CHAPTER - 4

RESULTS

4. RESULTS

4.1. Pharmacological Experiments

4.1.1. Splenic strip

4.1.1.1. Responses to NA and Adr and their modification by cocaine and alpha-adrenoceptor blockers

NA (0.85×10^{-9} M x 3.82×10^{-8} M) and Adr (0.27×10^{-9} M - 1.74×10^{-8} M) elicited dose-related contractile effects (Fig. 11).

Cocaine had no effect on the concentration-response curves of NA and Adr (Fig. 12).

The concentration-response curves for the contractile effects of NA and Adr were shifted to the right in a parallel manner by phentolamine (Fig. 11;Table 4 a and b). From the dose-ratios obtained with different concentrations of phentolamine, pA plots were constructed (Fig. 13). The pA_2 values of phentolamine were 8.0 with NA and 8.06 with Adr. The corresponding slope values of the plots were -0.79± 0.25 and -0.60± 0.29.

Phenoxybenzamine (0.32×10^{-6} M) blocked responses to NA (0.85×10^{-6} M - 3.82×10^{-8} M; n = 6) and Adr (0.27×10^{-9} M - 1.74×10^{-8} M; n = 6) completely and irreversibly.

4.1.1.2. Responses to tyramine and their modification by some blockers

Tyramine elicited reproducible concentration-related (1.07×10^{-5} M - 3.46×10^{-3} M) contractile response (Fig. 14; Table 4c).

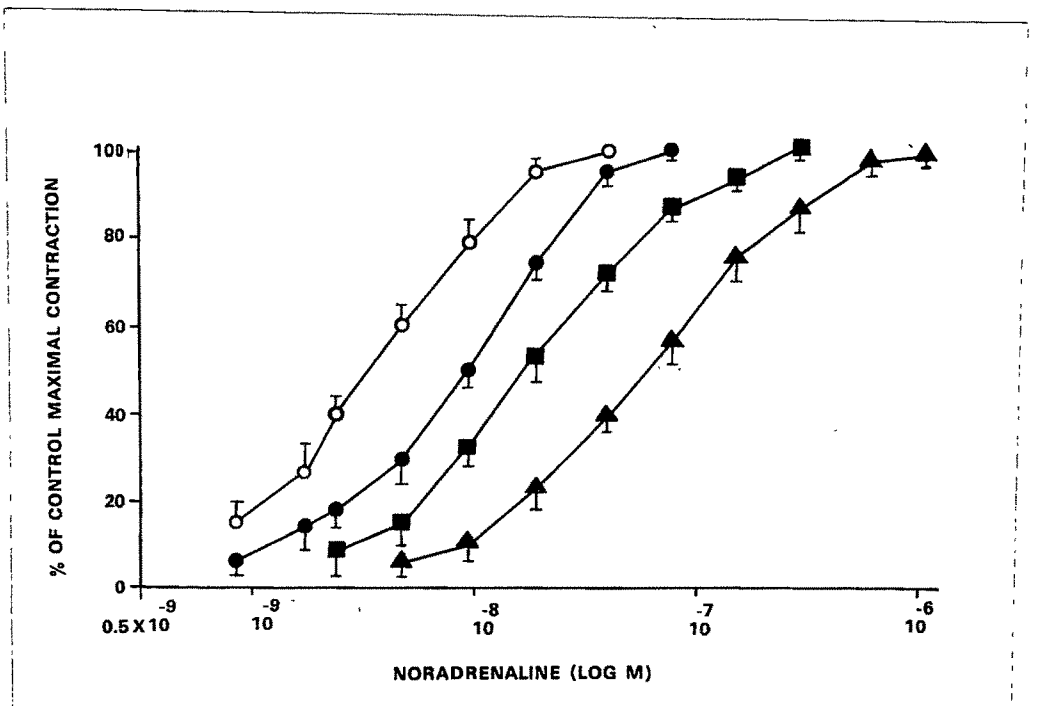
Phentolamine, reserpine and cocaine significantly inhibited the concentration-response curves of tyramine in a non-competitive manner (Fig. 14). In the same concentrations phentolamine blocked responses to NA competitively (Fig. 11a) but reserpine and cocaine had no effect on the concentration-response curves of NA (Fig. 12a).

Fig. 11

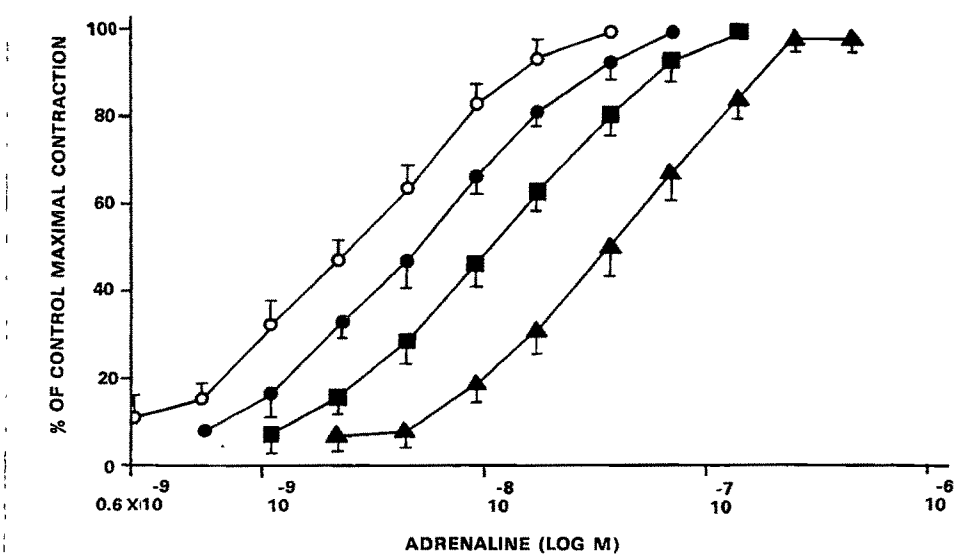
Concentration-response curves for the contractile effect on goat isolated spleen of noradrenaline (a) and adrenaline (b). (o——o) depicts control responses and (●——●), (■——■) and (▲——▲) depict responses obtained in the presence of 1.26×10^{-8} M; 6.3×10^{-8} M and 6.3×10^{-7} M phentolamine respectively. Vertical lines indicate S.E.M. (n = 6-8 for each curve).

Fig. 11

(a)


% OF CONTROL MAXIMAL CONTRACTION
100
80
60
40
20
0
0.5 X 10 -9
10 -9
10 -8
10 -7
10 -6
NORADRENALINE (LOG M)

(b)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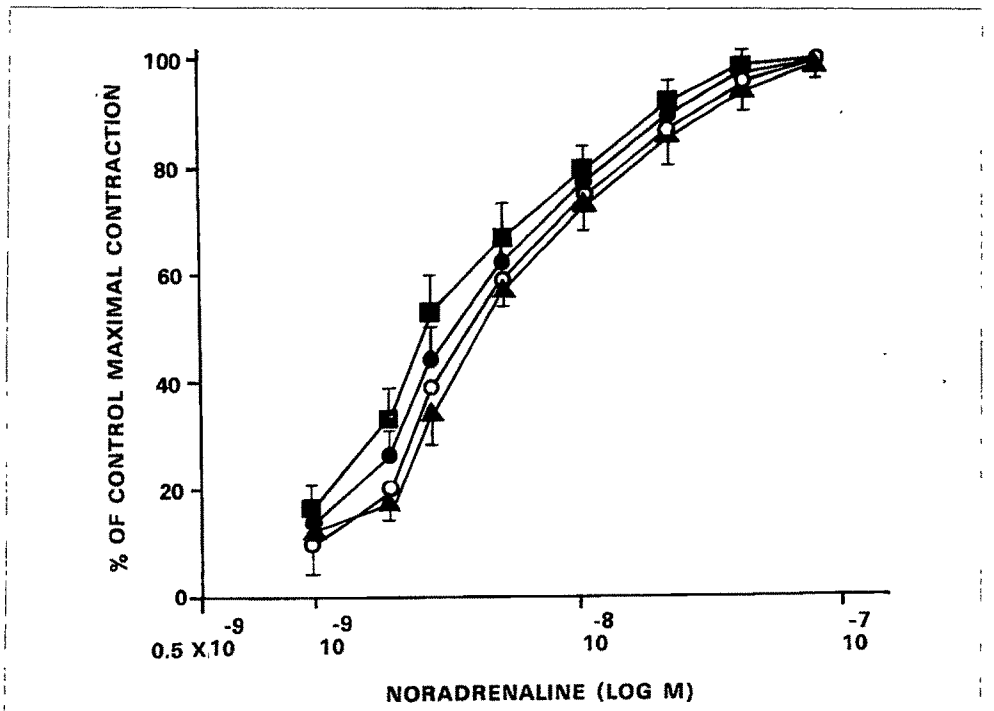
0.6 X 10 -9
10 -9
10 -8
10 -7
10 -6
ADRENALINE (LOG M)

Fig. 12

Concentration-response curves for the contractile effects on goat isolated spleen of noradrenaline (a) and adrenaline (b). (o——o) depicts control responses and (●——●) and (■——■) depict responses obtained in the presence of 1×10^{-5} M and 3×10^{-5} M cocaine respectively. In addition (▲——▲) in (a) depicts responses obtained in the presence of reserpine (5.43×10^{-6} M). Vertical lines indicate S.E.M. ($n = 6$-8 for each curve).

