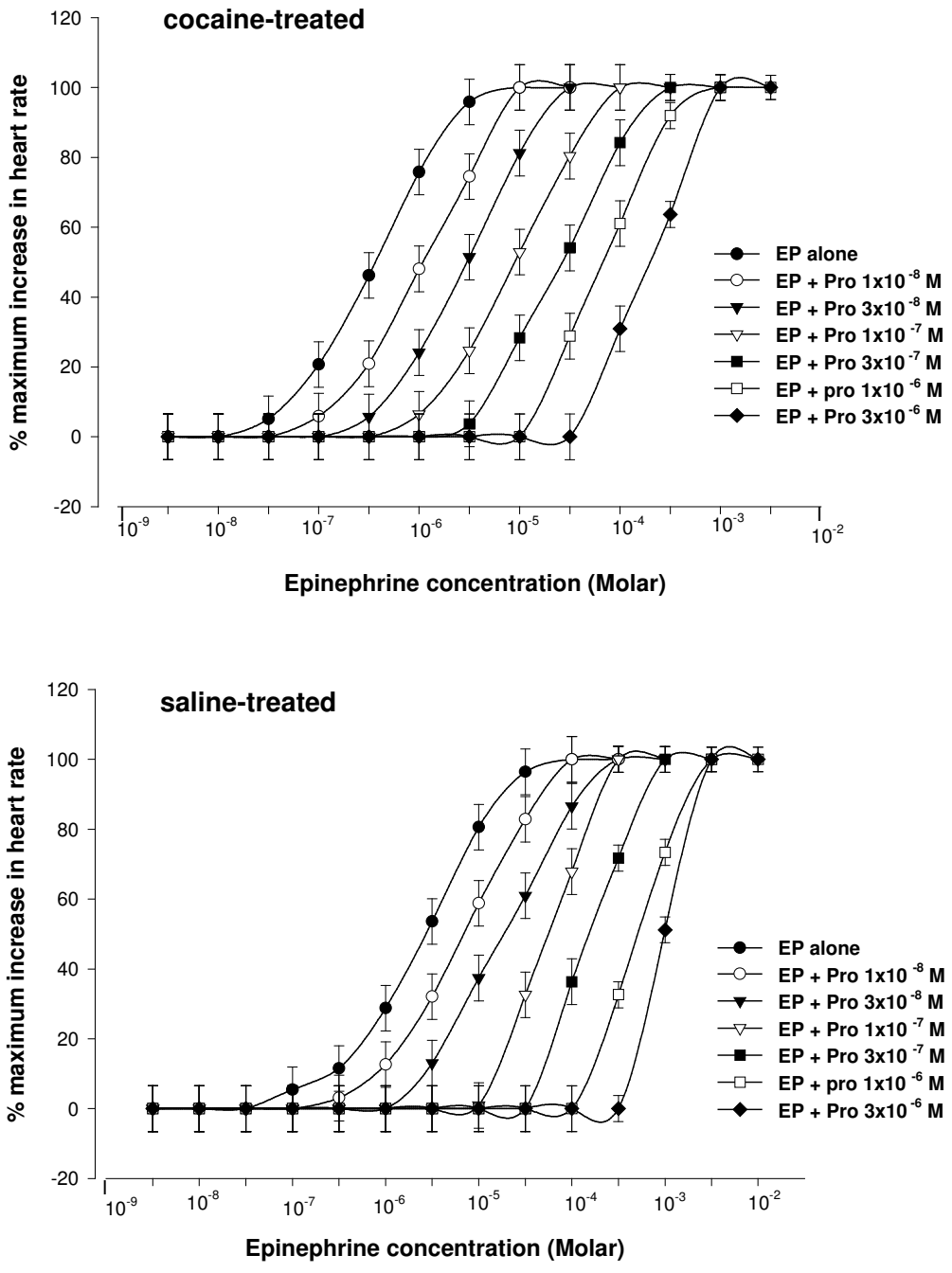


Figure 29 Effects of propranolol (Pro) on the cumulative-concentration response curves of *positive chronotropic effects* of epinephrine (EP) on isolated atria of cocaine-treated (above)

and saline-treated (below) guinea-pigs. Symbols represent means and vertical bars represent standard error of means (n = 8).

