ANALYSIS OF MODE OF ACTION OF SOME NICOTINIC BLOCKING DRUGS

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Abstract—Hexamethonium and tetraethylammonium (TEA) produced parallel shifts (equilibrium blockade) of nicotine and dimethylphenylpiperazinium dose-response curves at all dose levels on isolated rabbit and guinea pig ileum preparations, while mecamylamine, pempidine, chlorisondamine and pentolinium produced parallel shifts at lower doses and nonparallel shifts (nonequilibrium blockade) at higher dose levels. Hexamethonium and TEA protected against the nonequilibrium blockade by mecamylamine, pempidine, chlorisondamine and pentolinium. Dose ratios for the combination of hexamethonium with TEA, with chlorisondamine or with pentolinium were consistent with competitive type of blockade. Dose ratios for the combination of hexamethonium with mecamylamine or with pempidine were not consistent with the noncompetitive type of blockade. It is concluded, that hexamethonium and TEA act as equilibrium competitive ganglion blockers while mecamylamine, pempidine, chlorisondamine and pentolinium act as nonequilibrium competitive ganglion blockers.

Nicotinic ganglion blockers are agents which block the stimulant actions of nicotine or dimethyl phenylpiperazinium (DMPP) or acetyl choline at the ganglia. Chemically, these agents may be divided into two groups. Some of the quaternary onium compounds which comprise the first group include hexamethonium, tetraethylammonium (TEA), chlorisondamine and pentolinium. Mecamylamine and pempidine which are amines fall into the second group. Hexamethonium and TEA block the stimulant actions of nicotine or acetyl choline or DMPP (1–3) on the ganglia competitively. Mecamylamine and pentolinium (4, 5) act in a manner similar to hexamethonium. A different site (6) or a noncompetitive site (7) of action has however been proposed for mecamylamine. According to van Rossum (2) mecamylamine and pempidine act by a combination of competitive and noncompetitive antagonism and chlorisondamine acts purely as a competitive antagonist. Finally, Barnett and Benforado (8) observed that hexamethonium produced a combination of surmountable (competitive) and nonsurmountable (noncompetitive) inhibition and mecamylamine produced purely nonsurmountable inhibition of the effects of nicotine.

Thus the reports on the mode of action of the ganglion blocking agents are conflicting. In the present study, hexamethonium, tetraethylammonium, mecamylamine, pempidine, chlorisondamine and pentolinium were taken up for investigation. The reports on the

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competitive blocking action of hexamethonium and tetraethylammonium against nicotine and DMPP were confirmed. We, therefore, used these two agents as tools in some of the experiments designed to characterise the mode of action of other agents.

MATERIALS AND METHODS

Young adult rabbits weighing 1.2-2.5 kg and guinea pigs weighing 250-400 g were sacrificed by a sharp blow on the back of the neck and were exsanguinated by incising the neck vessels. The abdomen was opened and the terminal part of ileum near the ileocecal junction was removed to facilitate the preparation of two strips each approx. 2.5-3 cm long. The ileum preparations were suspended in 33 ml organ bath containing Tyrode solution of the following composition: NaCl, 136; KCl, 2.7; CaCl₂, 2.6; NaHCO₃, 20; NaH₂PO₄, 0.4; dextrose, 5.5 (mMoles per liter of distilled water).

The preparations were maintained at 37°C±1°C and the solution was bubbled with oxygen (guinea pig) or air (rabbit). The pH of the solution was 7.6. The contractions of the rabbit ileum were recorded with isotonic and auxotonic levers and those of the guinea pig ileum with an isotonic lever. The isotonic and auxotonic levers were subjected to 1 g tension and the magnification was 10-fold. The recording was made on smoked kymograph paper. The preparations were allowed to stabilize for 20 to 30 min before addition of the drugs.