FIG. 12

(a)


% OF CONTROL MAXIMAL CONTRACTION
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0.5 X10 -9
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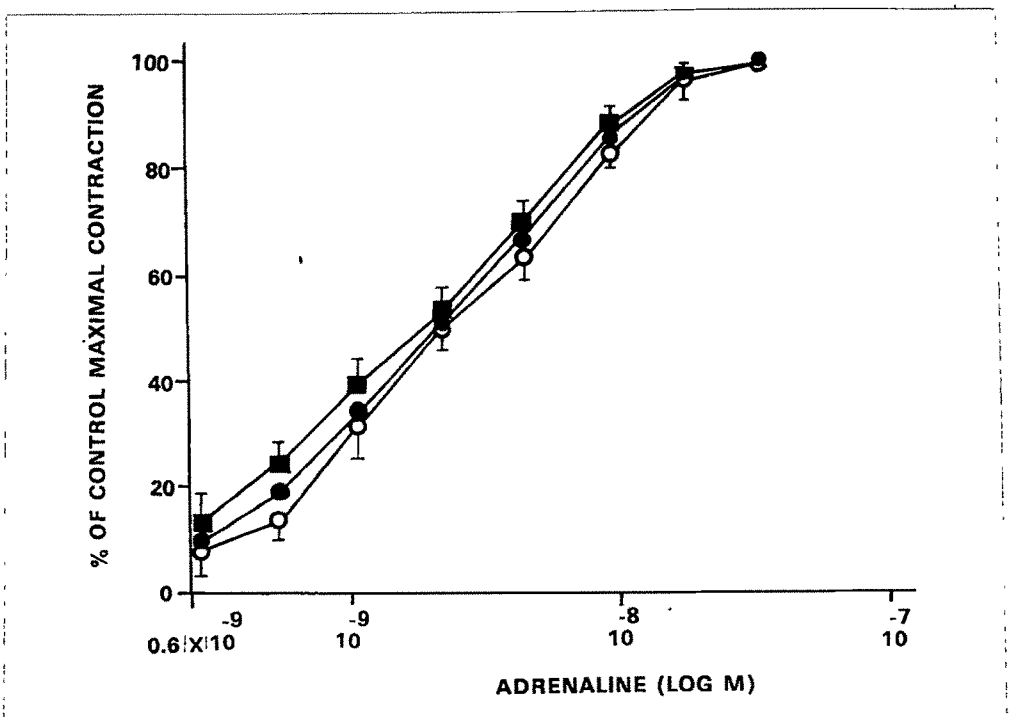
NORADRENALINE (LOG M)

(b)


% OF CONTROL MAXIMAL CONTRACTION
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0.6 X10 -9
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10 -8
10 -7
ADRENALINE (LOG M)

Table 4 a. EC_{50} values of NA and Adr obtained with goat isolated spleen in the absence and presence of phentolamine. The dose-ratio is the ratio of EC_{50} values in the presence of blocker to that in its absence.

Agonist	Blocker	n	EC_{50} ± S.E.M.	Dose-ratio
Noradrenaline	-	8	$3.39 \pm 0.25 \times 10^{-9}$ M	-
"	Phentolamine (1.26×10^{-8} M)	6	$9.55 \pm 0.17 \times 10^{-9}$ M *	2.87
"	Phentolamine (6.30×10^{-8} M)	6	$1.62 \pm 0.03 \times 10^{-8}$ M *	4.80
"	Phentolamine (6.30×10^{-7} M)	6	$4.58 \pm 0.08 \times 10^{-8}$ M *	14.51
Adrenaline	-	8	$2.29 \pm 0.26 \times 10^{-9}$ M	-
"	Phentolamine (1.26×10^{-8} M)	6	$5.21 \pm 0.14 \times 10^{-9}$ M *	2.28
"	Phentolamine (6.30×10^{-8} M)	6	$1.07 \pm 0.03 \times 10^{-8}$ M *	4.79
"	Phentolamine (6.30×10^{-7} M)	6	$3.06 \pm 0.05 \times 10^{-8}$ M *	14.40

n = Number of observations

* = Significant ($P < 0.01$)

Table 4 b. EC_{50} values of NA and Adr obtained with goat isolated spleen in the absence and presence of cocaine and reserpine. The dose-ratio is the ratio of EC_{50} values in the presence of blocker to that in its absence.

Agonist	Blocker	n	$EC_{50} \pm$ S.E.M.	Dose-ratio
Noradrenaline	-	8	$3.31 \pm 0.24 \times 10^{-9}$ M	-
"	Cocaine (1×10^{-5} M)	6	$2.28 \pm 0.27 \times 10^{-9}$ M	-
"	Cocaine (3×10^{-5} M)	6	$2.23 \pm 0.31 \times 10^{-9}$ M	-
"	Reserpine (5.43×10^{-6} M)	7	$3.89 \pm 0.41 \times 10^{-9}$ M	-
Adrenaline	-	8	$2.18 \pm 0.23 \times 10^{-9}$ M	-
"	Cocaine (1×10^{-5} M)	7	$2.08 \pm 0.16 \times 10^{-9}$ M	-
"	Cocaine (3×10^{-5} M)	6	$1.82 \pm 0.31 \times 10^{-9}$ M	-

n = Number of observations

Fig. 13

pA plots for the alpha-adrenoceptor blocking effects of phentolamine obtained with goat isolated spleen using noradrenaline (●——●) and adrenaline (▲——▲) as the agonists. The negative log molar concentration of phentolamine is plotted on the abscissa and log (dose ratios -1) as the ordinate. The points were obtained by regression analysis (n = 6 for each plot). The regression coefficients for the lines obtained with noradrenaline and adrenaline were -0.74 ± 0.25 and -0.60 ± 0.29 respectively and are not significantly different from the theoretical value of unity for competitive antagonism.

FIG. 13

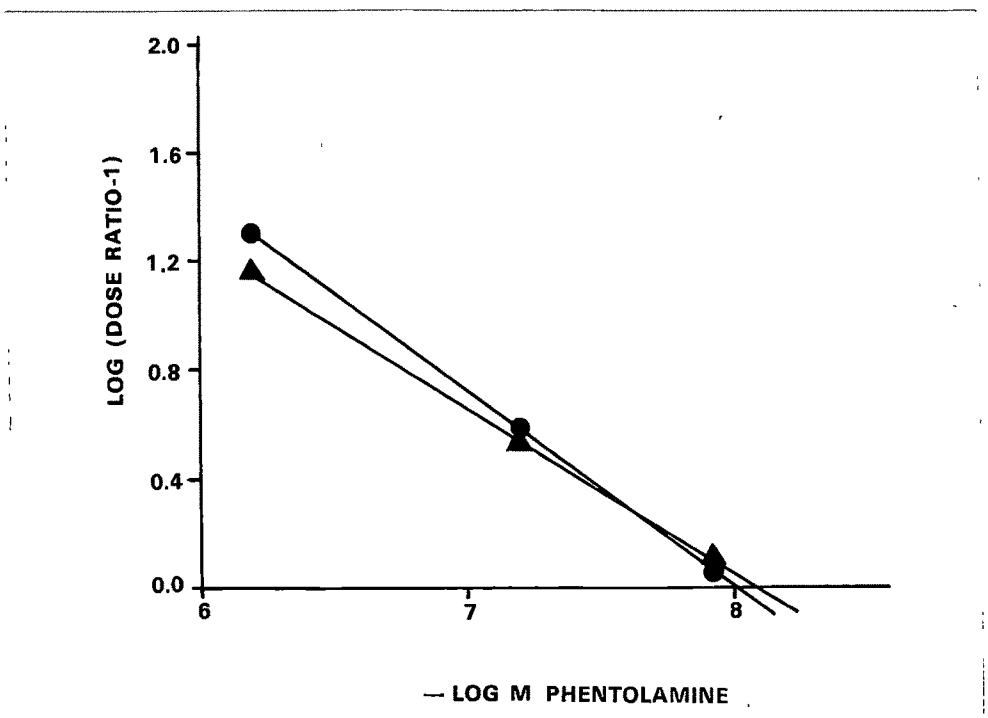
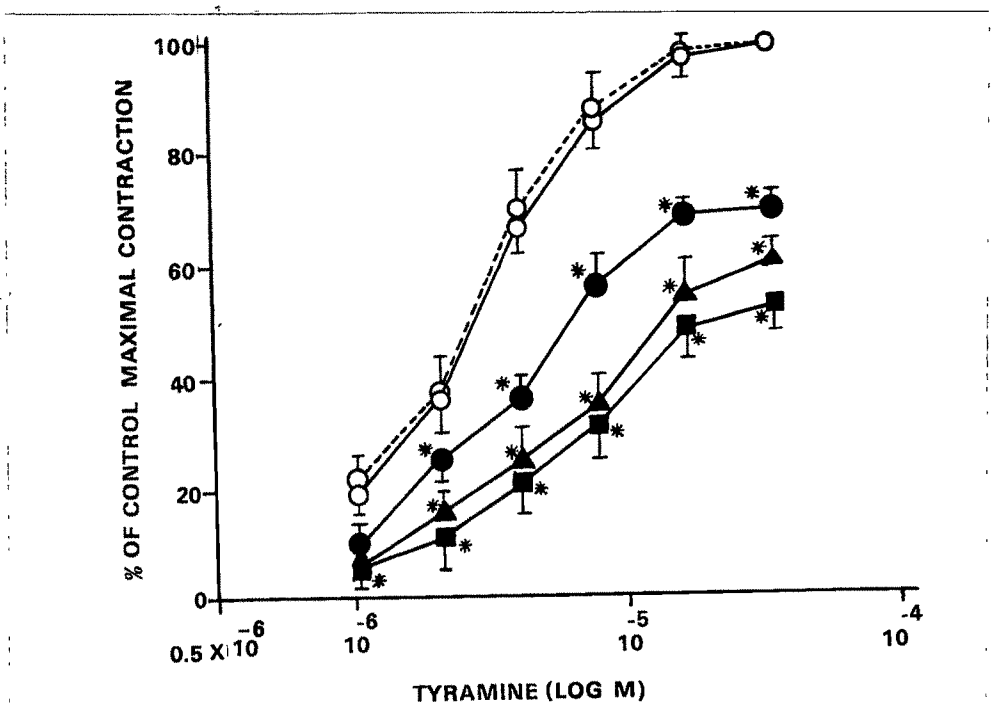


Fig. 14

Concentration-response curves for the contractile effects on goat isolated spleen of tyramine. The control response depicted by (○———○) was first elicited. This was followed by a rest period of 60 min and another control response depicted by (○- - - -○) was elicited. (●———●), (■———■) and (▲———▲) depict responses elicited in the presence of phentolamine (1.26×10^{-8} M), cocaine (1×10^{-5} M) and reserpine (5.43×10^{-6} M) respectively. Vertical lines depict S.E.M. (n = 6-8 for each curve). The asterisks denote $P < 0.01$.

FIG. 14


% OF CONTROL MAXIMAL CONTRACTION
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0
0.5 X 10^-6
10^-6
10^-5
10^-4
TYRAMINE (LOG M)

Table 4 c. EC_{50} values of tyramine with goat isolated spleen in the absence and presence of some blockers. The dose-ratio is the ratio of EC_{50} values in the presence of blocker to that in its absence.