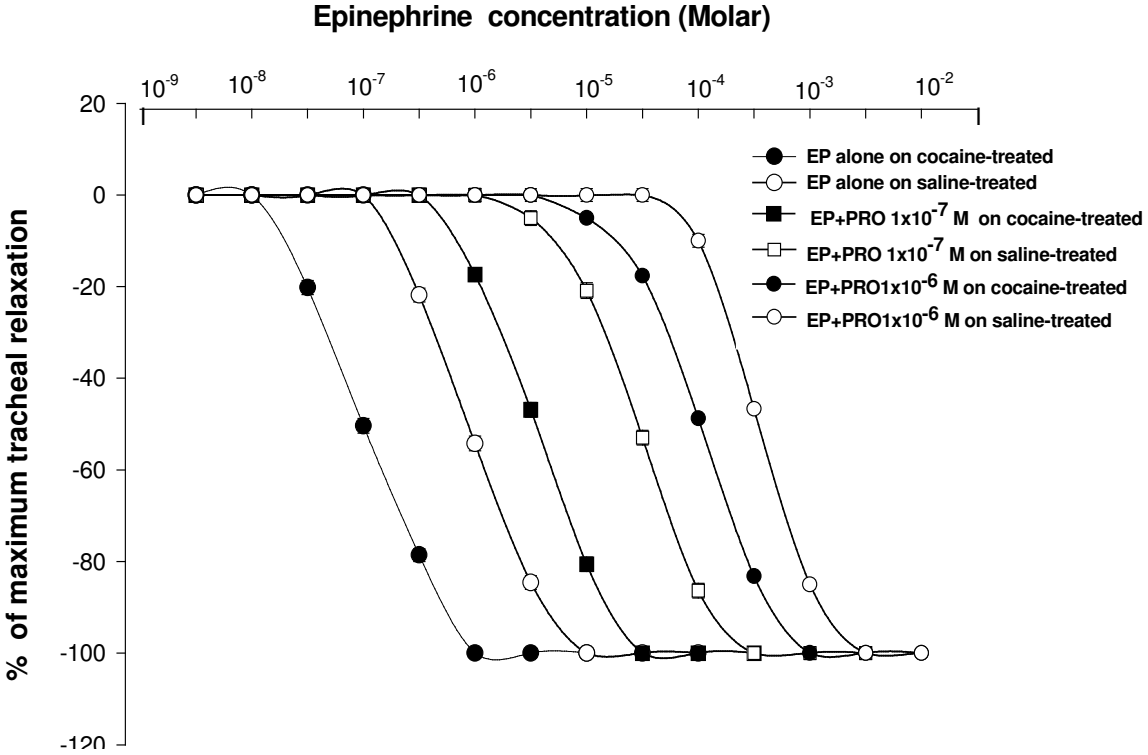


Figure 30 Effects of some concentrations (10^{-7}-10^{-6} M) of propranolol on the cumulative-concentration stimulation curves of epinephrine *on tracheal relaxation* on isolated trachea of cocaine-treated group compared to those of saline-treated

group. Symbols represent means and vertical bars represent standard error of means (n = 8).