1. **Quantitation of the blocking activity of ganglion blockers**

Ileum preparations from both rabbit and guinea pig were employed for this purpose. Two preparations from the same ileum were set up simultaneously. Nicotine and dimethylphenylpiperazinium (DMPP) were used as the agonists. After recording response to a dose of an agonist (contact time 30 sec) the preparation was given a washout. For obtaining control dose response curves, a response-wash cycle of 8 min was maintained and at least 4 doses of the agonist were employed.

The blockers were tested at four dose levels and were kept in the bath for 15 min before eliciting response to a dose of the agonist in the presence of the blocker. The preparation was now washed and following recovery from the blocker, the response to a higher dose of the agonist was obtained. In this manner dose-response curve in the presence of a dose of blocker was obtained. Not more than two doses of a blocker were tested in one preparation. Since two preparations from the same ileum were set up simultaneously, it was possible to test all the four doses on the same ileum. Three preparations were employed for each dose. Recovery of control responses to the agonists occurred after 15-20 min of washout of hexamethonium and 5-10 min of washout of tetraethylammonium. Recovery from the lower two doses of mecamylamine, pempidine, chlorisondamine and pentolinium occurred after 15-20 min but that from the higher two doses of mecamylamine, pempidine and pentolinium occurred after 50-70 min. Recovery from the higher two doses of chlorisondamine occurred after 2-5 hr.

Agonist dose-response curves were constructed by plotting the log dose on the abscissa and % of the maximal contraction on the ordinate.
Dose ratio, which is the ratio of equiactive doses of the agonist before and after the antagonist (9) was determined from the horizontal distance between the parallel dose-response lines; and when the lines were not parallel, it was calculated from the horizontal distance between dose-response curves at 50% of the maximal response.

The method of Arunlakshana and Schild (10) was used for constructing pA₂ plots. For this purpose log (dose ratio -1) was plotted on the ordinate against the negative log of the molar concentration of the antagonist on the abscissa. The intercept of the regression line with the abscissa (at zero level) gave the pA₂ value which was used for calculating the Kₐ value since pA₂ = -logKₐ. Antagonism was considered as competitive if the slope value (b) of the regression line was not significantly (p>0.05) different from the theoretical value of slope of -1 for competitive antagonism.

The Kₐ values of mecamylamine, pempidine, chlorisondamine and pentolinium under equilibrium conditions were calculated from the following formula (11):

\[ K_a = \frac{B}{\text{dose ratio} - 1} \]

where B is the concentration of the ganglion blocker studied. The K'ₐ values of these agents under nonequilibrium conditions were calculated from the formula:

\[ \log K'_{a} = pD'_{1} - pD_{x} - \log(x - 1) \]

where x is the ratio of the maximal responses to a given dose of the agonist in the absence and presence of a concentration of the ganglion blocker, the negative logarithm of which is pD₅ (12).

2. Experiments with combination of blockers

If two blockers compete for the same site, dose ratio for the combination (DR₁₊₂) will be equal to DR₁ + DR₂ - 1. If they act at different loci, (DR₁₊₂) = DR₁ × DR₂ (13). Experiments were therefore, made with the rabbit ileum (auxotonic lever) employing a combination of hexamethonium and other blockers. Nicotine or DMPP were used as agonists. Other details were as described under “quantitation of blocking action of ganglion blockers”.

3. Protection experiments

Ariens et al. (14) have shown that reversible competitive antagonists can afford excellent protection of parasympathetic receptors in guinea pig ileum against dibenamine blockade. Therefore, hexamethonium and TEA were used for protecting nicotinic ganglion receptors. Two preparations from the same ileum, one of which was protected and the other unprotected were set up simultaneously. After recording control agonist (nicotine; contact time, 1 min) dose-response curves, hexamethonium or TEA were kept in one of the baths for 15 min followed by mecamylamine or pempidine or chlorisondamine or pentolinium or atropine for another 15 min. The preparations were then given a washout. Ten min after the washout, responses to increasing doses of the agonist were elicited every 10 min. The specificity of the protecting action of hexamethonium or TEA was checked against the antimuscarinic action of atropine with muscarine as the agonist.