Agonist	Blocker	n	$EC_{50} \pm$ S.E.M.	Dose-ratio
Tyramine	-	8	$2.81 \pm 0.09 \times 10^{-5}$ M	-
"	Phentolamine (1.26×10^{-8} M)	7	$6.63 \pm 0.08 \times 10^{-5}$ M *	2.30
"	Cocaine (1×10^{-5} M)	6	$3.02 \pm 0.08 \times 10^{-4}$ M *	10.50
"	Reserpine (5.43×10^{-5} M)	6	$1.45 \pm 0.06 \times 10^{-4}$ M *	5.04

n = Number of observations

* = Significant ($P < 0.01$)

4.1.1.3. Effect of desensitization with naphazoline, tyramine and NA

Prior exposure to high concentrations of naphazoline (8.13×10^{-4} M) or tyramine (0.83×10^{-3} M) abolished submaximal responses to naphazoline (2.44×10^{-7} M) and tyramine (5.66×10^{-5} M), but had no effect on those to NA (2.4×10^{-8} M). On the other hand, prior exposure to a high concentration of NA (1.83×10^{-4} M) abolished submaximal responses to NA, but had no effect on those to a naphazoline and tyramine (Fig. 15).

4.1.1.4. Effect of ISO

ISO (9×10^{-7} M - 3.6×10^{-6} M) failed to produce any effect per se or any relaxation of KCl-induced spasm (n = 5 each). Higher concentrations of ISO (1.63×10^{-5} M - 2.58×10^{-4} M) per se produced concentration-related contractions (Fig. 16). Phenoxybenzamine (0.32×10^{-6} M) blocked the contractile responses to ISO (1.63×10^{-5} M - 2.58×10^{-4} M; n = 5) completely and irreversibly.

4.1.1.5. Responses to ACh and their modification by physostigmine, atropine and hexamethonium

ACh (1.68×10^{-7} M - 3.38×10^{-5} M) produced concentration-related contractions (Fig. 17).

Physostigmine (7.72×10^{-6} M) did not potentiate ACh-induced contractions. On the other hand, the ACh-induced concentration-response curve was shifted rightwards by physostigmine. The shift was parallel and there was no change in the maxima (Fig. 17; Table 5). The EC_{50} values of ACh without and with physostigmine were $1.31 \pm 0.21 \times 10^{-6}$ M and $2.69 \pm 0.12 \times 10^{-6}$ M) respectively ($P < 0.01$).

Fig. 15

The histograms represent contractile responses of goat isolated spleen to 2.44×10^{-7} M naphazoline (▬), 5.66×10^{-5} M tyramine (▭) and 2.4×10^{-8} M noradrenaline (▩). At A, B and C the tissues were exposed for 60 min to 8.13×10^{-4} M naphazoline, 0.83×10^{-3} M tyramine and 1.84×10^{-4} M noradrenaline respectively before reeliciting responses to the agonists. In the high concentrations used all the three agonists produced equivalent maximal responses. Vertical lines indicate S.E.M. ($n = 6$-8 for each histogram). The asterisks denate $P < 0.01$.

FIG. 15

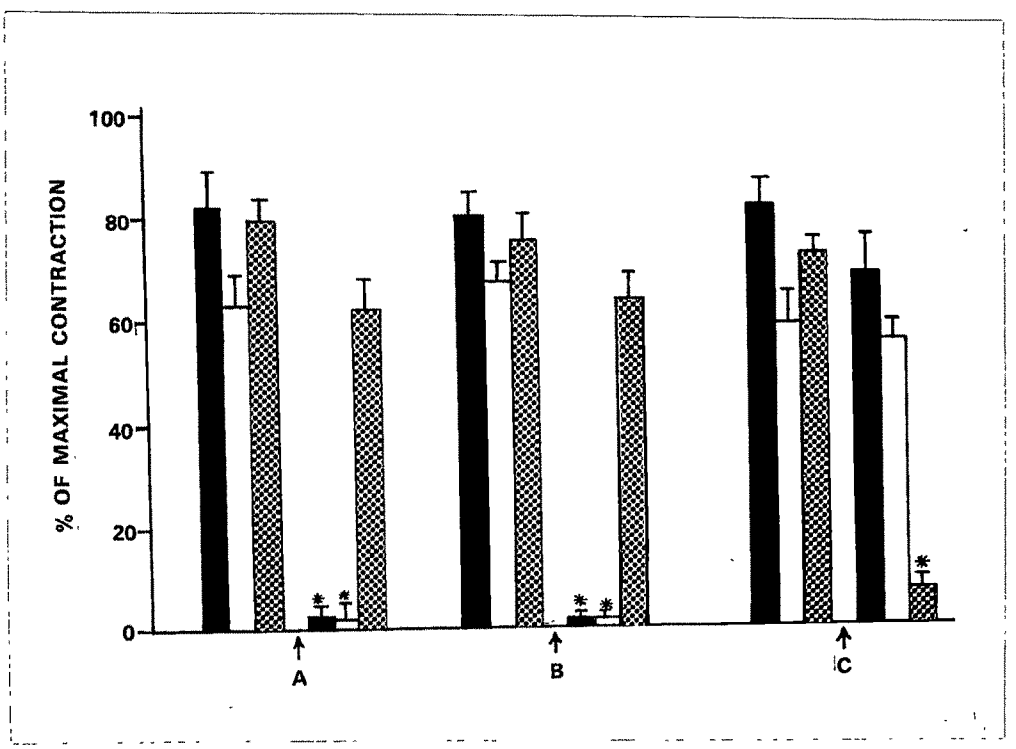


Fig. 16

Concentration-response curve for the contractile effect on goat isolated spleen of isoprenaline. Vertical lines indicate S.E.M. (n = 5).

FIG. 16

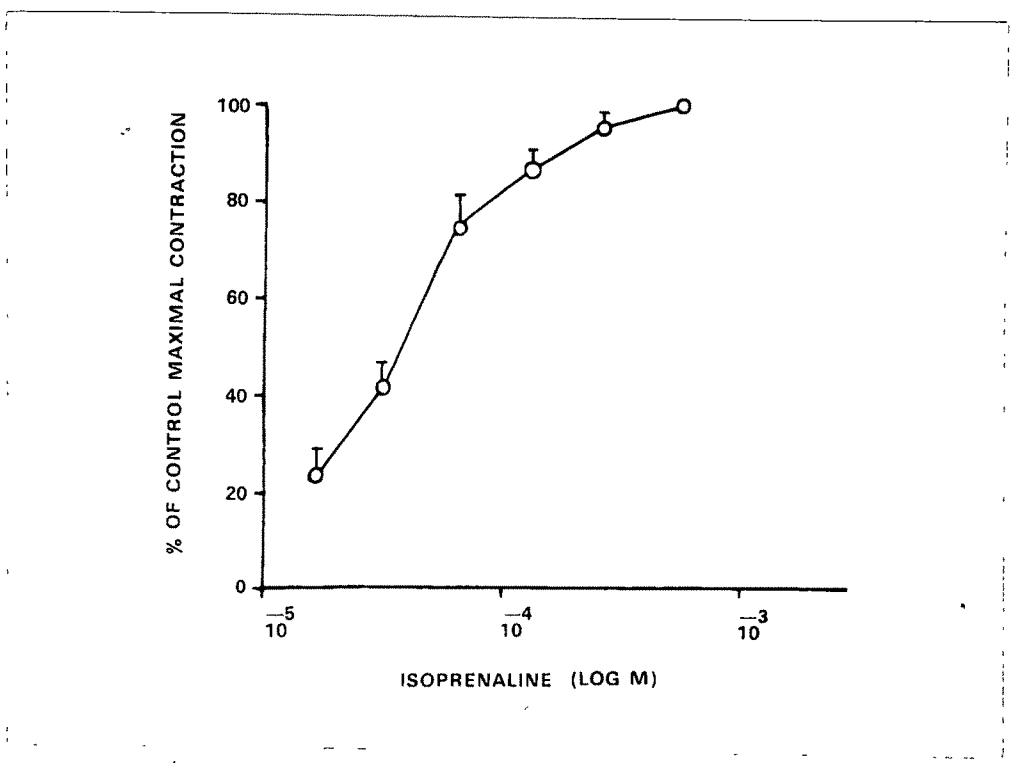

% OF CONTROL MAXIMAL CONTRACTION
100
80
60
40
20
0
10^{-5}
10^{-4}
10^{-3}
ISOPRENALINE (LOG M)

Fig. 17

Concentration-response curves for the contractile effects on goat isolated spleen of acetylcholine. (o——o) depicts control responses and (▲——▲) depicts responses obtained in the presence of 7.72×10^{-6} M physostigmine. Vertical lines indicate S.E.M. (n = 6-8 for each curve). The asterisks denote $P < 0.01$.

FIG. 17

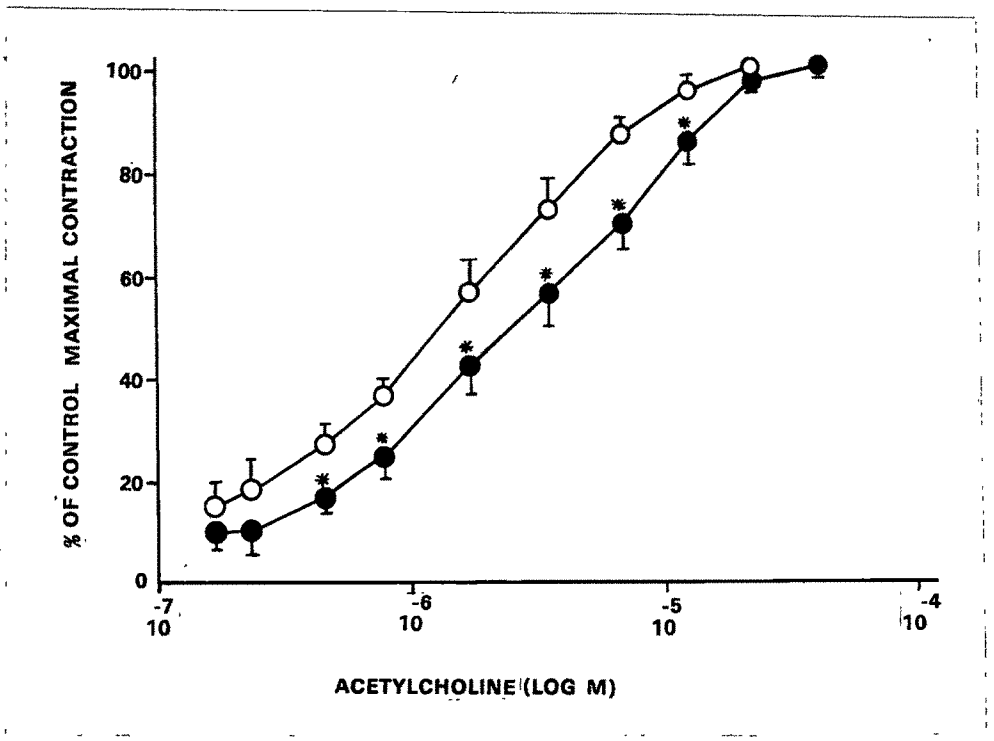

% OF CONTROL MAXIMAL CONTRACTION
100
80
60
40
20
0
-7
10
-6
10
-5
10
-4
10
ACETYLCHOLINE (LOG M)

Table 5. EC_{50} values of ACh obtained with goat isolated spleen in the absence and presence of atropine or physostigmine. Dose-ratio is the ratio of EC_{50} values of agonist in the presence of antagonist to that in its absence.

