RESULTS

(A) NICOTINIC GANGLIONIC BLOCKERS :

DOG NICTITATING MEMBRANE:

Supramaximal stimulation of the preganglionic fibres of the superior cervical ganglion elicited frequency dependent contractile responses of the nictitating membrane. When frequency-response curves were plotted with the per centage of maximal contraction on the ordinate and log of frequency on the abscissa, bell-shaped curves were obtained. Hexamethonium (4.95×10^{-8}, 1.58×10^{-7}, 4.95×10^{-7}, and 1.58×10^{-6}); tetraethylammonium (7.69×10^{-7}, 1.92×10^{-6}, 3.84×10^{-6}, and 7.69×10^{-6}; Fig. 4 a); mecamylamine (6.02×10^{-7}, 1.96×10^{-6}, 6.02×10^{-6}, and 1.96×10^{-5}; Fig. 4 b); pempidine (2.06×10^{-7}, 6.45×10^{-7}, 2.06×10^{-6}, and 6.45×10^{-6}); chlorisondamine (8.84×10^{-8}, 2.76×10^{-7}, 8.84×10^{-7}, and 2.76×10^{-6}): and pentolinium (1.33×10^{-7}, 4.16×10^{-7}, 1.33×10^{-6}, and 4.16×10^{-6}) elicited graded inhibition of the frequency-response curves as indicated by successive shifts of the curves to the right. The blocking action of hexamethonium and tetraethylammonium lasted for 15-20 min. The blocking action of the lower two doses of the other agents lasted for about 30 min and those of the two higher doses lasted for about 1 hr.

Hexamethonium and tetraethylammonium in all the doses studied produced parallel shifts of the frequency-response curves. There was no reduction of the maximal responses and no flattening of the curves, suggesting competitive antagonism. The degree of the antagonism was

LEGEND FOR FIG. 4 a

Frequency-response curves of the contractile responses of the dog nictitating membrane stimulated preganglionically with supra-maximal square wave pulses of 2.5 msec duration at variable frequencies for 30 sec every 10 min. Per centage of the maximal contraction is plotted on the ordinate and log frequency per sec on the abscissa. ●——● indicates control frequency-response curve. X——X, O——O, ■——■ and ▲——▲ indicate frequency-response curves after 7.69×10^{-7}, 1.92×10^{-6}, 3.84×10^{-6} and 7.69×10^{-6} M/ganglion respectively of tetraethylammonium given by close intra-arterial injection into the blood supply of the superior cervical ganglion. Responses to nerve stimulation were elicited 15 min after the injection of the blocker.

LEGEND FOR FIG. 4 b

Frequency-response curves of the contractile responses of the dog nictitating membrane stimulated preganglionically with supra-maximal square wave pulses of 2.5 msec duration at variable frequencies for 30 sec every 10 min. Per centage of the maximal contraction is plotted on the ordinate and log frequency per sec on the abscissa. ●——● indicates control frequency-response curve. X——X, O——O, ■——■ and ▲——▲ indicate frequency-response curves after 6.02×10^{-7}, 1.96×10^{-6}, 6.02×10^{-6} and 1.96×10^{-5} M/ganglion respectively of mecamylamine given by close intra-arterial injection into the blood supply of the superior cervical ganglion. Responses to nerve stimulation were elicited 15 min after the injection of the blocker.

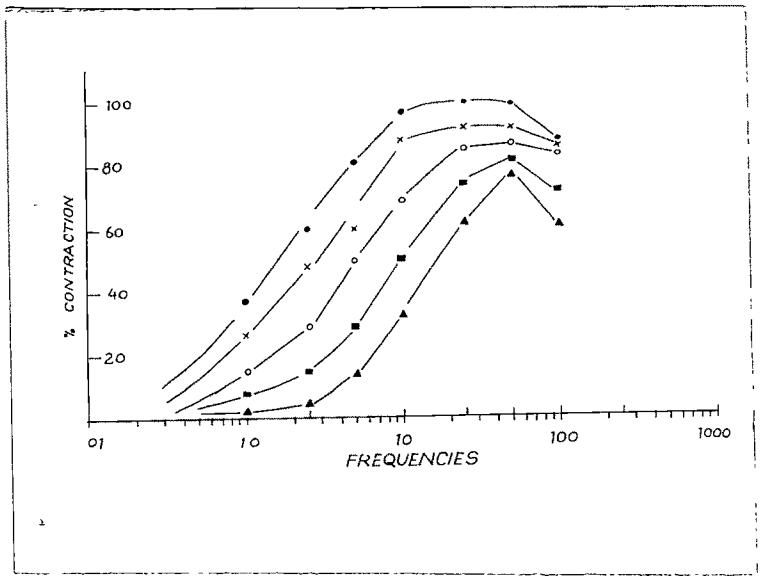
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Fig 4 a

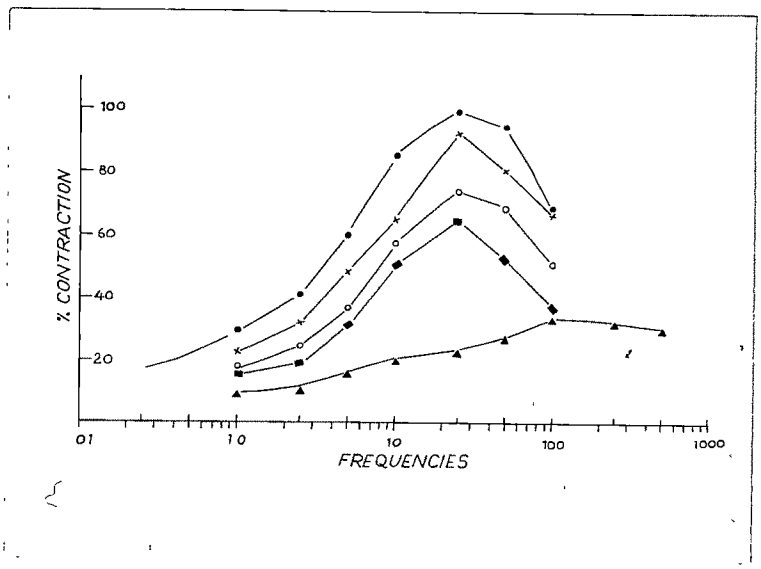
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Fig 4 b

calculated in terms of dose ratio, which is the ratio of the equieffective strength of the frequencies after and before the addition of the antagonist (Gaddum et al, 1955). The log of the dose ratio was given by the horizontal distance between the parallel lines and when the lines were not parallel, it was calculated from the horizontal distance between the frequency-response curves at 50% of the maximal response. The dose ratios for a given concentration of an antagonist were closely similar, indicating that variations in the sensitivity of a tissue to the antagonists were small and were independent of the variations in sensitivity to the frequency of stimulation. Mecamylamine (1.96×10^{-7}, and 6.02×10^{-7}); pempidine (2.06×10^{-7}, and 6.45×10^{-7}); chlorisondamine (8.83×10^{-8}, and 2.76×10^{-7}) and pentolinium (1.33×10^{-7}, and 4.16×10^{-7}) caused parallel shifts of the frequency-response curves. However, with higher doses of these four antagonists, the shift of the frequency-response curves was not parallel and there was reduction of the maximal responses with flattening of the curves. A parallel shift of the frequency-response curves does not constitute exclusive proof of competitive antagonism (Arunlakshana & Schild, 1959). Arunlakshana & Schild (1959) introduced the method of pA plot; for this plot the logarithm of $(x - 1)$ is plotted against the molar concentrations of B, where x is the dose ratio and B the molar concentration of the antagonist. The intercept of regression line with the abscissa (at zero level) gives the pA_2 value. The pA_{10} value is calculated from the regression line. In order for antagonism to be competitive, the slope value of the regression line should be unity. The data obtained with the dog

nictitating membrane were analysed in this manner and are summarized in Table 3. The mean pA_2, and the mean pA_{10} values were obtained from the individual pA_2 and pA_{10} values respectively. An examination of Table 3 reveals that taking pA_2 as a measure of the potency of the ganglion blockers, the order of potency was hexamethonium > chlorisondamine > mecamylamine > pempidine ≒ pentolinium > tetraethylammonium. The slope values of the regression lines for hexamethonium and tetraethylammonium were close to the theoretical value of unity recommended for competitive antagonism ($p > 0.05$), whereas the slope values of the regression lines for mecamylamine, pempidine, chlorisondamine and pentolinium were significantly different from the theoretical value ($p < 0.05$). Thus, at the superior cervical ganglion of the dog, hexamethonium, tetraethylammonium and lower doses of mecamylamine, pempidine, chlorisondamine and pentolinium acted competitively; while in higher doses the latter four blockers did not act competitively.