Statistical tests are those described by Snedecor (15).
The following drugs were used: Nicotine, 1, 1-dimethyl-4-phenylpiperazinium iodide (DMPP), hexamethonium chloride, tetraethylammonium bromide (TEA), mecamylamine hydrochloride, pempidine tartrate, chlorisondamine dimethochloride, pentolinium tartrate, muscarine iodide and atropine sulphate.

RESULTS

Nicotine \((5.6 \times 10^{-5} - 1.9 \times 10^{-5} \text{ M})\) and DMPP \((1.5 \times 10^{-6} - 3.0 \times 10^{-5} \text{ M})\) elicited graded contractile responses in the isolated rabbit ileum. The dose-response curves of the two agonists were bell-shaped, more so with nicotine. In order to avoid autoinhibition due to agonists, the influence of antagonists was studied on the ascending limb of the dose-response curves and extremely high doses of the agonists were avoided. There was no difference between records obtained with the isotonic or the auxotonic levers.

When responses of the guinea pig ileum to nicotine and DMPP were recorded with the isotonic lever, dose-response curves were more steep than those seen in the rabbit ileum. Thus in the absence of antagonists a narrow dose range of nicotine \((1.8 \times 10^{-7} - 1.8 \times 10^{-6} \text{ M})\) and DMPP \((1.6 \times 10^{-6} - 1.1 \times 10^{-5} \text{ M})\) could be used for constructing the dose-response curves. With auxotonic lever, autoinhibition ("fade") with nicotine \((1.8 \times 10^{-7} - 5.8 \times 10^{-7} \text{ M})\) and DMPP \((7.5 \times 10^{-6} - 2.2 \times 10^{-5} \text{ M})\) was greater than with the isotonic lever (12 experiments), therefore, the antagonists were studied only with the latter.

1. Quantitation of the blocking activity of ganglion blockers

Hexamethonium, TEA, mecamylamine, pempidine, chlorisondamine and pentolinium shifted the dose-response curves of nicotine and DMPP to the right. Hexamethonium

![Fig. 1. Dose-response curves of contractions of isolated rabbit ileum (isotonic lever) to nicotine. • - • are control responses. The other responses (left to right) were elicited in the presence of increasing concentrations of hexamethonium \((2.5 \times 10^{-3} \text{ M}, 5 \times 10^{-3} \text{ M}, 1 \times 10^{-4} \text{ M} \text{ and } 2 \times 10^{-4} \text{ M})\). Note that all concentrations of the blocker produced parallel shifts in the dose-response curve.](image)
Fig. 2. Dose-response curves of contractions of isolated guinea pig ileum (isotonic lever) to DMPP. ●—● are control responses. The other responses (from left to right) were elicited in the presence of increasing concentrations of tetraethylammonium (2.5 × 10^{-5} M, 7.7 × 10^{-5} M, 2.5 × 10^{-4} M and 7.7 × 10^{-4} M). Note that all concentrations of the blocker, produce parallel shifts of the dose-response curve.

Fig. 3. Dose-response curves for contractions of isolated guinea pig ileum (isotonic lever) to nicotine. ●—● are control responses. The other responses (from left to right) were elicited in the presence of increasing concentrations of pempidine (6.5 × 10^{-5} M, 2.1 × 10^{-4} M, 6.5 × 10^{-4} M and 2.1 × 10^{-3} M) or chlorisodamine (8.8 × 10^{-6} M, 2.8 × 10^{-5} M, 8.8 × 10^{-5} M and 2.8 × 10^{-4} M). Note that the lower two concentrations of each blocker produced parallel shifts, while the higher two concentrations depressed the maximal responses.
FIG. 4. Dose-response curves for contractions of isolated guinea pig ileum (isotonic lever) to DMPP. ● are control responses. The other responses (from left to right) were elicited in the presence of increasing concentrations of pentolinium (6.7 x 10^-6 M, 2.1 x 10^-5 M, 6.7 x 10^-4 M and 2.1 x 10^-3 M) or mecamylamine (6.0 x 10^-6 M, 1.9 x 10^-5 M, 6.0 x 10^-4 M and 1.9 x 10^-3 M). Note that the lower two concentrations of each blocker produced parallel shifts, while the higher two concentrations depressed the maximal responses.