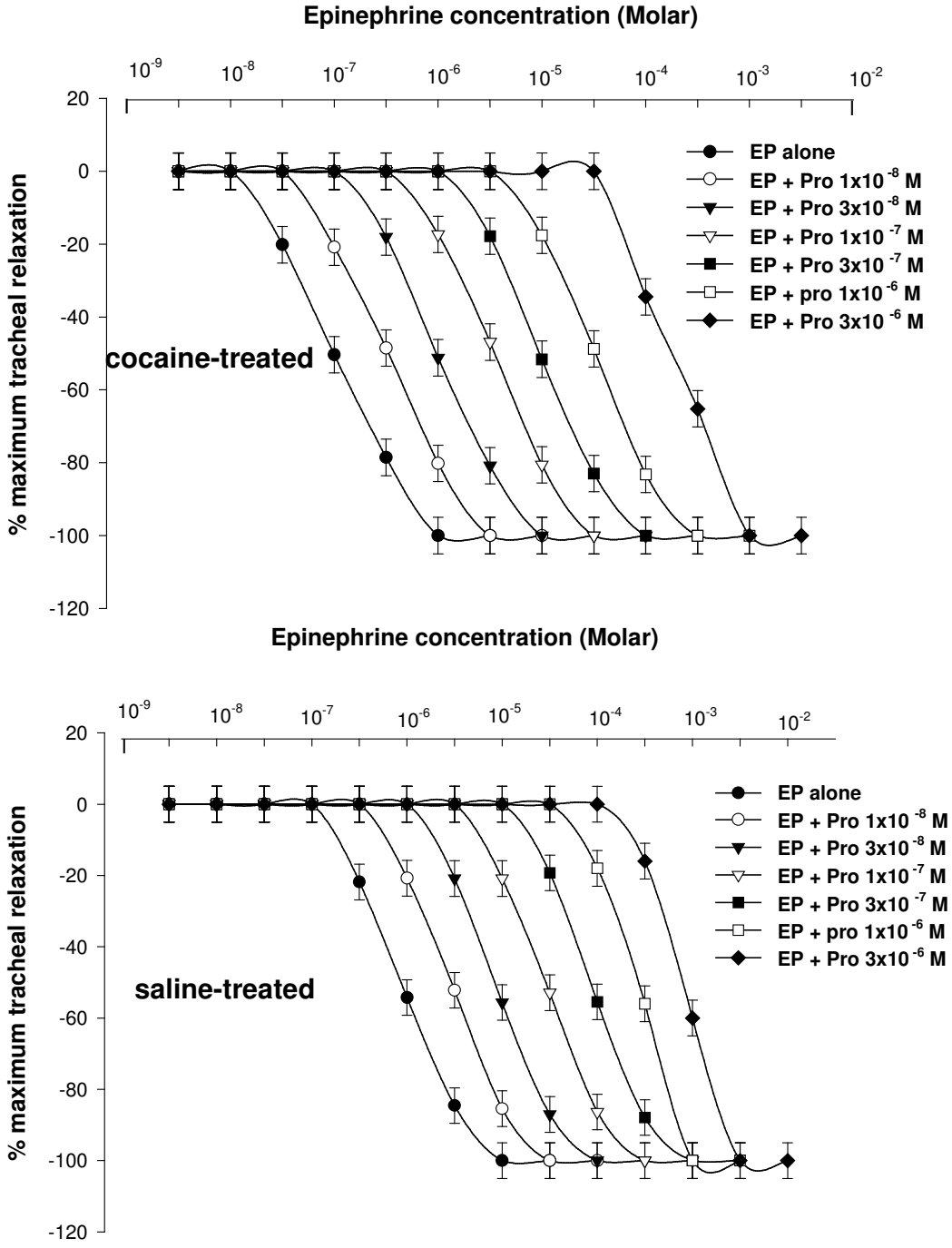


Figure 31 Effects of propranolol (Pro) on the cumulative-concentration response curves of *tracheal relaxing effects* of epinephrine (EP) on isolated trachea of cocaine (above)- and saline-treated (below)guinea-pigs. Symbols represent means and vertical bars represent standard error of means (n = 8).