Agonist	Blocker	n	EC_{50} ± S.E.M.	Dose-ratio
Acetylcholine	-	4	$1.25 \pm 0.21 \times 10^{-6}$ M	-
"	Atropine (4.32×10^{-8} M)	4	$2.57 \pm 0.13 \times 10^{-6}$ M *	2.04
"	Atropine (1.44×10^{-7} M)	4	$7.64 \pm 0.09 \times 10^{-6}$ M *	6.06
"	Atropine (4.32×10^{-7} M)	4	$6.86 \pm 0.04 \times 10^{-5}$ M *	54.5
Acetylcholine	-	8	$1.31 \pm 0.20 \times 10^{-6}$ M	-
"	Physostigmine (7.72×10^{-6} M)	6	$2.69 \pm 0.12 \times 10^{-6}$ M *	2.05
"	Hexamethonium (2.76×10^{-5} M)	5	$1.29 \pm 0.31 \times 10^{-5}$ M	-

n = Number of observations

* = Significant ($P < 0.01$)

Atropine (4.32×10^{-8} M, 1.44×10^{-7} M, 4.32×10^{-7} M) produced parallel shift of the concentration-response curve of ACh to the right without affecting the maximal responses (Fig. 18; Table 5). The slope value for the pA plot was -1.08 ± 0.34 ($n = 4$) and was not significantly different from the theoretical slope value of unity for competitive antagonism. The pA_2 value of atropine was 7.6 (Fig. 19).

The responses to ACh were not affected by hexamethonium (2.76×10^{-5} M, $n = 5$; Table 5).

4.1.1.6. Responses to 5-HT and their modification by phentolamine, cyproheptadine, lignocaine, cocaine and reserpine

5-HT (3.94×10^{-6} M - 6.31×10^{-5} M) elicited concentration-related contractions (Fig. 20). The subsequent concentration-response curves elicited at intervals of 60 min became smaller and smaller in magnitude indicating tachyphylaxis (Fig. 20; $n = 6$).

EC_{50} concentration of 5-HT (7.89×10^{-6} M) administered at intervals of 30 min produced reproducible responses ($45 \pm 7.1\%$ NA maximal response). Phentolamine (6.30×10^{-6} M) significantly blocked responses to 5-HT (7.89×10^{-6} M) and NA (2.4×10^{-8} M; Fig. 21) while cyproheptadine (7.0×10^{-6} M), xylocaine (2.61×10^{-4} M) and reserpine (5.43×10^{-6} M) significantly blocked response only to 5-HT (7.89×10^{-6} M). Cocaine (3×10^{-5} M) did not alter significantly responses to 5-HT (7.89×10^{-6} M) and NA (2.4×10^{-8} M; Fig. 21).

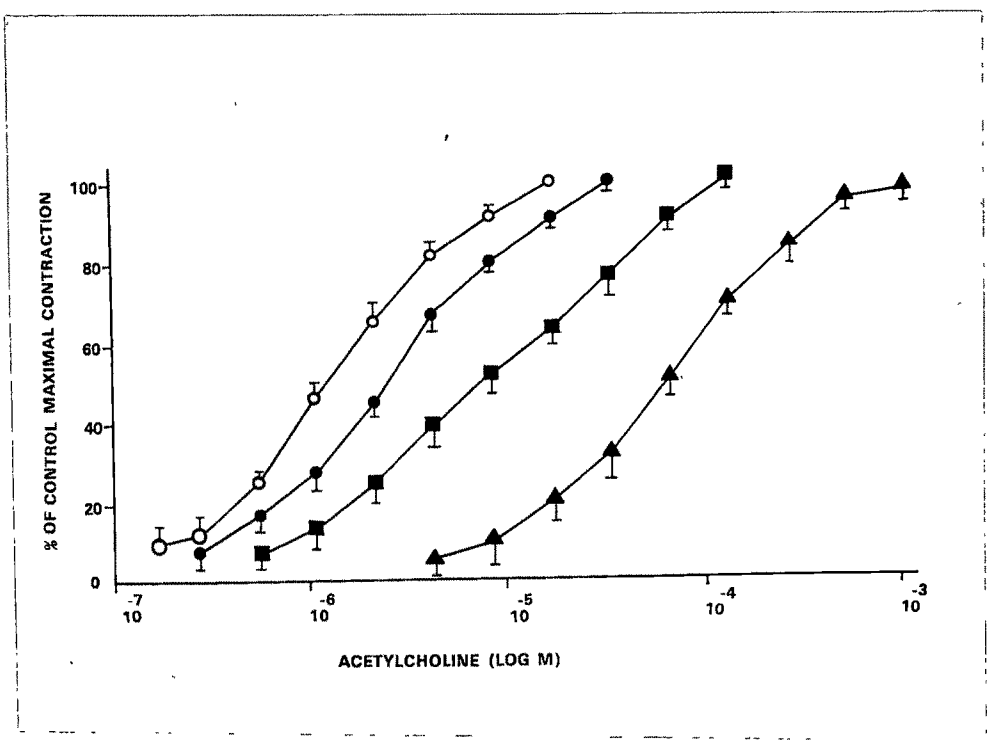
4.1.1.7. Effect of Ca^{++}-free physiological solution on responses to NA, tyramine, 5-HT and KCl

In Ca^{++}-free medium countaining EDTA (1.10×10^{-5} M) responses to 5-HT

Fig. 18

Concentration-response curves for the contractile effects on goat isolated spleen of acetylcholine. (o——o) depicts control responses and (●——●), (■——■) and (▲——▲) depict responses obtained in the presence of 4.32×10^{-8} M, 1.44×10^{-7} M and 4.32×10^{-7} M atropine respectively. Vertical lines indicate S.E.M. ($n = 4$ for each curve).

FIG. 18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-4
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-3
10
ACETYLCHOLINE (LOG M)

Fig. 19

pA plot for the muscarinic-receptor blocking effects of atropine obtained with goat isolated spleen using ACh as the agonist. The negative log molar concentration of atropine is plotted on the abscissa and log (dose ratio -1) as the ordinate. The points were obtained by regression analysis (n = 4). The regression coefficient for the line was -1.08 ± 0.34 and is not significantly different from the theoretical value of unity for competitive antagonism.

FIG. 19

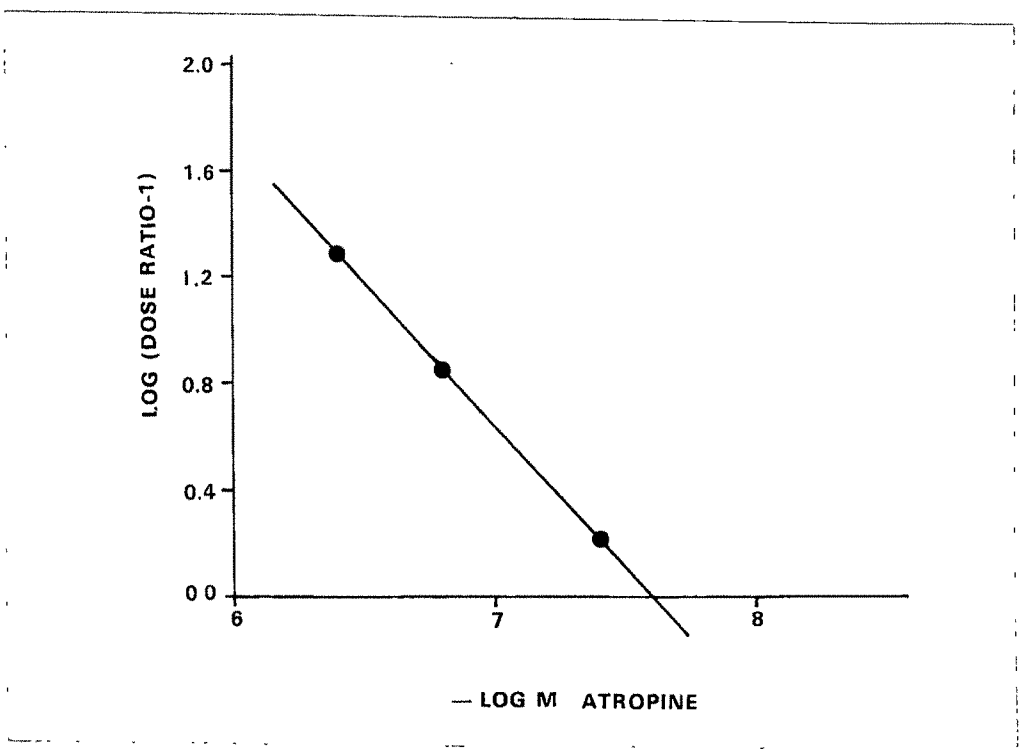


Fig. 20

Concentration-response curves for the contractile effect on goat isolated spleen of 5-HT (3.94×10^{-6} M - 6.31×10^{-5} M). In each panel, the first addition of 5-HT was begun at dots with the lowest dose and increments in doses were made in an arithmetical progression. A rest period of 60 min was allowed between each panel. Note the tachyphylaxis. Time mark, 1 minute.