ISOLATED RABBIT ILEUM :

Isotonic lever:

Nicotine (5.55×10^{-8}, 1.85×10^{-7}, 5.55×10^{-7}, 1.85×10^{-6}, and 5.55×10^{-6}) and dimethylphenylpiperazinium (DMPP) (1.5×10^{-6}, 3.0×10^{-6}, 6.0×10^{-6}, 7.5×10^{-6}, and 1.5×10^{-5}) elicited graded contractile responses. DMPP was comparatively a weaker agonist for this preparation. The dose-response curves of these two agonists were bell-shaped. The curves with nicotine were more bell-shaped than those with DMPP. In order to avoid autoinhibition due to nicotine and DMPP, the influence of the antagonists was studied on the ascending

TABLE 3

Dog nictitating membrane (stimulated indirectly preganglionically; ganglionic blockers given into the arterial blood supply of the superior cervical ganglion). Values of pA_2, pA_{10}, $pA_2 - pA_{10}$, and slope (-b) were obtained from pA plots. Probability (p) is of significance of the experimental value of (-b) from that of the theoretical value of unity for competitive antagonism.

Ganglionic blocker	Mean $pA_2 \pm$ S.E.M.	Mean $pA_{10} \pm$ S.E.M.	Mean $pA_2 - pA_{10}$	Mean slope (b) $\pm$ S.E.M.	p
Hexamethonium (12)	7.60 ± 0.084	6.80 ± 0.063	0.80	0.93 ± 0.053	> 0.05
Tetraethyl-ammonium (12)	5.96 ± 0.074	5.32 ± 0.052	0.64	0.91 ± 0.043	> 0.05
Mecamylamine (12)	6.51 ± 0.057	5.12 ± 0.044	1.39	0.43 ± 0.067	< 0.05
Pempidine (12)	6.46 ± 0.072	5.35 ± 0.083	1.11	0.51 ± 0.073	< 0.05
Chlorisond-amine (12)	6.70 ± 0.063	5.40 ± 0.054	1.30	0.43 ± 0.069	< 0.05
Pentolinium (12)	6.46 ± 0.089	5.63 ± 0.075	0.83	0.68 ± 0.072	< 0.05

Figures in parentheses indicate the number of experiments.

limb of the dose-response curves. The dose-response curves were plotted by taking the per cent of the maximal response on the ordinate and the log dose of nicotine or DMPP, in the presence and absence of various doses of antagonists, on the abscissa. Hexamethonium (2.47×10^{-5}, 4.95×10^{-5}, 9.90×10^{-5} and 1.98×10^{-4}; Fig. 5 a); tetraethylammonium (7.69×10^{-5}, 2.5×10^{-4}, 7.69×10^{-4}, and 2.5×10^{-3}); mecamylamine (6.02×10^{-7}, 1.50×10^{-6}, 6.02×10^{-6}, and 1.93×10^{-5}); pempidine (6.45×10^{-7}, 2.06×10^{-6}, 6.45×10^{-6}, and 2.06×10^{-5}); chlorisondamine (2.76×10^{-7}, 6.90×10^{-7}, 1.38×10^{-6}, and 6.90×10^{-6}) and pentolinium (2.08×10^{-6}, 6.66×10^{-6}, 2.08×10^{-5}, and 6.66×10^{-5}) shifted the dose-response curves of nicotine to the right. Similarly, hexamethonium (4.95×10^{-5}, 9.90×10^{-5}, 1.98×10^{-4}, and 4.95×10^{-4}); tetraethylammonium (7.69×10^{-5}, 2.46×10^{-4}, 7.69×10^{-4}, and 2.46×10^{-4}); mecamylamine (1.93×10^{-7}, 6.02×10^{-7}, 1.93×10^{-6}, and 6.02×10^{-6}); pempidine (6.45×10^{-7}, 2.06×10^{-6}, 6.45×10^{-6} and 2.06×10^{-5}); chlorisondamine (6.90×10^{-8}, 1.38×10^{-7}, 2.76×10^{-7}, and 6.90×10^{-7}) and pentolinium (2.08×10^{-6}, 6.66×10^{-6}, 2.08×10^{-5}, and 6.66 x 10; Fig. 5 b) shifted the dose-response curves of DMPP to the right. Hexamethonium and tetraethylammonium in all the doses studied caused parallel shifts of the dose-response curves with no reduction of the maximal responses and no flattening of the curves. Mecamylamine (6.02×10^{-7} and 1.50×10^{-6}); pempidine (6.45×10^{-7} and 2.06×10^{-6}); chlorisondamine (2.76×10^{-7} and 6.90×10^{-7}) and pentolinium (2.08×10^{-6} and 6.66×10^{-6}) caused parallel shifts of the dose-response curves of nicotine. Similarly, mecamylamine (1.93×10^{-7} and 6.02×10^{-7}); pempidine (6.45×10^{-7} and 2.06×10^{-6});

LEGEND FOR FIG. 5 a

Responses of the isolated rabbit ileum (recorded with isotonic lever) to nicotine (at dots) 5.55×10^{-8} M at (1); 1.85×10^{-7} M at (2); 5.55×10^{-7} M at (3); 1.85×10^{-6} M at (4); 5.55×10^{-6} M at (5); 1.85×10^{-5} M at (6); 5.55×10^{-5} M at (7) and 1.85×10^{-4} M at (8). Hexamethonium was given (4.95×10^{-5} M) at (A) and (1.98×10^{-4} M) at (B). The drum was stopped at ___ for 15 min. Contact time for nicotine was 30 sec and that for hexamethonium was 15 min.

LEGEND FOR FIG. 5 b

Dose-response curves of the contractile responses (recorded with isotonic lever) of the isolated rabbit ileum to DMPP (abscissa, log of DMPP; ordinate, per centage of maximal contraction). ●——● indicates control dose-response curve; x——x, o——o, ■——■ and ▲——▲ indicate dose-response curves after 2.08×10^{-6} M, 6.66×10^{-6} M, 2.08×10^{-5} M and 6.66×10^{-5} M respectively of pentolinium. Contact time for DMPP was 30 sec and that for pentolinium was 15 min.

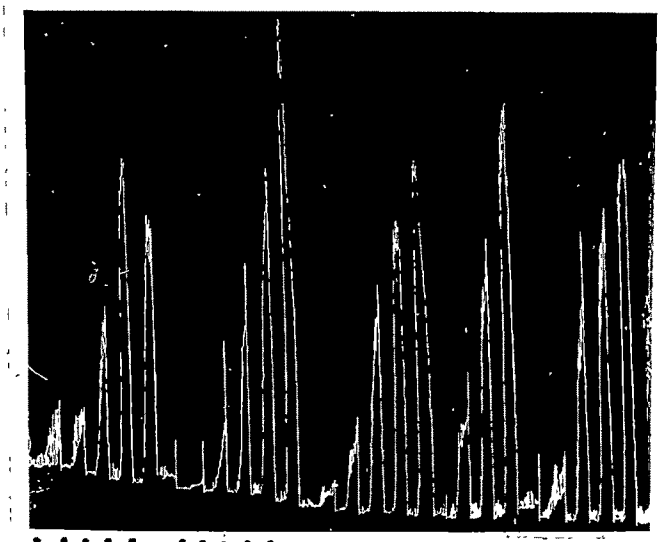
A
B
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Fig. 5a.