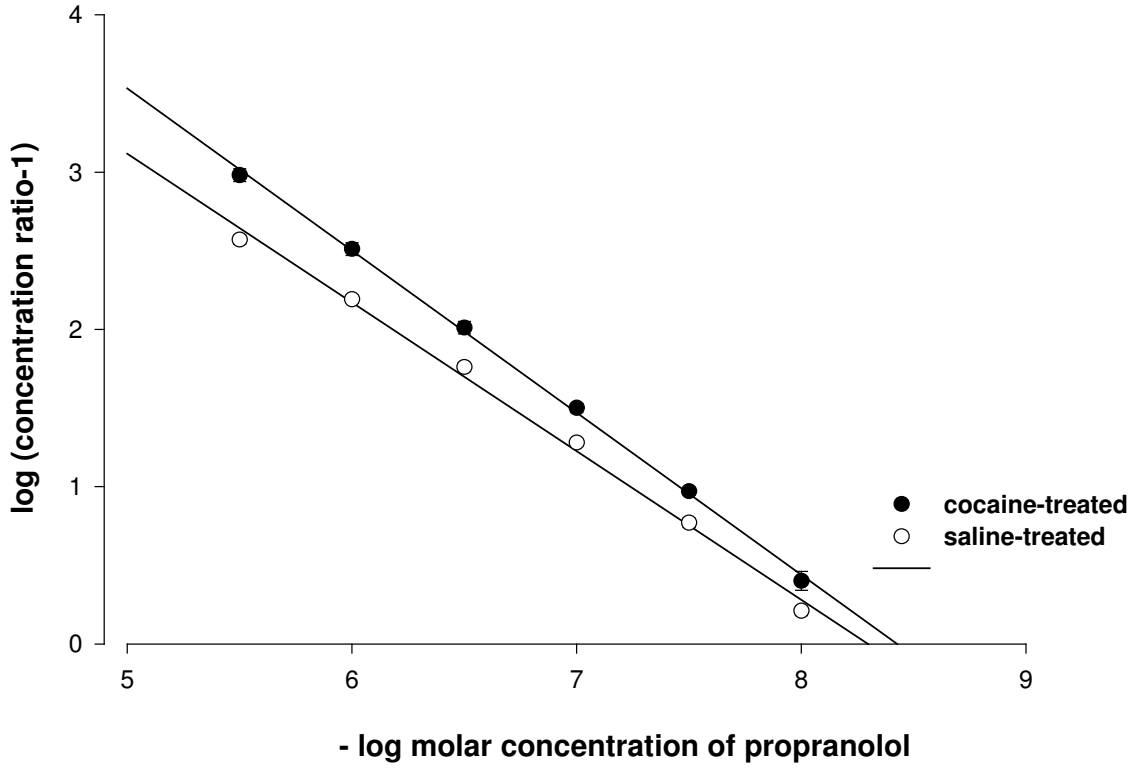


Figure 32 Schild plot for determination of pA_2 values of the antagonism of propranolol on the *positive inotropic response* to epinephrine of cocaine- and saline-treated guinea-pigs

according to the responses in Fig. 27. The pA2 values of propranolol in cocaine- and saline-treated were 8.42 and 8.30, respectively, slope of the regression line were 1.03 and 0.94 respectively. Each point is presented as a mean ± S.E.M of eight experiments.