Fig. 20

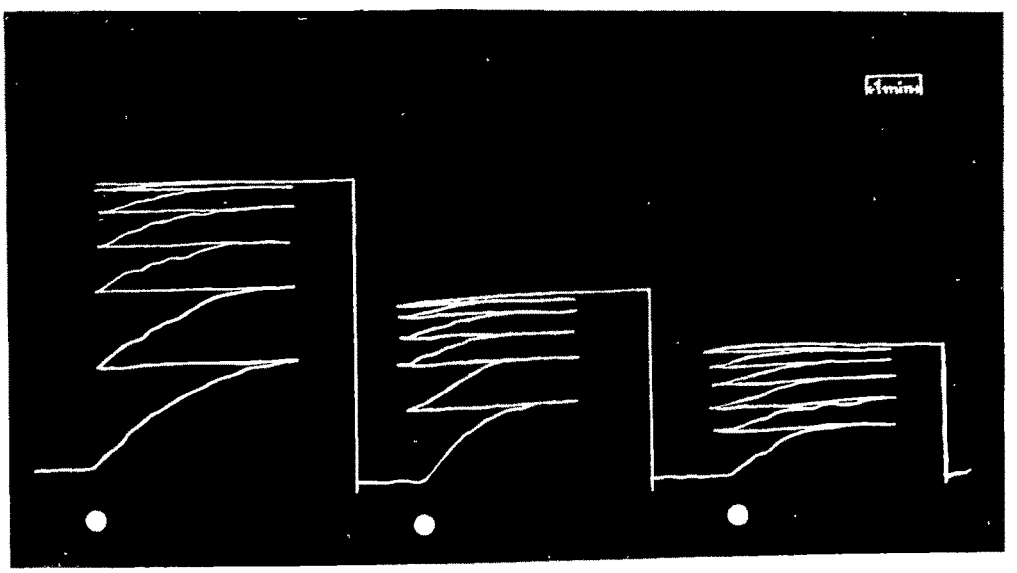
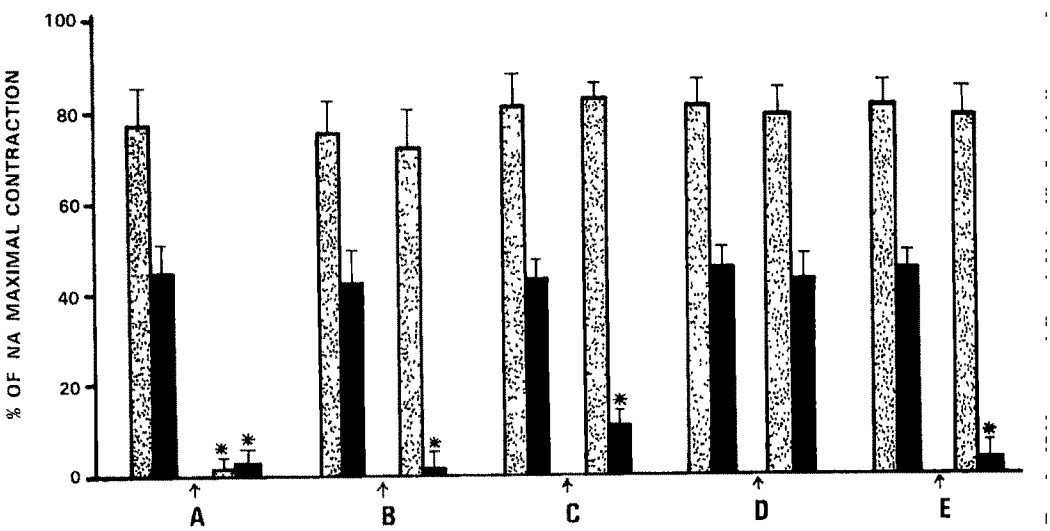


Fig. 21

The histograms represent contractile responses of goat isolated spleen to 2.40×10^{-8} M noradrenaline (▒▒▒) and 7.89×10^{-6} M 5-hydroxytryptamine (■■■). At A, B, C, D and E the tissues were exposed for 10 min to 6.30×10^{-6} M phentolamine, 7.0×10^{-6} M cyproheptadine, 2.61×10^{-4} M xylocaine, 3×10^{-5} M cocaine and 5.43×10^{-6} M reserpine respectively before reeliciting responses to the agonists. Each histogram represents % of the control NA maximal response. Vertical lines indicate S.E.M. ($n = 5$-6 for each histogram). The asterisks denote $P < 0.01$.

FIG. 21



(7.29×10^{-5} M) and KCl (2.69×10^{-2} M) were significantly inhibited compared to control responses (with normal Ca^{++}; 2.52×10^{-6} M) while those to NA (7.74×10^{-8} M) and tyramine (5.46×10^{-5} M) were not altered significantly (Fig. 22).

4.1.1.8. Responses to histamine and their modification by antazoline, metiamide, atropine, hexamethonium and phentolamine

Histamine (1.63×10^{-7} M - 5.21×10^{-5} M) produced concentration-related contractions (Fig. 23).

Antazoline (1.99×10^{-9} M, 7.97×10^{-9} M and 1.99×10^{-8} M) produced parallel shifts of the concentration-response curves of histamine to the right without affecting the maximum response (Fig. 23). The EC_{50} was increased significantly (Table 6).

The slope value of the pA plot was 1.14 ± 0.23 ($n = 4$) and was not significantly different from the theoretical value of unity for competitive antagonism. The pA_2 value of antazoline was 8.84 (Fig. 24).

Responses to histamine (1.63×10^{-7} M - 5.21×10^{-5} M) were not blocked by atropine (1.44×10^{-5} M), hexamethonium (2.76×10^{-5} M) and phentolamine added together to the bath (6.30×10^{-6} M; Table 6; $n = 6$).

4.1.2. Aortic strip

4.1.2.1. Responses to NA and their modification by cocaine, reserpine and phentolamine

NA (0.42×10^{-8} M - 1.92×10^{-7} M) elicited concentration-related contractile effects (Fig. 25).

Fig. 22

The histograms represent contractile responses of goat isolated spleen to 7.74×10^{-8} M NA (▒▒▒), 5.46×10^{-5} M tyramine (□), 7.38×10^{-6} M 5-hydroxytryptamine (■) and 2.69×10^{-2} M KCl (▨) obtained in Krebs-Henseleit medium containing 2.52 mM Ca. At arrow the tissue was preincubated for 15 min with Ca-free medium containing EDTA (1.10×10^{-5} M) followed by the addition of the agonists in the presence of Ca -free medium. Vertical lines indicate S.E.M. (n = 7 observations for each histogram). The asterisks denote $P < 0.01$.

FIG. 22

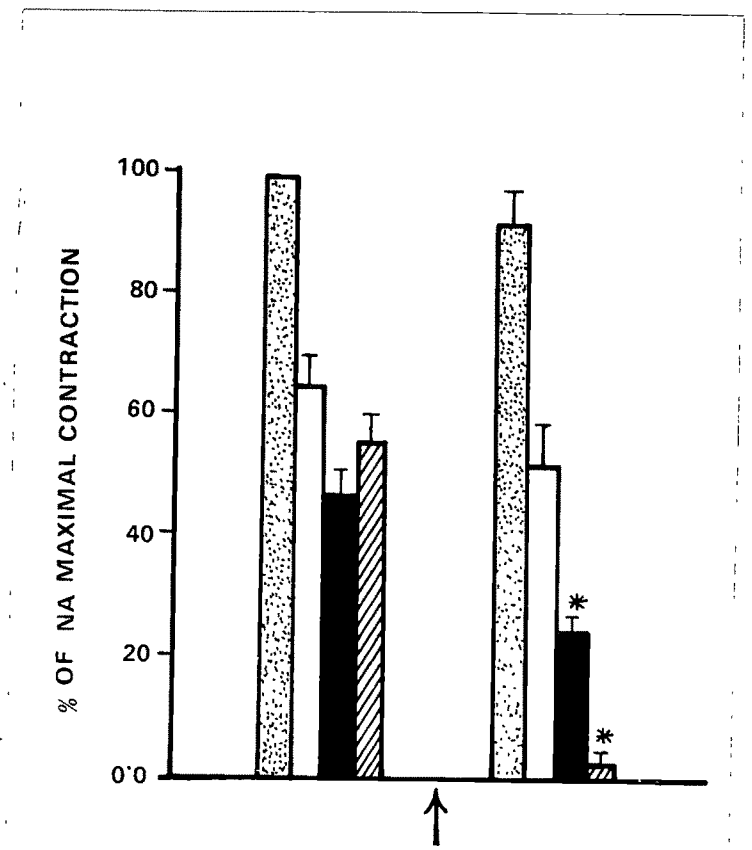
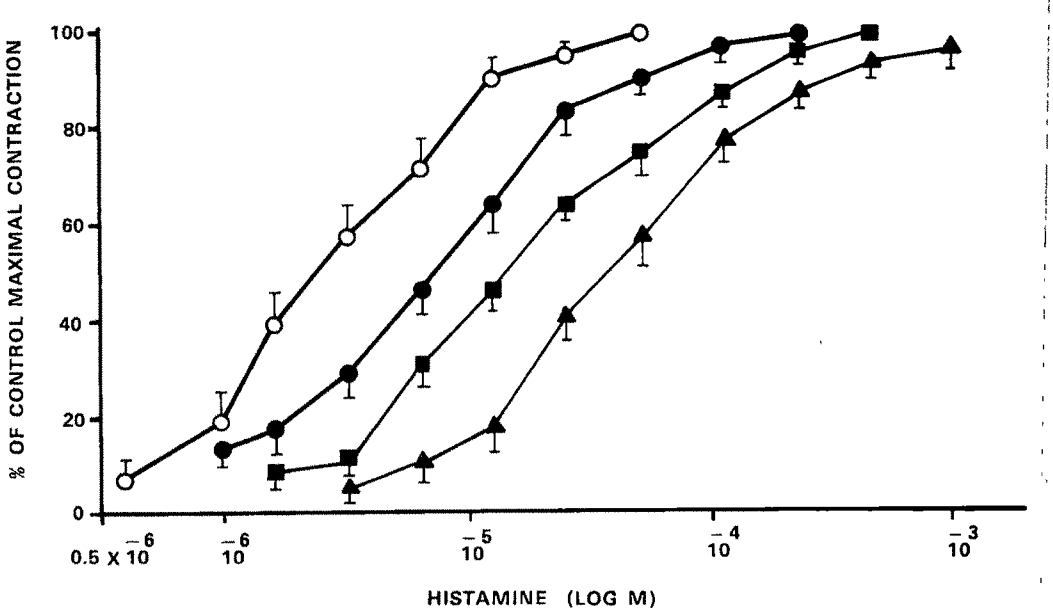


Fig. 23

Concentration-response curves for the contractile effects on goat isolated spleen of histamine. (○——○) depicts control responses and (●——●), (■——■) and (▲——▲) depict responses obtained in the presence of 1.99×10^{-9} M, 7.97×10^{-9} M and 1.99×10^{-8} M antazoline respectively. Vertical lines indicate S.E.M. (n = 5-6 for each curve).