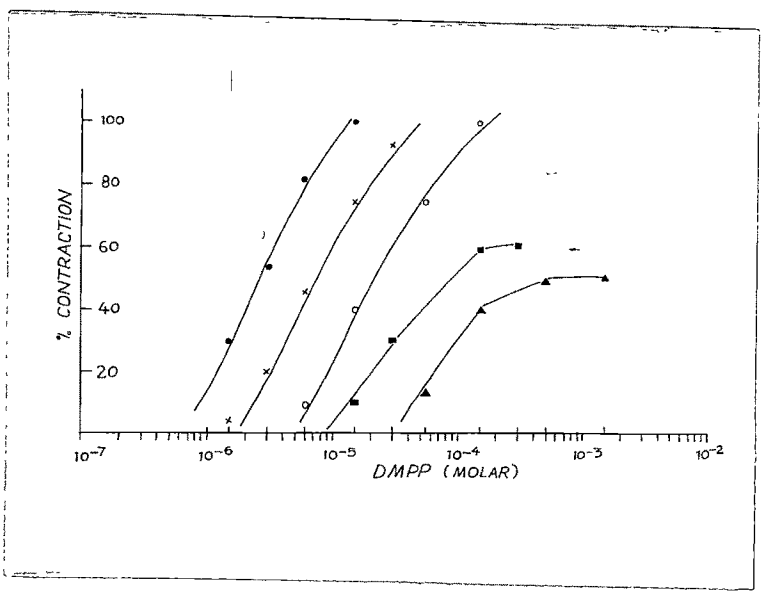
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Fig 5 b

chlorisondamine (6.90×10^{-8}, and 1.38×10^{-7}) and pentolinium (2.08×10^{-6}, and 6.66×10^{-6}) caused parallel shifts of the dose-response curves of DMPP. With the higher doses of the antagonists the shifts were not parallel and there was reduction of the maximal responses and flattening of the curves excluding competitive antagonism. pA_2, pA_{10}, $pA_2 - pA_{10}$ and slope values were calculated according to the method of Arunlakshana & Schild (1959) as described under the dog nictitating membrane. The data are summarized in Tables 4 and 5. An examination of the Tables 4 and 5 reveals that taking pA_2 values as measure of potency of the ganglion blockers, the order of potency was, mecamylamine > pempidine > chlorisondamine > pentolinium > hexamethonium > tetraethylammonium with nicotine as the agonist and chlorisondamine > mecamylamine > pempidine pentolinium > hexamethonium > tetraethylammonium with DMPP as the agonist. The slope values of pA plots for hexamethonium and tetraethylammonium were close to the theoretical value of 1 recommended for competitive antagonism ($p > 0.05$). The slope values of pA plots for mecamylamine, pempidine, chlorisondamine, and pentolinium were significantly different from the theoretical value of 1 ($p < 0.05$). Thus, hexamethonium and tetraethylammonium acted competitively against both nicotine and DMPP, whereas mecamylamine, pempidine, chlorisondamine, and pentolinium did not act competitively.

Auxotonic lever:

Nicotine (5.55×10^{-7}, 1.85×10^{-6}, 5.55×10^{-6} and 1.85×10^{-5}); and dimethylphenylpiperazinium (DMPP) (3.75×10^{-6}, 7.50×10^{-6}, 1.13×10^{-5}, 1.50×10^{-5}, and 3.0×10^{-5}) elicited graded contractile

TABLE 4

Isolated rabbit ileum (isotonic lever; agonist, nicotine). Values of pA_2, pA_{10}, $pA_2 - pA_{10}$, and slope(b) were obtained from pA plots. Probability (p) is of significance of experimental value of (b) from that of theoretical value of unity for competitive antagonism.

Ganglionic blocker	Mean $pA_2 \pm$ S.E.M.	Mean $pA_{10} \pm$ S.E.M.	Mean pA_2-mean pA_{10}	Mean slope ± (b) S.E.M.	p
Hexamethonium (12)	4.55 ± 0.073	3.90 ± 0.085	0.65	1.13 ± 0.073	> 0.05
Tetraethyl-ammonium (12)	4.35 ± 0.098	3.66 ± 0.086	0.70	1.05 ± 0.048	> 0.05
Mecamylamine (12)	6.68 ± 0.080	5.59 ± 0.084	1.09	0.68 ± 0.035	< 0.05
Pempidine (12)	6.46 ± 0.096	4.78 ± 0.007	1.68	0.45 ± 0.135	< 0.05
Chlorisond-amine (12)	6.22 ± 0.133	5.68 ± 0.076	0.54	1.41 ± 0.073	< 0.05
Pentolinium (12)	5.50 ± 0.098	4.96 ± 0.075	0.54	1.49 ± 0.074	< 0.05

Figures in parentheses indicate the number of experiments.

TABLE 5

Isolated rabbit ileum (isotonic lever; agonist, DMPP). Values of pA_2, pA_{10}, $pA_2 - pA_{10}$, and slope (b) were obtained from pA plots. Probability (p) is of significance of experimental value of (b) from that of the theoretical value of unity for competitive antagonism.

Ganglionic blocker	Mean pA_2 ± S.E.M.	Mean pA_{10} ± S.E.M.	Mean pA_2-mean pA_{10}	Mean slope (b) ± S.E.M.	p
Hexamethonium (12)	4.68 ± 0.067	3.92 ± 0.049	0.76	0.97 ± 0.074	> 0.05
Tetraethyl-ammonium (12)	4.21 ± 0.079	3.50 ± 0.053	0.71	1.05 ± 0.052	> 0.05
Mecamylamine (12)	6.34 ± 0.076	5.30 ± 0.097	1.04	0.69 ± 0.074	< 0.05
Pempidine (12)	6.16 ± 0.033	5.06 ± 0.057	1.10	0.66 ± 0.063	< 0.05
Chlorisond-amine (12)	6.49 ± 0.075	6.01 ± 0.083	0.49	1.52 ± 0.074	< 0.05
Pentolinium (12)	5.63 ± 0.063	5.07 ± 0.075	0.56	1.52 ± 0.050	< 0.05

Figures in the parentheses indicate numbers of experiments.

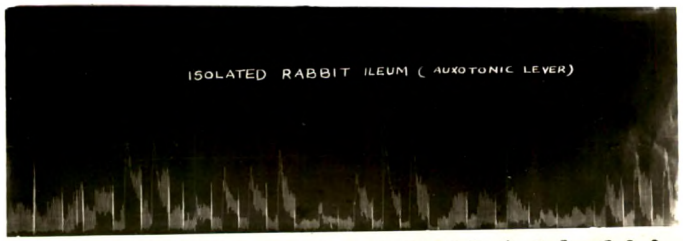
responses with different preparations. The dose-response curves were plotted as described for isotonic lever. The preparations could be used for 3-4 hr without the appearance of fade. In order to avoid auto-inhibition care was taken not to expose the tissue to extremely high doses of the agonists. Hexamethonium (1.57×10^{-5}, 4.85×10^{-5}, 9.90×10^{-5} and 1.98×10^{-4}); tetraethylammonium (2.46×10^{-5}, 7.69×10^{-5}, 2.46×10^{-4} and 7.69×10^{-4}); mecamylamine (6.02×10^{-7}, 1.50×10^{-6}, 6.02×10^{-6} and 1.92×10^{-5}; Fig. 6 a); pempidine (6.45×10^{-7}, 2.06×10^{-6}, 6.45×10^{-6} and 2.06×10^{-5}); chlorisondamine (2.76×10^{-7}, 8.83×10^{-7}, 2.76×10^{-6} and 8.83×10^{-6}) and pentolinium (1.33×10^{-6}, 4.16×10^{-6}, 1.33×10^{-5} and 4.16×10^{-5}) shifted the dose-response curves of nicotine to the right. Similarly, hexamethonium (4.95×10^{-6}, 1.58×10^{-5}, 4.95×10^{-5} and 1.58×10^{-4}); tetraethylammonium (2.46×10^{-5}, 7.69×10^{-5}, 2.46×10^{-4} and 7.69×10^{-4}); mecamylamine (6.02×10^{-7}, 1.50×10^{-6}, 6.02×10^{-6} and 1.93×10^{-5}); pempidine (6.45×10^{-7}, 1.61×10^{-6}, 6.45×10^{-6} and 2.06×10^{-5}); chlorisondamine (8.83×10^{-8}, 2.76×10^{-7}, 8.83×10^{-7} and 2.76×10^{-6}; Fig. 6 b) and pentolinium (1.33×10^{-6}, 4.16×10^{-6}, 1.33×10^{-5} and 4.16×10^{-5}) shifted the dose-response curves of DMPP to the right. pA_2, pA_{10}, $pA_2 - pA_{10}$ and slope values were calculated as described for the isotonic lever and the data are presented in Tables 6 and 7. The analysis of the data reveals that the antagonism exhibited by the six ganglion blockers was similar in nature to that obtained with the isotonic lever. The order of potency with nicotine as the agonist was, mecamylamine > chlorisondamine > pempidine > pentolinium > hexamethonium > tetraethylammonium and that with DMPP as the agonist was mecamylamine chlorisondamine > pempidine > pentolinium > hexamethonium >