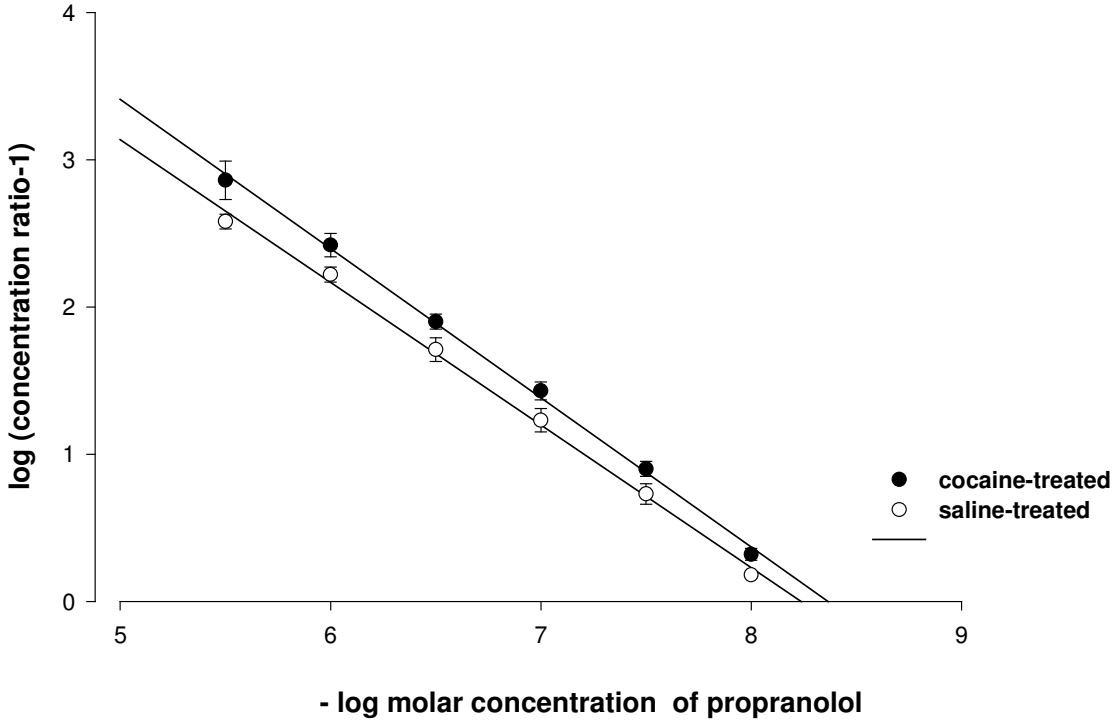


Figure 33 Schild plot for determination of pA_2 values of the antagonism of propranolol on the *positive chronotropic response* to epinephrine of cocaine and saline-treated guinea-

pigs according to the responses in Fig. 29. The pA2 values of propranolol in cocaine- and saline-treated were 8.37 and 8.24, respectively, slope of the regression line were 1.01 and 0.97 respectively. Each point is presented as a mean ± S.EM of eight experiments.

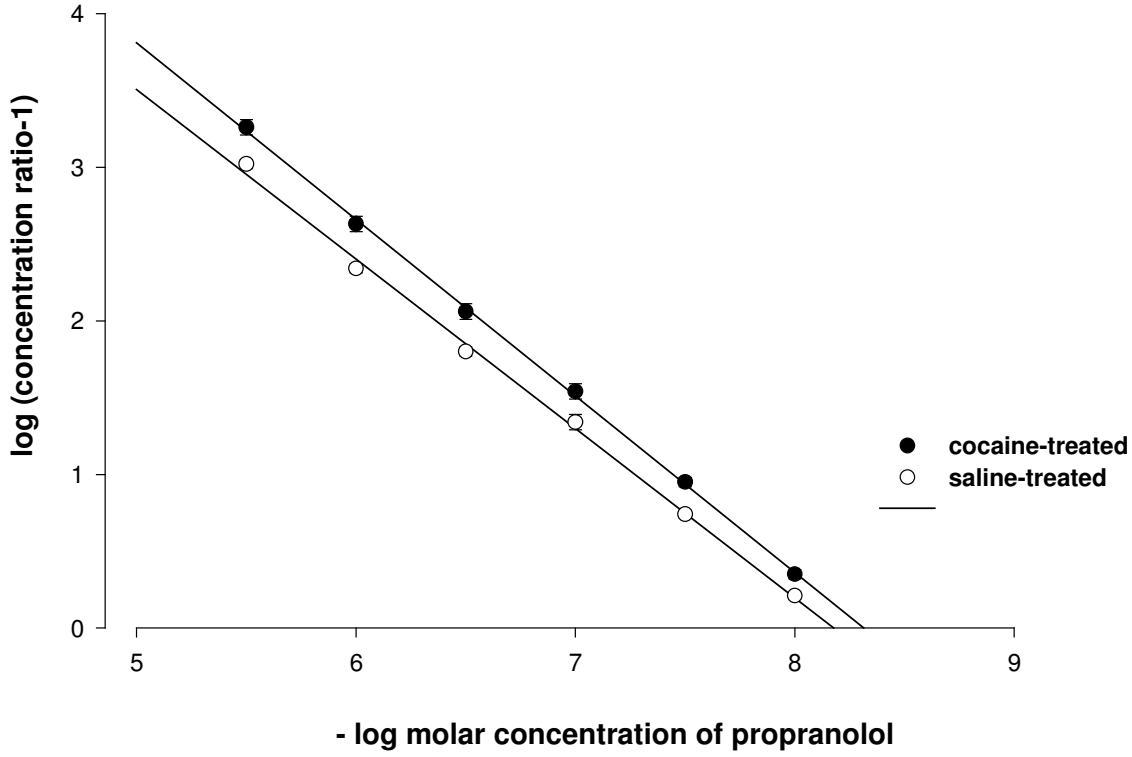
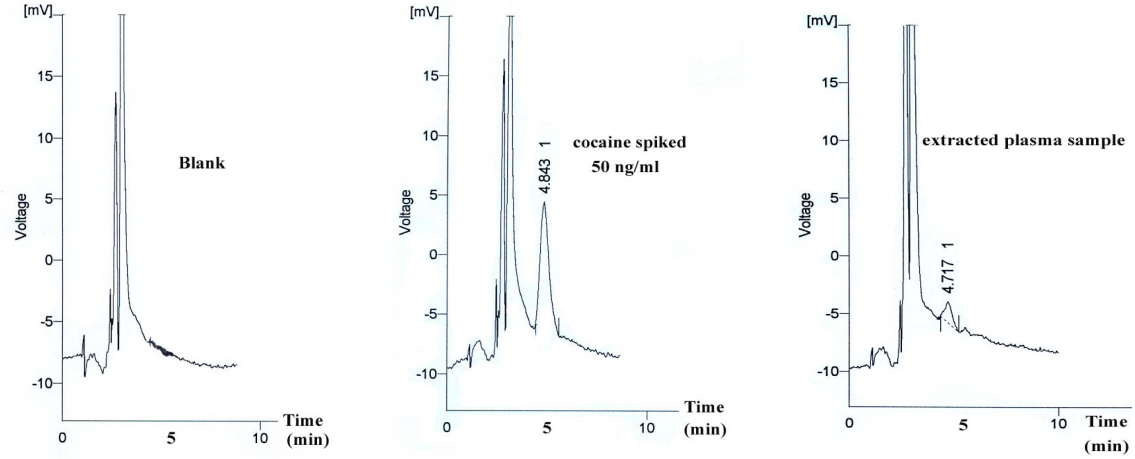