FIG. 23


% OF CONTROL MAXIMAL CONTRACTION
100
80
60
40
20
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0.5 X 10^-6
10^-6
10^-5
10^-4
10^-3
HISTAMINE (LOG M)

Table 6. EC_{50} values of histamine with goat isolated spleen in the absence and presence of some blockers. The dose-ratio is the ratio of EC_{50} values of agonist in the presence of blocker to that in its absence.

Agonist	Blocker	n	EC_{50} ± S.E.M.	Dose-ratio
Histamine	-	6	$2.39 \pm 0.08 \times 10^{-6}$ M	-
"	Antazoline (1.99×10^{-9} M)	6	$7.52 \pm 0.11 \times 10^{-6}$ M **	2.73
"	Antazoline (7.97×10^{-9} M)	5	$1.43 \pm 0.03 \times 10^{-5}$ M *	6.01
"	Antazoline (1.99×10^{-8} M)	6	$3.52 \pm 0.06 \times 10^{-5}$ M *	14.75
Histamine	Atropine (1.44×10^{-6} M), Hexamethonium (2.76×10^{-5} M), Phentolamine (6.30×10^{-6} M)	6	$2.46 \pm 0.09 \times 10^{-6}$	-

n = Number of observations

* = Significant ($P < 0.01$)

** = Significant ($P < 0.05$)

Fig. 24

pA plots for the H_1-receptor blocking effect of antazoline obtained with goat isolated spleen using histamine as the agonist. The negative log molar concentration of antazoline is plotted on the abscissa and log (dose ratio -1) as the ordinate. The points were obtained by regression analysis (n = 5). The regression coefficient for the line was -1.14 ± 0.23 and is not significantly different from the theoretical value of unity for competitive antagonism.

FIG. 24

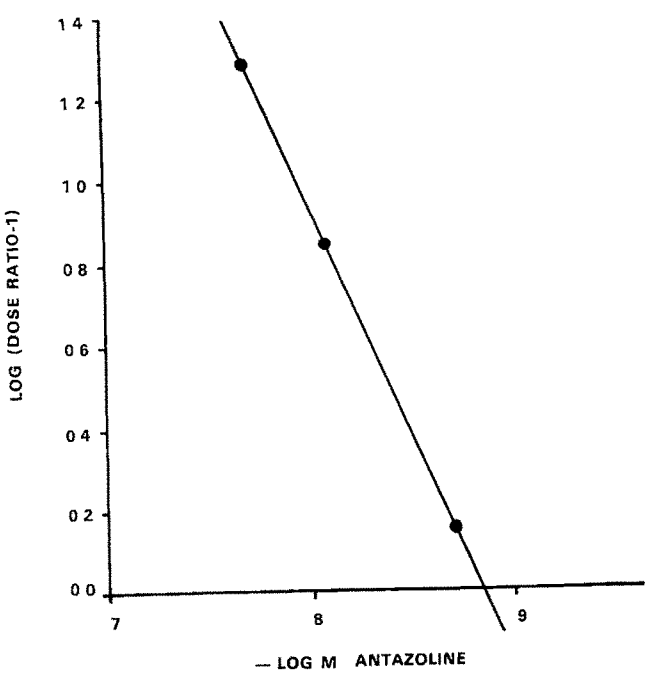
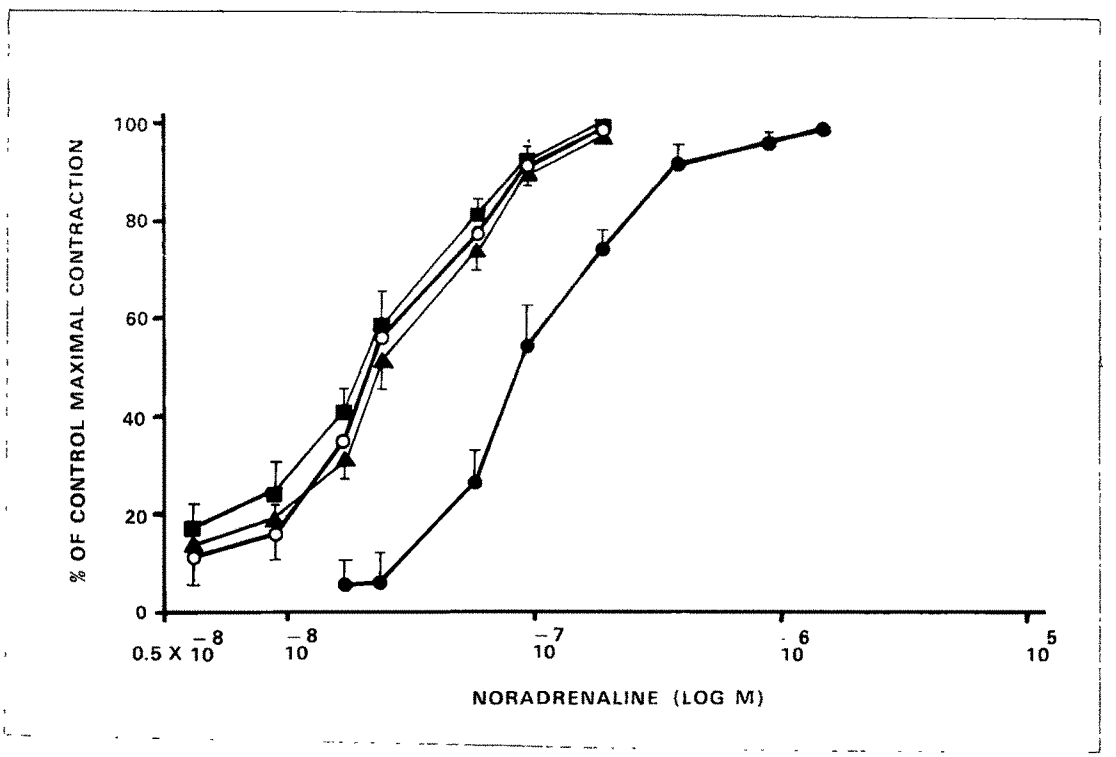


Fig. 25

Concentration-response curves for the contractile effect on goat isolated aortic strip of noradrenaline. (○——○) depicts control responses and (■——■), (▲——▲) and (●——●) depict responses obtained in the presence of 3×10^{-5} M cocaine, 5.43×10^{-6} M reserpine and 6.3×10^{-8} M phentolamine respectively. Vertical lines indicate S.E.M. (n = 6-8 for each curve).

FIG. 25


% OF CONTROL MAXIMAL CONTRACTION
100
80
60
40
20
0
0.5 X 10⁻⁸
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
NORADRENALINE (LOG M)

Cocaine (3.0×10^{-5} M) or reserpine (5.43×10^{-6} M) did not alter significantly the concentration-response curve of NA (Fig. 25) and the EC_{50} values were not significantly changed (Table 7).

Significant rightward shift without any change in the slope or maxima of concentration-response curve of NA was obtained with 6.3×10^{-8} M phentolamine (Fig. 25). The EC_{50} of NA was increased 4-fold (Table 7).

4.1.2.2. Responses to tyramine

No measurable response to tyramine (4.24×10^{-5} - 3.39×10^{-6} M) was obtained, precluding further experiments.

4.1.3. Tracheal chain

4.1.3.1. Responses to ISO and their modification by propranolol, phenoxybenzamine, normetanephrine and decreased bath temperature

ISO (2.25×10^{-9} M - 1.44×10^{-7} M) produced concentration-related relaxant effects in tracheal chain preparations contracted with pilocarpine (3.70×10^{-6} M; Fig. 26).

Significant rightward shifts of the concentration-response curves of ISO (2.25×10^{-9} M - 1.44×10^{-7} M) were obtained in the presence of propranolol (3.33×10^{-8} M, 9.99×10^{-8} M and 3.33×10^{-7} M; Fig. 26). The EC_{50} of ISO was increased significantly (Table 8). There was no effect on the slope or the maximum responses. The slope value for the pA plot was 0.84± 0.13 and was not significantly different from the theoretical value of unity for competitive antagonism. The pA_2 value of propranolol was 7.78 (Fig. 27).

Since phenoxybenzamine blocked the spasm-inducing action of pilocarpine, but not KCl, the latter agent was used to induce spasm in these experiments.

Table 7. EC_{50} values of noradrenaline with goat isolated aorta in the absence and presence of some blockers. The dose-ratio is the ratio of EC_{50} values in the presence of blocker to that in its absence.

Agonist	Blocker	n	EC_{50} ± S.E.M.	Dose-ratio
Noradrenaline	-	8	$2.14 \pm 0.16 \times 10^{-8}$ M	-
"	Cocaine (3×10^{-5} M)	6	$1.91 \pm 0.19 \times 10^{-8}$ M	-
"	Reserpine (5.43×10^{-6} M)	6	$2.30 \pm 0.18 \times 10^{-8}$ M	-
"	Phentolamine (6.30×10^{-8} M)	7	$8.70 \pm 0.07 \times 10^{-8}$ M *	4.06