LEGEND FOR FIG. 6 a

Responses of the isolated rabbit ileum (recorded with auxotonic lever) to nicotine (at dots) 5.55×10^{-7} M at (1); 1.85×10^{-6} M at (2); 5.55×10^{-6} M at (3); 1.85×10^{-5} M at (4); 5.55×10^{-5} M at (5); 1.85×10^{-4} M at (6); 5.55×10^{-4} M at (7) and 1.85×10^{-3} M at (8). Mecamylamine (1.50×10^{-6} M) and (1.92×10^{-5} M) was given at A and B respectively. The drum was stopped at --- for 15 min and at ____ for 50 min.

LEGEND FOR FIG. 6 b

Responses of the isolated rabbit ileum (recorded with auxotonic lever) to DMPP (at dots) 3.75×10^{-6} M at (1); 7.50×10^{-6} M at (2); 1.13×10^{-5} M at (3); 1.50×10^{-5} M at (4); 3.0×10^{-5} M at (5); 5.20×10^{-5} M at (6); 1.50×10^{-4} M at (7) and 5.20×10^{-4} M at (8). Chlorisondamine (8.83×10^{-8} M) and (8.83×10^{-7} M) was given at A and B respectively. The drum was stopped at --- for 15 min. Contact time for nicotine was 30 sec and that for chlorisondamine was 15 min.



1 1 2 3 4 A 3 4 5 6 1 2 3 4 B 4 5 6 7 8 2 3 4

Fig. 6 a

2 1 3 4 A 3 3 3 4 5 1 2 3 4 B 3 4 5 6 7 8

Fig. 6 b.

TABLE 6

Isolated rabbit ileum (auxotonic lever; agonist, nicotine). Values of pA_2, pA_{10}, $pA_2 - pA_{10}$, and slope (b) were obtained from pA plots. Probability (p) is of significance of experimental value of (b) from that of the theoretical value of unity for competitive antagonism.

Ganglionic blocker	Mean pA_2 ± S.E.M.	Mean pA_{10} ± S.E.M.	Mean pA_2-mean pA_{10}	Mean slope (b) ± S.E.M.	p
Hexamethonium (12)	4.86 ± 0.083	4.09 ± 0.078	0.77	0.96 ± 0.074	> 0.05
Tetraethyl-ammonium (12)	4.18 ± 0.087	3.42 ± 0.098	0.76	0.97 ± 0.013	> 0.05
Mecamylamine (12)	6.89 ± 0.097	5.63 ± 0.076	1.26	0.59 ± 0.065	< 0.05
Pempidine (12)	6.42 ± 0.075	5.46 ± 0.087	0.96	0.74 ± 0.031	< 0.05
Chlorisond-amine (12)	6.55 ± 0.068	5.16 ± 0.073	1.39	0.56 ± 0.076	< 0.05
Pentolinium (12)	5.80 ± 0.054	5.23 ± 0.075	0.57	1.43 ± 0.097	< 0.05

Figures in the parentheses indicate the number of experiments.

TABLE 7

Isolated rabbit ileum (auxotonic lever; agonist, DMPP). Values of pA_2, pA_{10}, $pA_2 - pA_{10}$, and slope (b) were obtained from pA plots. Probability (p) is of significance of experimental value of (b) from that of theoretical value of unity for competitive antagonism.

Ganglionic blocker	Mean pA_2 ± S.E.M.	Mean pA_{10} ± S.E.M.	Mean pA_2-mean pA_{10}	Mean slope (b) ± S.E.M.	p
Hexamethonium (12)	5.01 ± 0.073	4.25 ± 0.085	0.76	0.96 ± 0.037	> 0.05
Tetraethyl-ammonium (12)	4.33 ± 0.089	3.58 ± 0.073	0.75	1.02 ± 0.063	> 0.05
Mecamylamine (12)	6.77 ± 0.076	5.38 ± 0.064	1.39	0.56 ± 0.067	< 0.05
Pempidine (12)	6.37 ± 0.087	5.06 ± 0.093	1.31	0.57 ± 0.054	< 0.05
Chlorisond-amine (12)	6.62 ± 0.076	5.42 ± 0.063	1.21	0.63 ± 0.076	< 0.05
Pentolinium (12)	6.17 ± 0.057	4.76 ± 0.069	1.41	0.55 ± 0.079	< 0.05

Figures in parentheses indicate the number of experiments.

tetraethylammonium.

Nickerson's (1957) approach to differentiate classical competitive antagonism from nonequilibrium antagonism was based on the premise that different factors control the temporal development and disappearance of the two types of blockade. The time course appears to be limited primarily by penetration into and escape from the biophase (K_a and K_b) in the case of competitive antagonists and by the very low value of K_2 in the case of nonequilibrium agents. The development of the blockade produced by a classical competitive agent ceases and is reversed as soon as the active drug is removed from the surrounding medium whereas the formation of the stable IR complex from active nonequilibrium inhibitor present in the biophase may continue for some time after washing.

Based on this approach, study was made with hexamethonium and chlorisondamine. Two identical pieces of rabbit ileum from the same animal were mounted simultaneously under similar experimental conditions (5 experiments). DMPP was used as the agonist. Hexamethonium was used as an agent acting competitively since it is reported in the literature and observed in the present study to be a competitive antagonist and chlorisondamine was used as an antagonist not acting competitively. Hexamethonium (4.95×10^{-5}) and chlorisondamine (2.76×10^{-7}) were each kept in contact with the tissue for 5 min, 10 min, 15 min and 45 min so that the equilibration time for both the drugs could be studied. The dose ratios of hexamethonium for 15 and 45 min of contact time were 8.80 ± 0.8 and 9.8 ± 0.4 respectively and those of chlorisondamine were 7.2 ± 0.6 and 8.0 ± 0.4 respectively.

The dose ratios of the two blockers for different periods of contact time were not statistically significantly different ($p > 0.05$) from each other. Next, the time taken for DMPP to elicit control responses following wash out of the antagonist was studied. After a contact time of 45 min, recovery with chlorisondamine was observed at the end of 75 min whereas that with hexamethonium was observed at the end of 45 min. In general, it was observed that the recovery of the responses to nicotine and DMPP following 15 min contact time with high doses of mecamylamine (1.92×10^{-5}); pempidine (2.06×10^{-5}); chlorisondamine (8.83×10^{-6}) and pentolinium (6.66×10^{-6}) occured after 50 to 70 min and that following 15 min of contact time with high dose of hexamethonium (4.95×10^{-4}) and tetraethylammonium (7.69×10^{-4}) occured after 15 min.

In another set of 5 experiments paired preparations were set up as described above. DMPP was used as the agonist. One piece was exposed to a single high dose of hexamethonium (4.95×10^{-5}) or chlorisondamine (8.83×10^{-6}) for 45 min. The other piece was exposed to the same dose in the form of three divided doses given cummulatively at intervals of 15 min (hexamethonium 5.8×10^{-6}; 5.8×10^{-6} and 3.79×10^{-5} and chlorisondamine 1.65×10^{-6}, 1.65×10^{-6} and 5.53×10^{-6}). The dose-ratios of hexamethonium given singly and cummulatively were 12.75 ± 0.36 and 11.69 ± 0.21 respectively. The dose-ratios of chlorisondamine given singly and cummulatively were 8.35 ± 0.21 and 19.56 ± 0.36 respectively.