Figure 34 Schild plot for determination of pA_2 values of the antagonism of propranolol on the *tracheal relaxing response* to epinephrine of cocaine- and saline-treated guinea-pigs

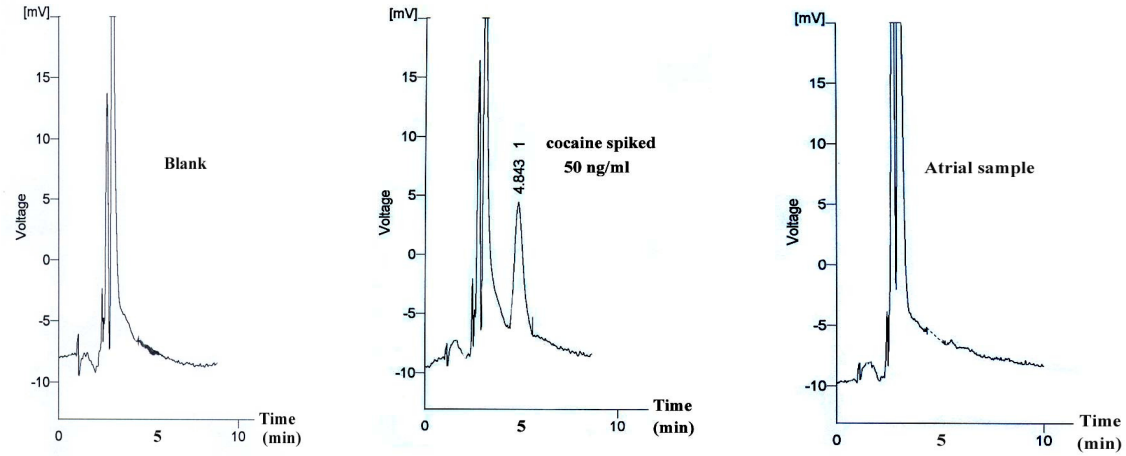
according to the response in Fig. 31. The pA2 values of propranolol in cocaine and saline-treated were 8.31 and 8.12, respectively, slope of the regression line were 1.15 and 1.11 respectively. Each point is presented as a mean ± S.E.M of eight experiments.

Figure 35 The representative chromatograms of cocaine in plasma, atria, ventricle, and tracheal tissues; (1) blank (2) standard cocaine spiked 50 ng/ml and (3) sample taken from plasma, atria, ventricular, and tracheal smooth muscle in cocaine-treated guinea-pig at 24 hr after cocaine cessation.