TABLE 1. Data on the $K_B$ and slope values calculated from $pA_x$ plots$^{(1)}$ for hexamethonium and tetraethylammonium obtained with isolated rabbit and guinea pig ileum preparations.

| Ganglionic blocker$^{(2)}$ | Species and recording lever | Agonist | Dissociation constants $K_B$ (x 10^-6 M) | Slope ± S.E.M. |
|---------------------------|-----------------------------|---------|----------------------------------------|----------------|
| Hexamethonium 0.16-20     | Rabbit (isotonic)           | Nicotine | 2.8 | -1.13 ± 0.07 |
|                           | Rabbit (auxotonic)          | Nicotine | 1.4 | -0.96 ± 0.04 |
|                           | Guinea pig (isotonic)       | Nicotine | 0.23 | -1.06 ± 0.08 |
| Tetraethylammonium 2.5-250| Rabbit (isotonic)           | Nicotine | 4.5 | -1.05 ± 0.05 |
|                           | Rabbit (auxotonic)          | Nicotine | 6.6 | -0.97 ± 0.01 |
|                           | Guinea pig (isotonic)       | Nicotine | 6.5 | 0.92 ± 0.07 |

$^{(1)}$ The $pA_x$ plots were obtained with -log molar concentrations of the blockers on the abscissa and log (dose ratio −1) on the ordinate. The slope values were determined by regression analysis.

$^{(2)}$ For each estimate of $pA_x$, four doses of a blocker were employed and 3 experiments were done with each dose.

$^{(3)}$ $K_B$ values were calculated from $pA_x = -\log K_B$
and TEA in all the doses and the four other blockers in two lower doses each caused parallel shifts of the dose-response curves to the right with no reduction of the maximal responses and no flattening of the curves. Dose ratios for hexamethonium ranged between 1.3 and 53.7 and those for TEA ranged between 1.3 and 223.9. Equilibrium dose ratios for the other four blockers ranged between 1.2 and 10.2. With the higher doses of each mecamylamine, pempidine, chlorisondamine and pentolinium, shifts of the agonist dose-response curves were not parallel and there was a reduction in the maximal responses and flattening of the curves. Figs. 1, 2, 3 and 4 represent data plotted for one set of experiments each with hexamethonium, TEA, mecamylamine, pempidine, chlorisondamine and pentolinium with the rabbit or guinea pig ileum.

The regression lines of pA₅₀ plots for hexamethonium and TEA were significantly different from zero (P<0.05). Slope values of the regressions were close to the theoretical values of unity (Table 1) for competitive antagonism (P>0.05). Since hexametho-

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**TABLE 2.** Dissociation constants (Kₐ and K'ₐ values) of ganglion blockers determined in isolated rabbit ileum (isotonic lever).