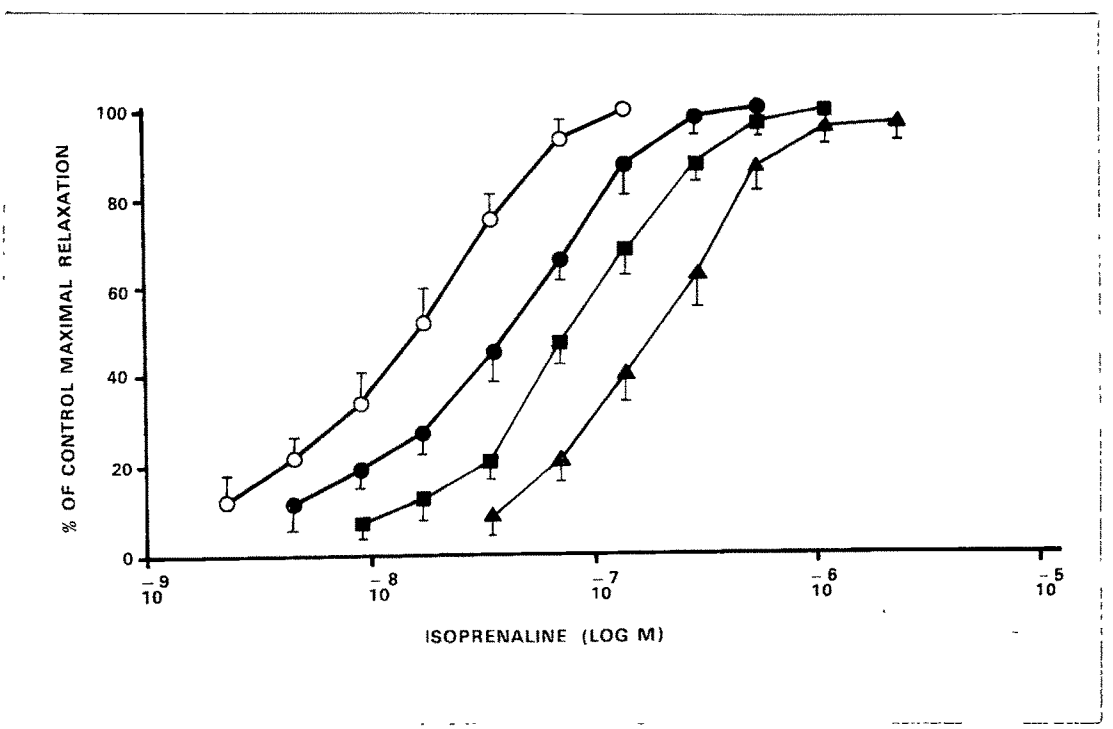
n = Number of observations

* = Significant ($P < 0.01$)

Fig. 26

Concentration-response curves for the relaxant effect of isoprenaline on goat isolated tracheal chain contracted with pilocarpine (3.70×10^{-6} M). (○——○) depicts control responses and (●——●), (■——■) and (▲——▲) depict responses obtained in the presence of 3.33×10^{-8} M, 9.99×10^{-8} M and 3.33×10^{-7} M propranolol respectively. Vertical lines indicate S.E.M. (n = 6-8 for each curve).

FIG. 26


% OF CONTROL MAXIMAL RELAXATION
100
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20
0
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
ISOPRENALINE (LOG M)

Table 8. EC_{50} values of isoprenaline obtained with goat isolated tracheal chain in the absence and presence of propranolol, normetanephrine and phenoxybenzamine on pilocarpine (3.70×10^{-6} M) or KCl (1.36×10^{-2} M) induced spasm. Dose-ratio is the ratio of EC_{50} in the presence of the blocker to that in its absence.

Spasmogen	Agonist	Blocker	n	$EC_{50} \pm$ S.E.M.	Dose-ratio
Pilocarpine (3.70×10^{-6} M)	Isoprenaline	-	8	$1.66 \pm 0.08 \times 10^{-8}$ M	-
	"	Propranolol (3.33×10^{-8} M)	7	$4.24 \pm 0.13 \times 10^{-8}$ M **	2.59
	"	Propranolol (9.99×10^{-8} M)	7	$7.47 \pm 0.23 \times 10^{-8}$ *	4.50
	"	Propranolol (3.33×10^{-7} M)	6	$1.93 \pm 0.03 \times 10^{-7}$ M *	11.66
Pilocarpine (3.70×10^{-6} M)	Isoprenaline	-	7	$1.58 \pm 0.06 \times 10^{-8}$ M	-
	"	Normetanephrine (2.73×10^{-5} M)	5	$0.54 \pm 0.03 \times 10^{-8}$ M **	-2.74
KCl (1.36×10^{-2} M)	Isoprenaline	-	6	$1.44 \pm 0.05 \times 10^{-8}$ M	-
	"	Phenoxybenzamine (3.27×10^{-6} M)	6	$1.24 \pm 0.09 \times 10^{-8}$ M	-

n = Number of observations

* = Significant ($P < 0.01$)

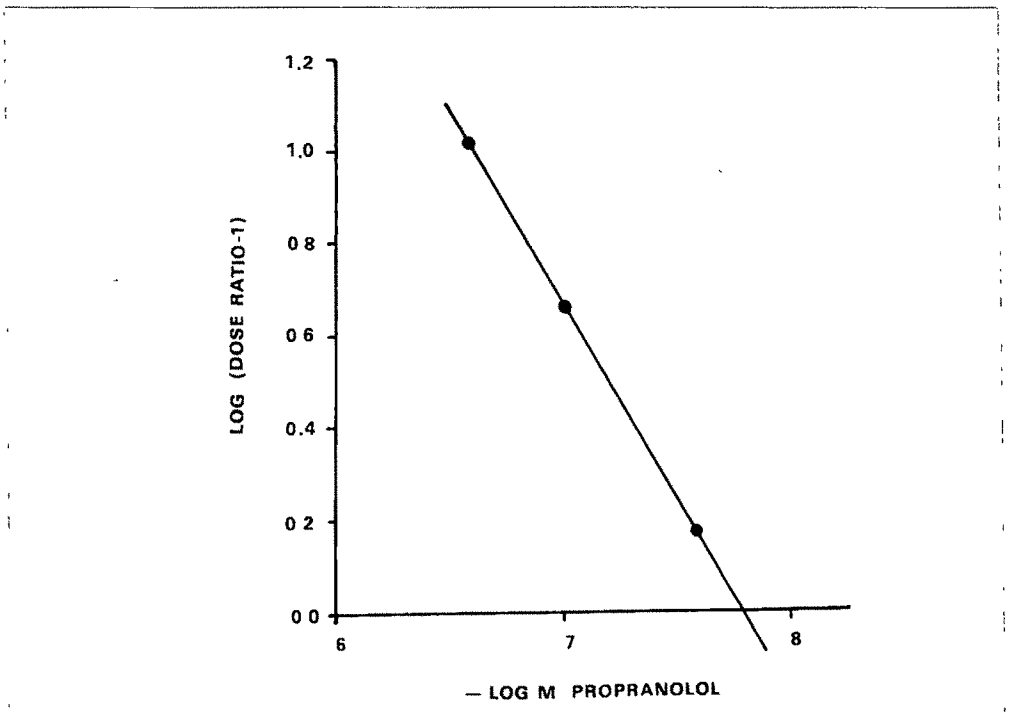
** = Significant ($P < 0.05$)

Minus sign preceding the dose-ratio indicates leftward shift of the dose-response curve.

Fig. 27

pA plot for the beta-receptor blocking effect of propranolol obtained with goat isolated tracheal chain using isoprenaline as the agonist on pilocarpine (3.70×10^{-6} M)-induced spasm. The netative log molar concentration of propranolol is plotted on the abscissa and log (dose ratio -1) as the ordinate. The points were obtained by regression analysis, (n = 6). The regression coefficient for the line was -0.84 ± 0.13 and is not significantly different from the theoretical value of unity for competitive antagonism.

FIG. 27



Concentration-response curve for the relaxant effect of ISO (2.25×10^{-9} - 1.44×10^{-7}M) against KCl (1.36×10^{-2} M) induced spasm was not significantly different from that against pilocarpine (3.70×10^{-6} M) induced spasm (Fig. 26 and Fig. 28).

Phenoxybenzamine (3.27×10^{-6} M) did not alter significantly the concentration-response curve of isoprenaline (Fig. 28; Table 8).

Normetanephrine (2.73×10^{-5} M) produced significant leftward shift of concentration-response curve of ISO (decreased EC_{50}) without any change in maxima (Fig. 29; Table 8).

Similarly reduction of bath temperature from 37 °C ± 0.5°C to 22 °C ± 0.5 °C produced significant leftward shift of the concentration-response curve of ISO without any change in maxima (Fig. 29; Table 8).

4.1.3.2. Responses to histamine and their modifications by metiamide and mepyramine

Submaximal response (80 ± 7.2 of NA maximal response) to histamine (1.0×10^{-5} M) was not significantly affected by H_2-receptor blocker metiamide (2.72×10^{-5} M) but completely blocked by H_1-receptor blocker mepyramine (2.50×10^{-6} M; Fig. 30).

4.1.3.3. Effect of histamine on KCl-induced spasm

Submaximal concentrations of histamine (1.0×10^{-5} M) did not produce any relaxation of the spasm induced by KCl (1.36×10^{-2} M; n = 5).

Fig. 28

Concentration-response curves for the relaxant effect of isoprenaline on goat isolated tracheal chain contracted with KCl (1.36×10^{-2} M). (●——●) depicts control responses and (▲——▲) depicts responses obtained in the presence of 3.27×10^{-6} M phenoxybenzamine. Vertical lines indicate S.E.M. (n = 6 for each curve).

FIG. 28

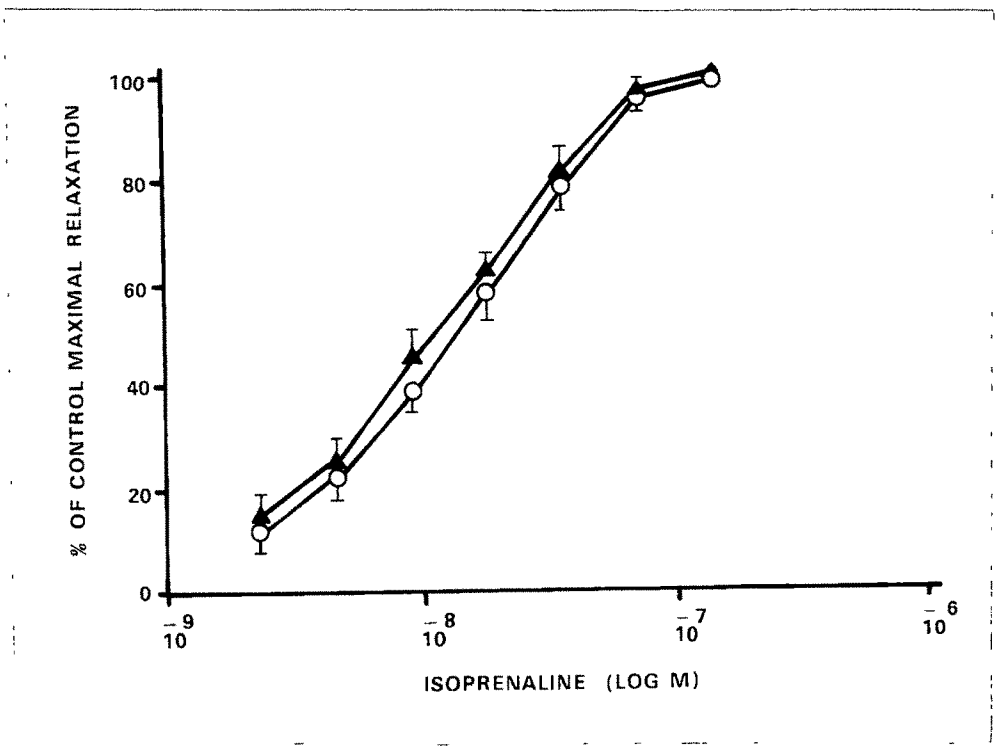

% OF CONTROL MAXIMAL RELAXATION
100
80
60
40
20
0
10^{-9}
10^{-8}
10^{-7}
10^{-6}
ISOPRENALINE (LOG M)

Fig. 29

Concentration-response curves for the relaxant effect of isoprenaline on goat isolated tracheal chain contracted with pilocarpine (3.70×10^{-6} M). (O——O) depicts control responses at $37 \pm 0.5^{\circ}$C and (●——●) depicts responses obtained in the presence of 2.73×10^{-5} M normetanephrine at $37 \pm 0.5^{\circ}$C. (■——■) depicts responses obtained at $22 \pm 0.5^{\circ}$C. Vertical lines indicate S.E.M. (n = 5-7 for each curve).