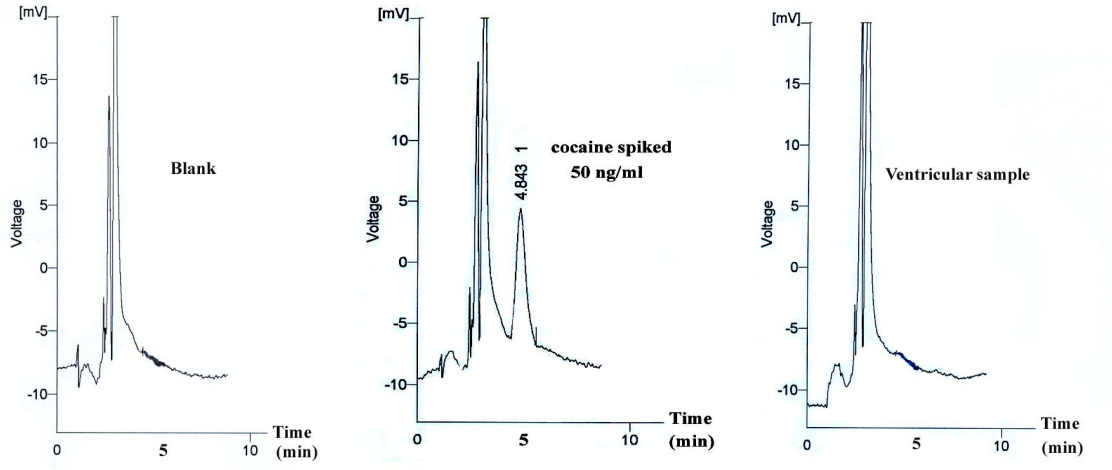
(1) (2) (3)

(b) Chromatogram of atria



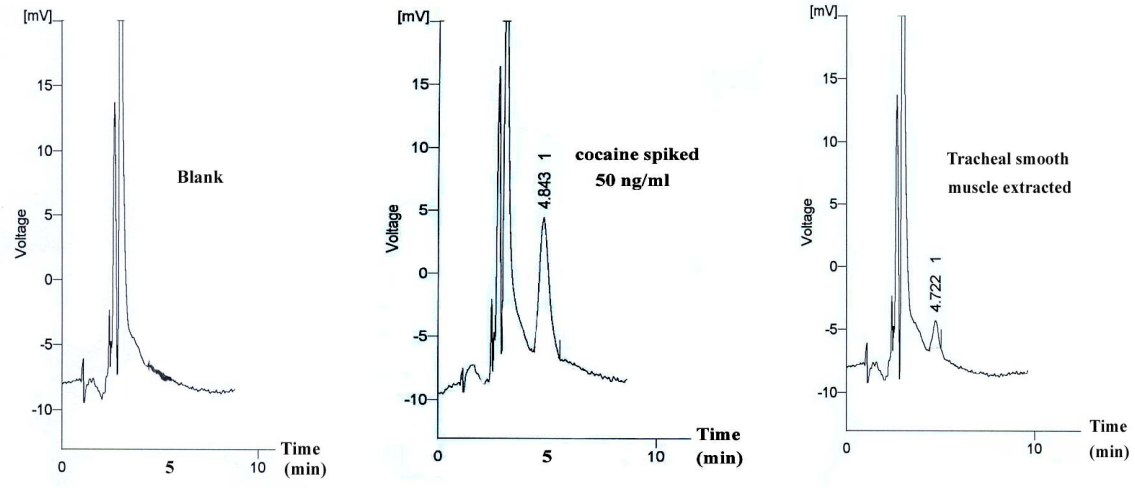
(1) **(2)** **(3)**

(c) Chromatogram of ventricle



(1) **(2)** **(3)**

(d) Chromatogram of tracheal smooth muscle



(1) **(2)** **(3)**

Table 9 The $D_{max50}$and pD_2 values of the *positive inotropic*, *chronotropic* and *tracheal relaxing effects* of epinephrine and salbutamol in isolated guinia-pigs atria and trachea

Table 10 The pA_2 values for propranolol on the inhibition of the responsiveness of positive inotropic, positive chronotropic and relaxing effects of exogenous epinephrine on the isolated atria and trachea of cocaine and saline-treated guinea-pig (n=8)

N	pA_2					
	Force of contraction		Heart Rate		Relaxation of trachea	
	treatment	control	treatment	control	treatment	control
1	8.37	8.29	8.27	8.24	8.27	8.16
2	8.42	8.28	8.35	8.31	8.28	8.16
3	8.38	8.28	8.36	8.29	8.35	8.16
4	8.41	8.22	8.47	8.30	8.34	8.20
5	8.49	8.34	8.47	8.18	8.30	8.19