In 5 experiments using paired preparations, hexamethonium and chlorisondamine which were found to be " competitive " and " not competitive " antagonists respectively were tested alone and in combination against DMPP. According to Paton and Rang (1965) when two antagonists which compete to occupy the same receptors are used together then the dose ratio (DR) of both is given by the formula, $(DR_1 + DR_2 - 1)$; but if the two antagonists occupy different receptor sites then the dose ratio (DR) is given by the formula, $(DR_1 \times DR_2)$ where DR_1 stands for the dose ratio of one and DR_2 stands for the dose ratio of the other. The individual dose-ratios of hexamethonium (4.95×10^{-5}) and chlorisondamine (2.76×10^{-6}) were 9.0 ± 0.6 and 4.0 ± 0.3 respectively. When both the antagonists were used together in the same concentrations, the dose ratio was 38 ± 1.5 which is very close to the product of the individual dose ratios $9 \times 4 = 36$, suggesting that the two blockers in the doses used did not compete with each other for the same receptor. The parallel shift of the dose-response curve produced by hexamethonium (4.95×10^{-5}) used alone was converted to a nonparallel shift with reduction of the maximal responses when used in combination with chlorisondamine (2.76×10^{-6}).

In the next series of experiments a higher dose of hexamethonium was used together with a series of doses of chlorisondamine. Hexamethonium (1.57×10^{-4}) and chlorisondamine (8.83×10^{-7}) or hexamethonium (1.57×10^{-4}) and chlorisondamine (8.83×10^{-8}) induced nonparallel shifts of the agonist dose-response curves and there was reduction of the maximal responses. However, hexamethonium (1.57×10^{-4})

and chlorisondamine (2.76×10^{-8}) induced a parallel shift of the dose-response curve and there was no reduction of the maximal responses. This indicates that hexamethonium (1.57×10^{-4}) could protect the specific receptors from the lower doses of chlorisondamine (2.76×10^{-8}) whereas the same dose of hexamethonium could not protect the receptors against the higher doses of chlorisondamine (8.83×10^{-8}; 8.83×10^{-7}; Fig. 6c). [illegible].

Antagonism of atropine against DMPP: DMPP was used as an agonist to study the effect of atropine against it. Atropine (1.1×10^{-7}, 3.47×10^{-7}, 1.1×10^{-6} and 3.47×10^{-6}) induced a nonparallel shift of the dose-response curve of DMPP and there was a reduction of the maximal responses with flattening of the curves indicating that the antagonism was not competitive. The data were analysed as outlined under dog nictitating membrane. The mean pA_2 value was 6.84 ± 0.071; the mean pA_{10} value was 5.25 ± 0.094; the mean pA_2 - the mean pA_{10} value was 1.59 and the mean slope value was 0.46 ± 0.16. Thus the antagonism of atropine against DMPP was not competitive.

ISOLATED GUINEA PIG ILEUM

Isotonic lever :

Nicotine and DMPP induced graded contractile responses of the terminal portion of the guinea pig ileum. The dose-response curves were more steep with nicotine than with DMPP and also more steep than those obtained with the isolated rabbit ileum. Thus a narrow dose range of nicotine (1.80×10^{-7}, 2.88×10^{-7}, 4.5×10^{-7}, 5.76×10^{-7}, 9.0×10^{-7}, and 1.80×10^{-6}) and DMPP (1.56×10^{-6},

LEGEND FOR FIG. 6 c

Dose-response curves of the contractile responses (recorded with auxotonic lever) of the isolated rabbit ileum to DMPP (abscissa, log M of DMPP; ordinate, per centage of maximal contraction). ●——● indicates control dose-response curve. o——o, x——x and ▲——▲ indicate dose-response curves after 1.57×10^{-4} M hexamethonium and 2.76×10^{-8} M chlorisondamine, 1.57×10^{-4} M hexamethonium and 8.83×10^{-8} M chlorisondamine, and 1.57×10^{-4} M hexamethonium and 8.83×10^{-7} M chlorisondamine respectively. Contact time for DMPP was 30 sec and that for the antagonists was 15 min.

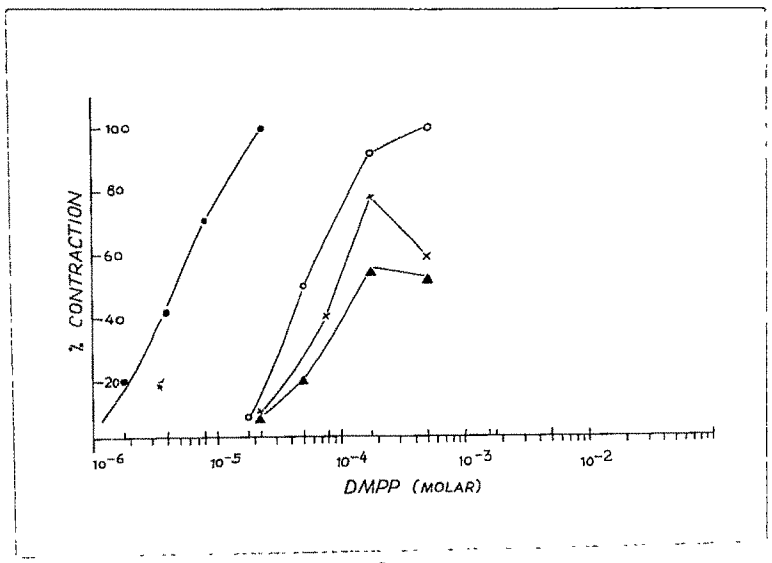


Fig 6 C

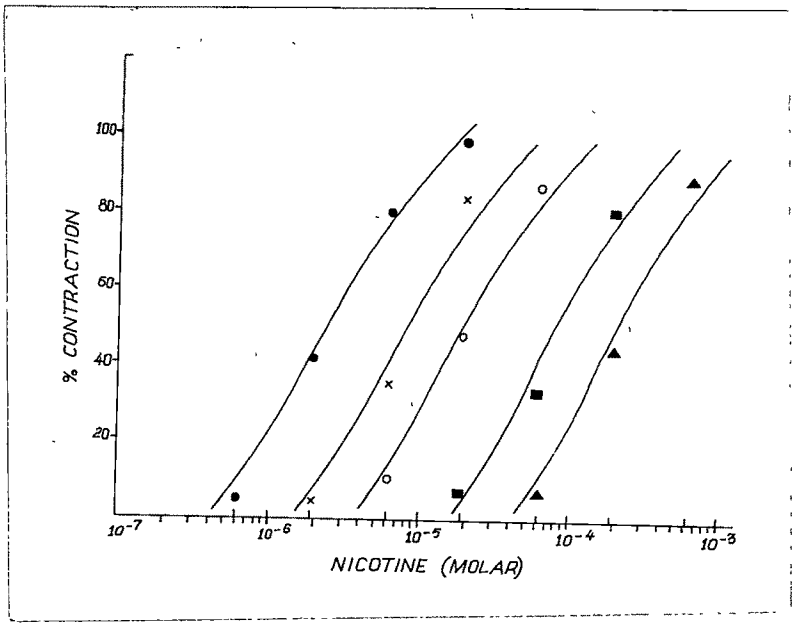
3.75×10^{-6}, 4.8×10^{-6}, 7.5×10^{-6} and 1.05×10^{-5}) could be used to construct the dose-response curves in the absence of the antagonists. Hexamethonium (4.95×10^{-6}, 1.58×10^{-5}, 4.95×10^{-5} and 1.58×10^{-4}) and tetraethylammonium (2.46×10^{-5}, 7.69×10^{-5}, 2.46×10^{-4} and 7.69×10^{-4}; Fig. 7 a) induced parallel shifts of dose-response curves of nicotine to the right. Similarly hexamethonium (1.58×10^{-6}, 4.95×10^{-6}, 1.58×10^{-5}, and 4.95×10^{-5}) and tetraethylammonium (2.46×10^{-5}, 7.69×10^{-5}, 2.46×10^{-4} and 7.69×10^{-4}) induced parallel shifts to the right of the dose-response curves of DMPP. There was no reduction of the maximal responses and no flattening of the curves. Lower doses of mecamylamine (6.02×10^{-7} and 1.92×10^{-6}); pempidine (6.45×10^{-7} and 2.06×10^{-6}; Fig. 7 b), chlorisondamine (8.83×10^{-8} and 2.76×10^{-7}) and pentolinium (6.66×10^{-7} and 2.08×10^{-6}) induced paralel shifts of the dose-response curves of nicotine and DMPP to right. With higher doses of mecamylamine (6.02×10^{-6} and 1.92×10^{-5}); pempidine (6.45×10^{-6} and 2.06×10^{-5}; Fig. 7 b); chlorisondamine (8.83×10^{-7} and 2.76×10^{-6}) and pentolinium (6.66×10^{-6} and 2.08×10^{-5}) the shifts of the dose-response curves to the right were nonparallel and there was reduction of the maximal responses and flattening of the curves. This indicated that antagonism by mecamylamine, pempidine, chlorisondamine and pentolinium was competitive in lower doses and not competitive in higher doses. The data were further analysed to calculate pA_2, pA_{10}, $pA_2 - pA_{10}$ and slope values as outlined for the dog nictitating membrane. The data are summarized in Tables 8 and 9. It is clear from the Tables that hexamethonium and tetraethylammonium acted competitively and the other