FIG. 29

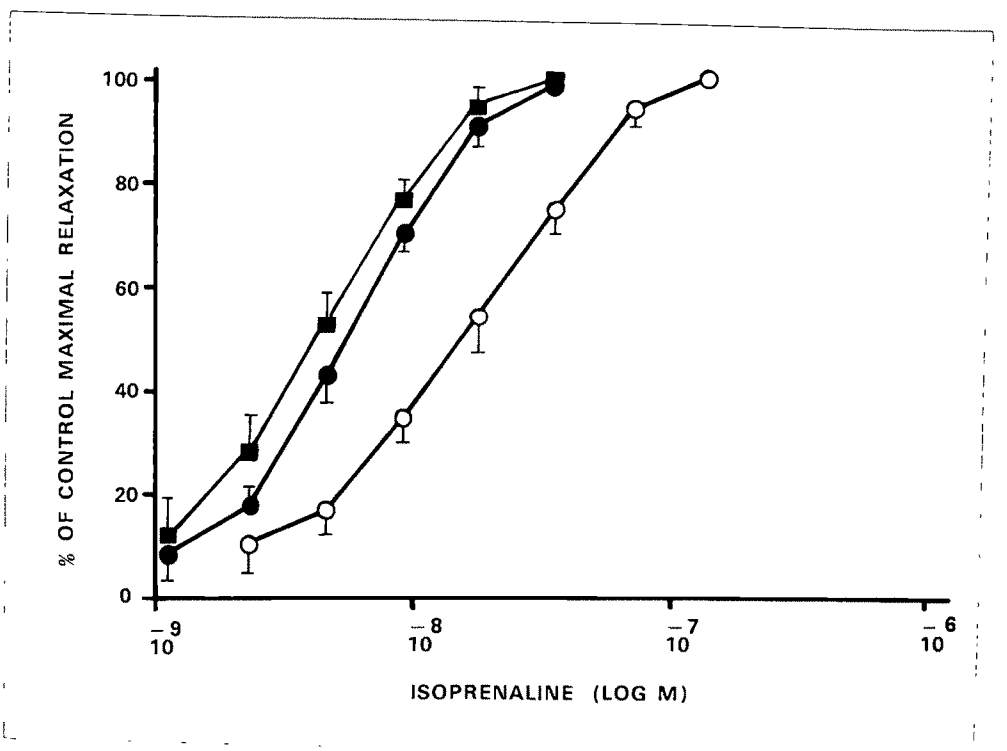
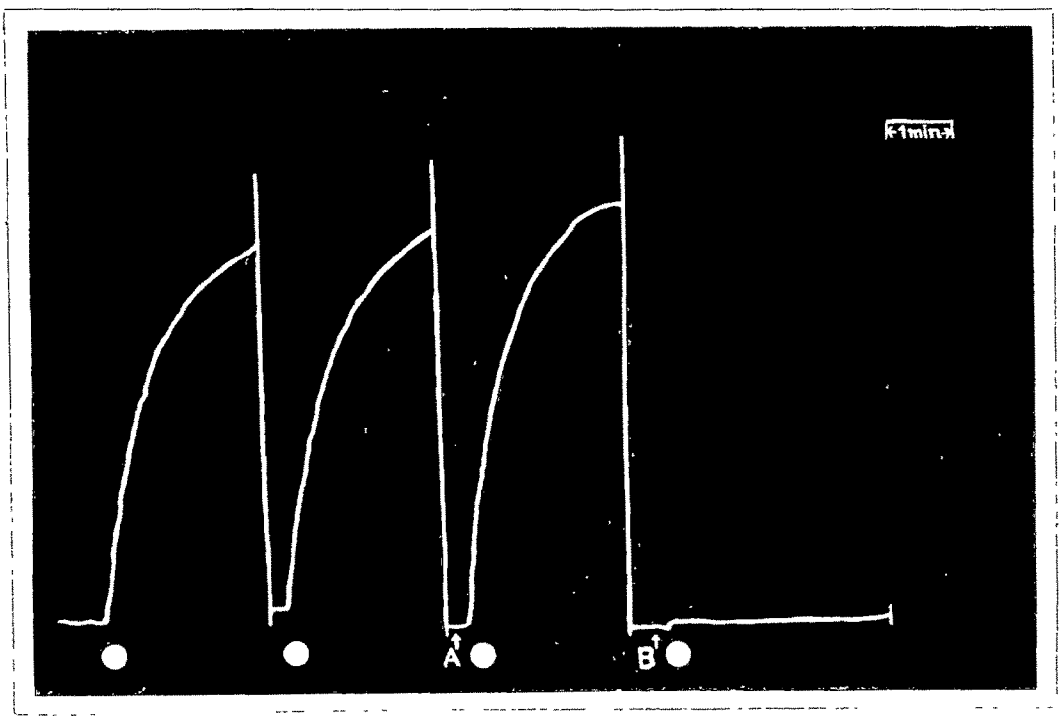

% OF CONTROL MAXIMAL RELAXATION
100
80
60
40
20
0
10^{-9}
10^{-8}
10^{-7}
10^{-6}
ISOPRENALINE (LOG M)

Fig. 30

Record of contractile effects on goat isolated tracheal chain of 1×10^{-5} M of histamine added at dots. At A and B the tissue was exposed to 2.72×10^{-5} M metiamide and 2.5×10^{-6} M mepyramine respectively. Note that the response to histamine is not affected by metiamide but completely blocked by mepyramine. Time mark, 1 minute.

FIG. 30



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4.2. Biochemical experiments

4.2.1. Uptake of NA

The control NA contents of the spleen, aorta and heart were 13.72± 0.18, 6.80±0.36 and 10.24±0.21 nmoles/g of tissue respectively (n = 6-8 each).

The results of incubation with different concentrations of NA at different incubation times are shown in Table 9. It is clear that in no case the splenic, aortic and heart tissues achieved concentrations which were higher than those in the medium. Preincubation with cocaine (1×10^{-5} M) did not influence the levels of NA in these tissues (Table 10).

4.2.2. AChE activity

The AChE activities (microequivalents of ACh hydrolysed) per 1 ml of 3.33% w/v of tissue hemogenates of guinea pig spleen, liver and intestine were 204±2.43, 124±2.43 and 141.7±4.34 (n = 6 for each respectively). There was almost total absence of AChE activity in goat spleen (3.37±0.49 microequivalents of ACh hydrolysed per 1 ml of 3.33% w/v of goat spleen hemogenate; n = 20).

Table 9. NA contents of different goat tissues after incubation with various concentrations of NA.

Concentration of NA used for incubation	Mean increase* in NA content (nmoles/g of tissue ± S.E.M.)**								
	Spleen			Aorta			Heart		
	1 min	5 min	15 min	1 min	5 min	15 min	1 min	5 min	15 min
5.91×10^{-7} M	0.12 ± 0.04	0.23 ± 0.03	0.30 ± 0.06	0.10 ± 0.03	0.19 ± 0.04	0.22 ± 0.03	0.16 ± 0.04	0.29 ± 0.04	0.32 ± 0.05
1.77×10^{-6} M	0.17 ± 0.06	0.38 ± 0.02	0.44 ± 0.07	0.15 ± 0.06	0.32 ± 0.05	0.36 ± 0.04	0.25 ± 0.06	0.41 ± 0.03	0.46 ± 0.07
5.91×10^{-6} M	1.60 ± 0.18	2.31 ± 0.42	2.31 ± 0.77	1.72 ± 0.18	2.37 ± 0.36	2.31 ± 0.88	2.25 ± 0.36	3.02 ± 0.58	3.02 ± 0.71
1.18×10^{-5} M	2.31 ± 0.36	2.54 ± 0.23	2.54 ± 0.12	1.83 ± 0.23	2.78 ± 0.18	2.79 ± 0.72	2.90 ± 0.23	3.49 ± 0.46	3.49 ± 0.77
2.36×10^{-5} M	2.90 ± 0.59	4.03 ± 0.59	4.0 ± 0.77	1.89 ± 0.18	3.49 ± 0.18	3.43 ± 0.72	3.02 ± 0.46	5.50 ± 0.36	5.50 ± 0.46

* The control NA contents of spleen, aorta and heart were (nmoles/g): 13.2 ± 0.18, 6.8 ± 0.36 and 10.24 ± 0.21 respectively.

** Each value is the mean of 6-8 observations.

Table10. Influence of preincubation for 15 min with cocaine (1×10^{-5} M) on the accumulation of NA by goat tissues incubated with different concentrations of NA.

Concentration of NA used for incubation	Mean increase in NA accumulation (mmoles/g of tissue±S.E.M.*)					
	Spleen		Aorta		Heart	
	Control	Cocaine	Control	Cocaine	Control	Cocaine
5.91×10^{-6} M	2.34 ± 0.19	2.42 ± 0.13	2.30 ± 0.43	2.38 ± 0.29	3.06 ± 0.20	3.16 ± 0.18
1.77×10^{-5} M	2.94 ± 0.14	3.01 ± 0.17	3.03 ± 0.32	3.09 ± 0.22	3.71 ± 0.30	3.79 ± 0.21

* Each value is the mean of 6-8 observations.