6	8.42	8.36	8.42	8.25	8.33	8.16
7	8.46	8.29	8.28	8.12	8.32	8.20
8	8.45	8.33	8.38	8.23	8.33	7.75
Mean	** 8.42	8.30	** 8.37	8.24	* 8.31	8.12
± S.E.	0.04	0.03	0.06	0.06	0.04	0.03

(*$P < 0.05$; **$P<0.01$ compared with the control groups)

Table 11 Comparison of the D_{max50} ratio (which indicated the degree of supersensitivity) of positive inotropic, positive chronotropic and tracheal relaxing effects of epinephrine (EP) and salbutamol(SAL) and cocaine concentration in plasma, cardiac, and tracheal tissues taken at 24 hr after cocaine cessation of 2.5 mg/kg of cocaine-treated guinea-pigs

D_{max50} ratio						Concentration of cocaine (Mean ±S.E.M)			
Inotropic		Chronotropic		Relaxing		Plasma (ng/ml)	Atria	Ventricle	Trachea (ng/g)
EP	SAL	EP	SAL	EP	SAL				
7.0	10.4	7.8	9.47	8.5	12.9	5.08±	ND	ND	2.80±

1	9	5		9	2	0.63			0.41

$[D]_{max50}$ ratio = $[D]_{max50}$ of EP or Sal of the control groups divided by those $[D]_{max50}$ of cocaine-treated group (see Table 4.1).
ND: Not detectable
The detection limit of cocaine in plasma, atrial, ventricular, and smooth muscle of trachea sample was 0.69 ng/ml, 17.5 ng/g, 3.8 ng/g, and 0.80 ng/g, respectively.

Table 12 Concentration of cocaine in *plasma* following 2.5 mg/kg of cocaine administration at 24 hr after cessation

N	Peak Area of Plasma (mV.sec) Repeatability 1	2	3	X	S.E.	Mean Conc. (ng/ml)	S.E
1	12.54	12.54	11.49	12.19	0.61	4.11	0.47
2	8.55	8.93	8.55	8.68	0.22	3.00	0.35
3	21.32	23.34	22.22	22.29	1.01	7.28	0.60

4	16.23	11.49	16.23	14.65	2.74	4.88	1.14
5	9.32	8.94	9.22	9.16	0.20	3.16	0.34
6	15.55	16.60	15.55	15.90	0.61	5.27	0.47
7	8.25	7.89	8.35	8.16	0.24	2.84	0.36
8	21.55	17.25	22.22	20.34	2.70	6.66	1.13
9	22.61	23.89	22.61	23.04	0.74	7.51	0.51
10	18.17	16.83	20.79	18.60	2.01	6.12	0.91
Mean				15.301	1.11	5.08	0.63
Equation	Peak Area = 3.188(concentration)-0.9						

Table 13 Concentration of cocaine in *tracheal smooth muscle* following 2.5 mg/kg of cocaine administration at 24 hr after cessation

N	Peak Area of Trachea (mV.sec)					Mean	Mean	
	Repeatability					Conc.	Conc.	S.E
	1	2	3	X	S.E.	(ng/ml)	ng/g	
1	8.46	8.54	8.52	8.51	0.04	2.16	2.51	0.30
2	8.32	8.11	8.09	8.17	0.13	2.05	2.39	0.32
3	9.21	10.38	9.21	9.60	0.68	2.49	2.91	0.49
4	9.55	8.34	8.32	8.73	0.70	2.22	2.59	0.50
5	6.55	7.46	6.52	6.85	0.53	1.64	1.91	0.45
6	8.89	8.79	7.25	8.31	0.92	2.09	2.44	0.57
7	8.28	8.15	8.20	8.21	0.07	2.06	2.40	0.30
8	8.69	8.34	8.69	8.57	0.20	2.17	2.53	0.35
9	9.78	9.19	9.78	9.58	0.34	2.49	2.90	0.39
10	9.56	9.38	8.56	9.17	0.53	2.36	2.75	0.45
Mean				8.57	0.41	2.40	2.80	0.41
Equation	Peak Area = 3.232(concentration)+1.544							