LEGEND FOR FIG. 7 a

Dose-response curves of the contractile responses (recorded with isotonic lever) of the isolated guinea pig ileum to nicotine (abscissa, log M of nicotine; ordinate, per centage of maximal contraction). ●——● indicates control dose-response curve. ×——×, o——o, ■——■ and ▲——▲ indicate dose-response curves after 2.46×10^{-5} M, 7.69×10^{-5} M, 2.46×10^{-4} M and 7.69×10^{-4} M respectively of tetraethylammonium. Contact time for nicotine was 30 sec and that for tetraethylammonium was 15 min.

LEGEND FOR FIG. 7 b

Dose-response curves of the contractile responses (recorded with isotonic lever) of isolated guinea pig ileum to nicotine (abscissa, log M of nicotine; ordinate, per centage of maximal contraction). ●——● indicates control dose-response curve. ×——×, o——o, ■——■ and ▲——▲ indicate dose-response curves after 6.45×10^{-7} M, 2.06×10^{-6} M and 2.06×10^{-5} M respectively of pempidine. Contact time for nicotine was 30 sec and that for pempidine was 15 min.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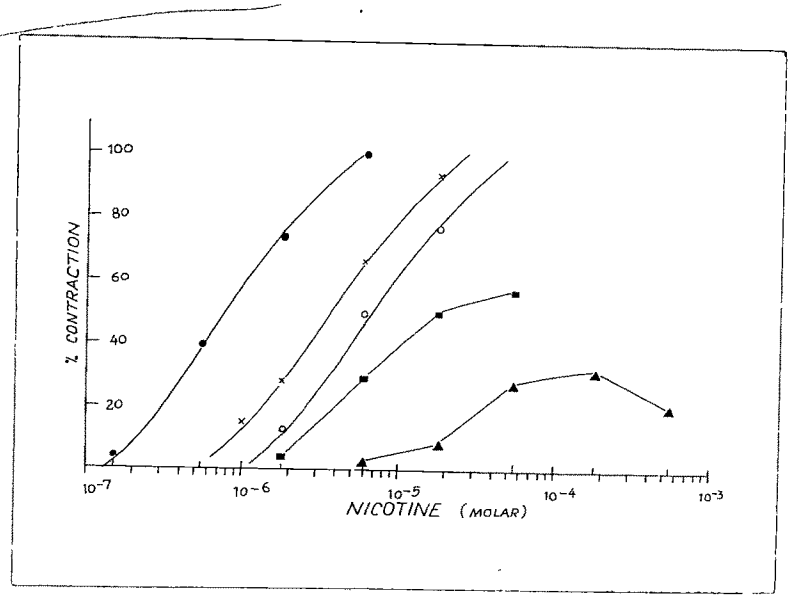
NICOTINE (MOLAR)

Fig 7 a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NICOTINE (MOLAR)

Fig 7 b

TABLE 8

Isolated guinea pig ileum (isotonic lever; agonist, nicotine). Values of pA_2, pA_{10}, $pA_2 - pA_{10}$, and slope (b) were obtained from pA plots. Probability (p) is of significance of experimental value of (b) from that of the theoretical value of unity for competitive antagonism.

Ganglionic blocker	Mean $pA_2 \pm$ S.E.M.	Mean $pA_{10} \pm$ S.E.M.	Mean pA_2-mean pA_{10}	Mean slope (b) $\pm$ S.E.M.	p
Hexamethonium (12)	5.64 ± 0.076	4.96 ± 0.096	0.68	1.06 ± 0.078	> 0.05
Tetraethyl-ammonium (12)	4.19 ± 0.094	3.38 ± 0.064	0.82	0.92 ± 0.067	> 0.05
Mecamylamine (12)	6.56 ± 0.037	5.46 ± 0.059	1.10	0.68 ± 0.073	< 0.05
Pempidine (12)	6.50 ± 0.087	5.28 ± 0.073	1.22	0.64 ± 0.073	< 0.05
Chlorisond-amine (12)	7.47 ± 0.074	6.36 ± 0.048	1.11	0.63 ± 0.069	< 0.05
Pentolinium (12)	6.35 ± 0.096	5.10 ± 0.084	1.25	0.58 ± 0.093	< 0.05

Figures in the parentheses indicate the number of experiments.

TABLE 9

Isolated guinea pig ileum (isotonic lever; agonist, DMPP). Values of pA_2, pA_{10}, $pA_2 - pA_{10}$, and slope (b) were obtained from pA plots. Probability (p) is of significance of experimental value of (b) from that of the theoretical value of unity for competitive antagonism.

Ganglionic blocker	Mean $pA_2 \pm$ S.E.M.	Mean $pA_{10} \pm$ S.E.M.	Mean pA_2-mean pA_{10}	Mean slope (b) ± S.E.M.	p
Hexamethonium (12)	5.77 ± 0.065	5.10 ± 0.074	0.67	1.13 ± 0.067	> 0.05
Tetraethyl-ammonium (12)	4.46 ± 0.074	3.65 ± 0.096	0.81	0.93 ± 0.067	> 0.05
Mecamylamine (12)	6.31 ± 0.065	5.80 ± 0.079	0.51	1.48 ± 0.036	< 0.05
Pempidine (12)	6.30 ± 0.071	5.00 ± 0.023	1.30	0.58 ± 0.075	< 0.05
Chlorisond-amine (12)	6.92 ± 0.037	5.72 ± 0.053	1.20	0.61 ± 0.035	< 0.05
Pentolinium (12)	6.63 ± 0.043	5.25 ± 0.054	1.38	0.53 ± 0.054	< 0.05

Figures in the parentheses indicate the number of experiments.

blockers did not act competitively.

With nicotine as the agonist the order of potency (pA_2 values) was chlorisondamine > mecamylamine > pempidine > pentolinium hexamethonium > tetraethylammonium and with the DMPP as the agonist the order of potency was chlorisondamine > pentolinium > mecamylamine > pempidine > hexamethonium > tetraethylammonium.

Auxotonic lever :

Nicotine (1.80×10^{-7}, 2.88×10^{-7}, 4.5×10^{-7} and 5.76×10^{-7}) and DMPP (7.5×10^{-6}, 1.1×10^{-5}, 1.56×10^{-5} and 2.2×10^{-5}) were used to construct the dose-response curves. The curves were more bell shaped than those with isotonic lever and the preparations showed "fade" very soon (12 experiments). Therefore, detailed investigation with the guinea pig ileum was carried out only with the isotonic lever.