| Ganglion blocker (x 10⁻⁷ M) | Mean Kₐ (x 10⁻⁷ M) or K'ₐ (x 10⁻⁷ M) ± S.E.M. | Mean Kₐ (x 10⁻⁷ M) or K'ₐ (x 10⁻⁷ M) ± S.E.M. |
|-----------------------------|-----------------------------------------------|-----------------------------------------------|
| Mecamylamine                | Agonist, nicotine                            | Agonist, DMPP                                 |
| 6                           | 2.5± 0.55                                    | 1.6± 0.11                                    |
| 19                          | 4.4± 0.82                                    | 1.8± 0.43                                    |
| 60                          | 80 ± 9                                        | 39 ± 10.3                                    |
| 190                         | 130 ± 14                                      | 70 ± 10                                      |
| Pempidine                   |                                               |                                              |
| 6.5                         | 4.4± 0.83                                    | 3.1± 0.44                                    |
| 21                          | 6.5± 0.66                                    | 6.2± 0.82                                    |
| 65                          | 120 ± 17                                      | 150 ± 9                                      |
| 210                         | 240 ± 23                                      | 290 ± 27                                      |
| Chlorisondamine             |                                               |                                              |
| 2.8                         | 7.8± 0.58                                    | 7.9± 0.12                                    |
| 6.9                         | 5.4± 1.17                                    | 5.5± 0.19                                    |
| 14                          | 13 ± 1.8                                      | 8.3± 1.7                                    |
| 69                          | 25 ± 0.7                                      | 7.4± 0.66                                    |
| Pentolinium                 |                                               |                                              |
| 21                          | 14 ± 3.5                                      | 15 ± 2.4                                    |
| 67                          | 13 ± 2.3                                      | 10 ± 2                                      |
| 210                         | 370 ± 110                                     | 620 ± 110                                    |
| 670                         | 300 ± 39                                      | 460 ± 185                                    |

(1) Number of observations for each dose of ganglion blocker = 3
(2) Kₐ values were calculated for the lower two doses of each ganglion blocker (equilibrium blockade) according to the formula: Kₐ = antagonist (M) / dose ratio 1.
(3) K'ₐ values were calculated for the higher two doses of each ganglion blocker (non-equilibrium blockade) according to the formula: log K'ₐ = pDx + log(x-1), where pDx is the negative log of the molar doses of antagonist and x is the ratio of maximum responses in the presence and absence of the antagonist.
nium and TEA acted competitively at any dose, the KB values for these agents were not calculated for each dose. KB values are shown in Table 1.

| TABLE 3. Dissociation constants (KB and K'B values) of ganglion blockers determined in isolated rabbit ileum (auxotonic lever). |
|---|---|---|
| **Ganglion blocker** (×10⁻⁷M) | **Mean KB(1) or K'B(2)±S.E.M.** (×10⁻⁷M) | **Mean KB(1) or K'B(2)±S.E.M.** (×10⁻⁷M) |
| Mecamylamine | Agonist, nicotine | Agonist, DMPP |
| 6 | 4.3±0.7 | >0.5 |
| 19 | 3.7±0.4 | 5.7±2.3 |
| 60 | 100±9 | 51±7.1 |
| 190 | 130±14 | 69±13 |
| Pempidine | 6.5 | 3.2±0.68 | >0.5 |
| | 21 | 3±0.47 | 11.7±1.2 |
| | 65 | 200±35 | >0.05 |
| | 210 | 310±24 | 180±39 |
| Chlorisondamine | 2.8 | 2.8±0.99 | >0.5 |
| | 8.8 | 3.6±0.92 | 2.3±0.19 |
| | 28 | 45±3.7 | 14±3.2 |
| | 88 | 50±7 | 23±4.8 |
| Pentolinium | 1.3 | 1.9±0.2 | >0.1 |
| | 4.2 | 2.5±0.13 | 2.7±0.47 |
| | 13 | 45±9 | 23±4.8 |
| | 42 | 46±11.4 | 33±2.9 |
| Atropine | 1.1 | — | 0.79±0 |
| | 3.5 | — | 0.5±0.37 |
| | 11 | — | 2±0.23 |
| | 35 | — | 24±1.3 |

(1) Number of observations for each dose of ganglion blocker = 3
(2) The two lower doses of mecamylamine, pempidine, chlorisondamine and pentolinium produced equilibrium blockade (KB values).
(3) The two higher doses of mecamylamine, pempidine, chlorisondamine and pentolinium produced non-equilibrium blockade (K'B values). All four doses of atropine produced non-equilibrium blockade.

For calculations of KB and K'B values see Table 2.

The individual KB values for each of the two lower doses and the individual K'B values for each of the two higher doses of mecamylamine, pempidine, chlorisondamine and pentolinium calculated from curves such as those in Figs. 3 and 4 were pooled and the mean values are given in Tables 2, 3 and 4. The KB values of these agents are 1 to 2 log units lower than those of hexamethonium and TEA. In a number of instances the KB values of these agents are up to 2 log units lower than their K'B values. The KB values obtained with the rabbit ileum in the two lower doses of a blocker were not significantly different except in the case of pempidine (against DMPP, Tables 2 and 3) and chlo-
Table 4. Dissociation constants ($K_B$ and $K'_B$ values) of ganglion blockers determined in the isolated guinea pig ileum (isotonic lever).

| Ganglion blocker $^{(1)}$ | Mean $K_B^{(2)}$ or $K_B^{(3)}$ $\pm$ S.E.M. $^{(2)}$ | Mean $K_B^{(4)}$ or $K_B^{(5)}$ $\pm$ S.E.M. $^{(2)}$ |
|---------------------------|-------------------------------------------------|-------------------------------------------------|
|                           | $^{(1)}$ or $^{(3)}$ or $^{(5)}$ $\times 10^{-2}$ M | $^{(1)}$ or $^{(3)}$ or $^{(5)}$ $\times 10^{-2}$ M |
| Mecamylamine              | Agonist, nicotine                               | Agonist, DMPP                                   |
| 6                         | $3.5 \pm 0.18$                                  | $7.3 \pm 2$                                    |
| 19                        | $3.7 \pm 0.91$                                  | $6.9 \pm 0.3$                                  |
| 60                        | $540 \pm 20.4$                                  | $180 \pm 26$                                   |
| 190                       | $300 \pm 126$                                   | $340 \pm 35$                                   |
| Pempidine                 | $4.1 \pm 0.9$                                   | $7.3 \pm 0.105$                                |
| 21                        | $3.4 \pm 0.3$                                   | $8.6 \pm 1.11$                                 |
| 65                        | $100 \pm 53$                                    | $190 \pm 4.7$                                  |
| 210                       | $330 \pm 76$                                    | $290 \pm 41$                                   |
| Chlorisondamine           | $0.6 \pm 0.09$                                  | $1.4 \pm 0.19$                                 |
| 2.8                       | $0.96 \pm 0.23$                                 | $1.9 \pm 0.3$                                  |
| 8.8                       | $10.5 \pm 3$                                    | $28 \pm 7.4$                                   |
| 28                        | $9 \pm 4.1$                                     | $15 \pm 5.1$                                   |
| Pentolinium               | $26 \pm 8.3$                                    | $9.4 \pm 2.1$                                  |
| 21                        | $9.6 \pm 3$                                     | $17 \pm 0.9$                                   |
| 67                        | $190 \pm 91$                                    | $230 \pm 80$                                   |
| 210                       | $130 \pm 27$                                    | $280 \pm 99$                                   |

(1) Number of observations for each dose $= 3$

(2) The lower two doses of each ganglion blocker produced equilibrium blockade ($K_B$ values).

(3) The higher two doses of each ganglion blocker produced nonequilibrium blockade ($K'_B$ values).

For calculations of $K_B$ and $K'_B$ values see Table 2.

In all cases of unprotected rabbit ileum preparations, maximal responses to nicotine were blocked by mecamylamine, pempidine, chlorisondamine and pentolinium with approx. a 60-90 min washout of the blockers from the bath fluid. In protected preparations maximal responses to the blockers could be elicited at this time (Figs. 5 and 6). In the case of atropine, the slope of the nicotine dose-response curve was completely changed (Fig. 7) in unprotected experiments. Hexamethonium or TEA produced dramatic protection in that the dose-response curve for protected experiments ran parallel to the con-
trol dose-response curve (Fig. 7). Hexamethonium or TEA gave a slight protection against the atropine blockade of contractile responses of the ileum to muscarine (Fig. 7).