ISOLATED GUINEA PIG HYPOGASTRIC NERVE VAS DEFERENS :

Isotonic lever :

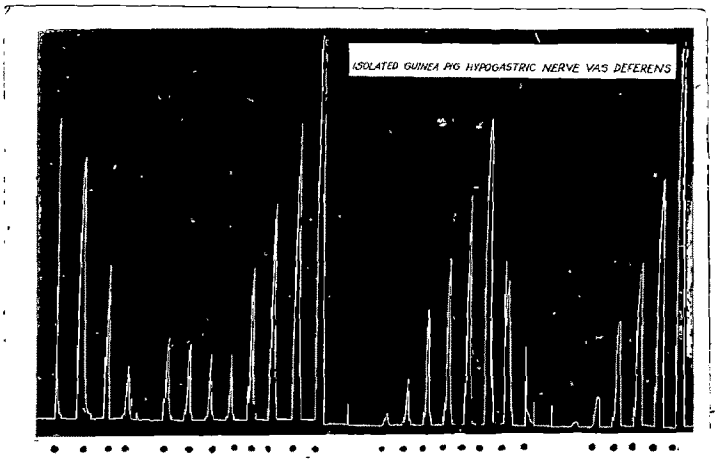
Hypogastric nerve when stimulated supramaximally (3 times the maximal voltage) at frequencies ranging from 1,2,5,7,10,25,50 and 100 per sec applied for 30 sec and pulse duration of 2.5 msec every 10 min elicited frequency-dependent responses of the smooth muscle. Frequency-response curves were obtained by plotting the log of the frequencies on the abscissa and per centage of the maximal contraction on the ordinate. Lower doses of all the six ganglion blockers i.e. hexamethonium (4.95×10^{-6} and 2.47×10^{-6} ; Fig. 8 a); tetraethylammonium (2.46×10^{-5} and 7.69×10^{-5} ; Fig. 8 b); mecamylamine (1.93×10^{-6} and 6.02×10^{-6}); pempidine (2.06×10^{-6} and 6.45×10^{-6} ; Fig. 8 c);

LEGEND FOR FIG. 8 a

Isolated guinea pig hypogastric nerve vas deferens preparation. Responses of vas deferens (at dots) stimulated indirectly through hypogastric nerve with supramaximal square wave pulses of 2.5 msec duration for 30 sec every 10 min. Frequencies (per sec) of stimuli were 7 at (1); 5 at (2); 3.5 at (3); 2 at (4); 10 at (5); 20 at (6); 60 at (7) and 120 at (8). Hexamethonium (4.95×10^{-6} M) was given at (A) and (1.98×10^{-4} M) at (B). Contact time for hexamethonium was 15 min.

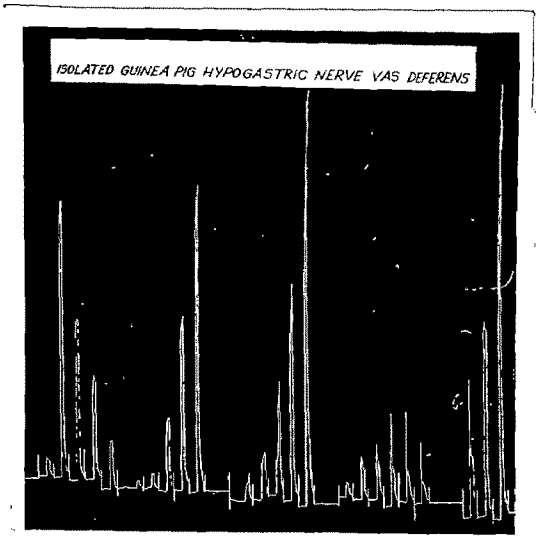
LEGEND FOR FIG. 8 b

Isolated guinea pig hypogastric nerve vas deferens preparation. Responses of vas deferens (at dots) stimulated indirectly through hypogastric nerve with supramaximal square wave pulses of 2.5 msec duration for 30 sec every 10 min. Frequencies (per sec) of stimulation were 7 at (1); 5 at (2); 3.5 at (3); 2 at (4); 10 at (5); 20 at (6); 60 at (7) and 120 at (8). Tetraethylammonium (7.69×10^{-5} M) was given at (A) and (7.69×10^{-4} M) at (B). Contact time for tetraethylammonium was 15 min.



1 2 3 4 A 2 2 2 2 1 5 6 7 B 4 3 2 1 5 6 7 8 4 3 2 1 5

Fig. 8a



A B

1 2 3 4 3 3 2 1 5 4 4 3 2 1 2 1 5 6 7 8 2 1 5

Fig. 8 b

LEGEND FOR FIG. 8 c

Isolated guinea pig hypogastric nerve vas deferens preparation. Responses of the vas deferens (at dots) stimulated indirectly through hypogastric nerve with supramaximal square wave pulses of 2.5 msec duration for 30 sec every 10 min. Frequencies (per sec) of stimulation were 2 at (1); 3.5 at (2); 5 at (3); 7 at (4); 10 at (5); 20 at (6); 60 at (7) and 120 at (8). Pempidine (2.06×10^{-6} M) was given at (A) and (6.45×10^{-5} M) at (B). Contact time for pempidine was 15 min.

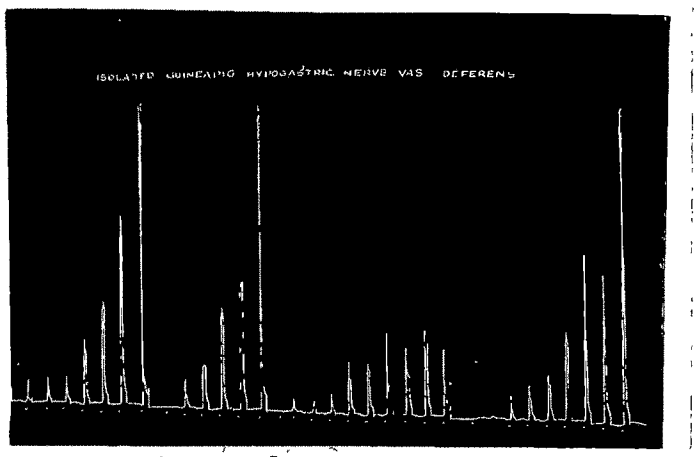

ISOLATED GUINEA PIG HYPOGASTRIC NERVE VAS DEFERENS
A
B
11 12345 3456 222445678 1234556

Fig. 8c

chlorisondamine (6.9×10^{-8} and 1.38×10^{-7}) and pentolinium (6.66×10^{-6} and 2.08×10^{-5}) produced parallel shifts to the right of the frequency-response curves. However, with higher doses of hexamethonium (9.9×10^{-5} and 1.98×10^{-4}; Fig. 8 a); tetraethylammonium (2.46×10^{-4} and 7.69×10^{-4}; Fig. 8 b); mecamylamine (1.93×10^{-5} and 6.02×10^{-5}); pempidine (2.06×10^{-5} and 6.45×10^{-5}; Fig. 8 c); chlorisondamine (2.76×10^{-7} and 6.9×10^{-7}) and pentolinium (4.16×10^{-5} and 6.66×10^{-5}) the shifts to the right were not parallel, and there was reduction of the maximal responses and flattening of the curves. The results suggested that in lower doses these agents acted competitively and in higher doses the antagonism was not competitive. Further analysis of the data was done as outlined for the dog nictitating membrane and the results are summarized in Table 10. The slope values of all the six antagonists were significantly different from the theoretical value of 1 ($p < 0.05$) suggesting that the six ganglion blockers did not act competitively at the peripheral ganglia situated on the hypogastric nerve innervating the vas. The order of potency (pA_2 values) was chlorisondamine > mecamylamine > pempidine > pentolinium > hexamethonium > tetraethylammonium.

The preparations when stimulated postganglionically by placing the shielded electrodes on the nerve 1-5 mm away from the vas exhibited responses at a time when responses to preganglionic stimulation had been completely blocked by the antagonists.

Auxotonic lever:

The data obtained with auxotonic lever were identical with

TABLE 10

Isolated guinea pig hypogastric nerve vas deferens (stimulated indirectly preganglionically). Values of pA_2, pA_{10}, $pA_2 - pA_{10}$, and slope (b) were obtained from pA plots. Probability (p) is of significance of the experimental value of (b) from that of the theoretical value of unity for competitive antagonism.

Ganglionic blocker	Mean $pA_2 \pm$ S.E.M.	Mean $pA_{10} \pm$ S.E.M.	Mean pA_2-mean pA_{10}	Mean slope (b) $\pm$ S.E.M.	p
Hexamethonium (12)	4.24 ±0.067	2.82 ±0.075	1.42	0.55 ± 0.073	< 0.05
Tetraethyl-ammonium (12)	3.44 ±0.093	2.07 ±0.075	1.37	0.51 ± 0.097	< 0.05
Mecamylamine (12)	5.53 ±0.075	4.52 ±0.067	1.01	0.72 ± 0.033	< 0.05
Pempidine (12)	5.39 ±0.066	4.30 ±0.037	1.08	0.67 ± 0.095	< 0.05
Chlorisondamine (12)	6.65 ±0.096	5.51 ±0.074	1.14	0.65 ± 0.032	< 0.05
Pentolinium (12)	4.96 ±0.040	3.64 ±0.065	1.32	0.58 ± 0.073	< 0.05

Figures in parentheses indicate the number of experiments.

those obtained with isotonic lever (12 experiments). Hence detailed study with this lever was not attempted.

(B) NON-NICOTINIC GANGLIONIC BLOCKERS:

CAT NICTITATING MEMBRANE:

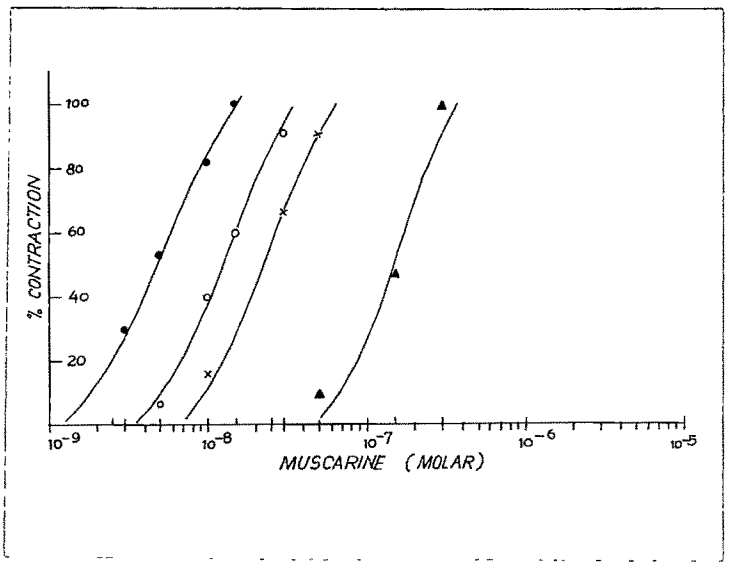
Muscarine and 4-(m-chlorophenyl-carbamoyloxy)-2-butynyl-trimethylammonium chloride (McN-A-343) elicited graded dose-related responses of the nictitating membrane when administered intra-arterially through the lingual artery into the blood supplying the superior cervical ganglion. In preliminary experiments it was observed that muscarine was more potent than McN-A-343. Muscarine (5.75×10^{-9}, 1.44×10^{-8}, 2.87×10^{-8} and 5.75×10^{-8}) and McN-A-343 (6.09×10^{-8}, 1.22×10^{-7}, 2.03×10^{-7}, 4.06×10^{-7}, 6.09×10^{-7} and 8.13×10^{-7}) were used to construct the dose-response curves by plotting the logs of their doses on the abscissa and the per centage of the maximal contraction on the ordinate. Atropine (3.4×10^{-10}, 8.5×10^{-10}, 1.7×10^{-9} and 3.4×10^{-9}; Figs. 9 a and 9 b) caused parallel shifts to the right (without suppression of the maximal responses and flattening of the curves) of the dose-response curves indicating competitive antagonism. From the pA plots pA_2, pA_{10}, $pA_2 - pA_{10}$ and slope values were computed. The data are summarized in Table 11. The slope values are close to the theoretical value of unity ($p > 0.05$). Thus atropine exhibited competitive antagonism against muscarine and McN-A-343 at the superior cervical ganglia of the cat.

LEGEND FOR FIG. 9 a

Dose-response curves of the contractile responses of the cat nictitating membrane to muscarine (injected intra-arterially into the blood supply of the superior cervical ganglion); abscissa, log M of muscarine; ordinate, per centage of maximal contraction. ●——● indicates control dose-response curve. o——o, ×——× and ▲——▲ indicate dose-response curves after 8.5×10^{-10} M, 1.7×10^{-9} M and 3.4×10^{-9} M respectively of atropine. Contractions were recorded 15 min after injection of atropine into the blood supply of the superior cervical ganglion.

LEGEND FOR FIG. 9 b

Dose-response curves of the contractile responses of the cat nictitating membrane to McN-A-343 (injected intra-arterially into the blood supply of the superior cervical ganglion); abscissa, log M of McN-A-343; ordinate, per centage of maximal contraction. ●——● indicates control dose-response curve. ×——×, o——o, ■——■ and ▲——▲ indicate dose-response curves after 3.4×10^{-10} M, 8.5×10^{-10} M, 1.7×10^{-9} M and 3.4×10^{-9} M respectively of atropine. Contractions were recorded 15 min after injection of atropine into the blood supply of the superior cervical ganglion.


% CONTRACTION
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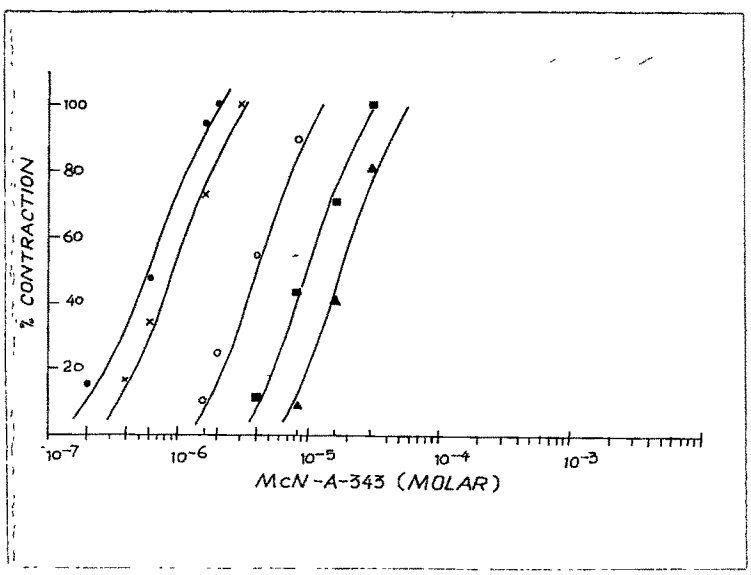
MUSCARINE (MOLAR)

Fig 9 a


% CONTRACTION
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10^{-5}
10^{-4}
10^{-3}
McN-A-343 (MOLAR)

Fig 9 b

TABLE 11

Cat nictitating membrane (isotonic lever; agonists, muscarine; McN-A-343). Values of pA_2, pA_{10}, $pA_2 - pA_{10}$, and slope (b) were obtained from pA plots. Probability (p) is of significance of experimental value of (b) from that of the theoretical value of unity for competitive antagonism.

Ganglionic blocker (muscarinic)	Mean $pA_2 \pm$ S.E.M.	Mean $pA_{10} \pm$ S.E.M.	Mean pA_2-mean pA_{10}	Mean slope (b) ± S.E.M.	p
Muscarine/ atropine (12)	9.60 ± 0.073	8.96 ± 0.087	0.64	1.11 ± 0.057	> 0.05
McN-A-343/ atropine (12)	9.48 ± 0.068	8.83 ± 0.078	0.66	1.16 ± 0.080	> 0.05

Figures in parentheses indicate the number of experiments.