Results with both guinea pig and rabbit ileum (1 experiment with each blocker) were similar.

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Fig. 5. Dose-response curves for contractions of paired (protected and unprotected) isolated rabbit ileum preparations to nicotine (auxotonic lever). Pempidine ($6.5 \times 10^{-5} \text{M}$) or chlorisondamine ($2.8 \times 10^{-6} \text{M}$) blocked the maximal responses of unprotected preparations. Maximal responses could be elicited in preparations protected by hexamethonium ($6.3 \times 10^{-4} \text{M}$). Three experiments were set up for each blocker. Vertical bars indicate S.E.M.

Fig. 6. Dose-response curves for contractions of paired (protected and unprotected) isolated rabbit ileum preparations to nicotine (auxotonic lever). Mecamylamine ($1.9 \times 10^{-5} \text{M}$) or pentolinium ($4.2 \times 10^{-4} \text{M}$) blocked the maximal responses of unprotected preparations. Maximal responses could be elicited in preparations protected by tetraethylammonium ($4.6 \times 10^{-4} \text{M}$). Three experiments were set up for each blocker. Vertical bars indicate S.E.M.
3. **Combination of antagonists**

Data are summarized in Table 5. It is clear that in the case of hexamethonium and TEA or hexamethonium and chlorisondamine or hexamethonium and pentolinium combinations, the results are consistent with \((DR_{1+2})=DR_1+DR_2-1\). In the case of a combination of hexamethonium with mecamylamine or pempidine, the results are neither

| Ganglion blockers | Concentration M | DR \pm S.E.M. | Observed DR for combination \pm S.E.M. | Theoretical DR after combination
|------------------|----------------|--------------|-------------------------------------|---------------------------------|
|                  |                | DR \pm S.E.M. | Competiton                          | Non-competition                 |
| Hexamethonium    | \(1 \times 10^{-4}\) | 9 \pm 0.4 | 11 \pm 0.6 | 10 | 18 |
| TEA              | \(2.5 \times 10^{-5}\) | 2 \pm 0.8 | (3) |                |
| Hexamethonium    | \(1 \times 10^{-4}\) | 9 \pm 0.4 | 9.6 \pm 2.7 | 18 | 90 |
| Mecamylamine     | \(6 \times 10^{-6}\) | 10 \pm 1.04 | (5) |                |
| Hexamethonium    | \(1 \times 10^{-4}\) | 9 \pm 0.4 | 26.4 \pm 8.6 | 66 | 522 |
| Pempidine        | \(2.1 \times 10^{-5}\) | 58 \pm 8.9 | (5) |                |
| Hexamethonium    | \(5 \times 10^{-6}\) | 8 \pm 1.6 | 13 \pm 8.5 | 12 | 40 |
| Chlorisondamine  | \(2.8 \times 10^{-6}\) | 5 \pm 0.8 | (4) |                |
| Hexamethonium    | \(1 \times 10^{-4}\) | 9 \pm 0.4 | 22.4 \pm 10.6 | 18 | 90 |
| Pentolinium      | \(1.3 \times 10^{-6}\) | 10 \pm 1 | (5) |                |

Figures in parentheses indicate the number of observations. Agonist used with hexamethonium and chlorisondamine combination was DMPP; in all other cases the agonist was nicotine.
consistent with \( (\text{DR}_{14}) = \text{DR}_1 - \text{DR}_2 - 1 \), nor with \( (\text{DR}_{14}) = \text{DR}_1 \times \text{DR}_2 \). Although the doses of mecamylamine, pempidine, chlorisondamine and pentolinium were such that when used alone, they depressed maximal agonist responses, in combination with hexamethonium, maximal responses were obtained in some experiments, but were depressed in others. This produced dose ratios with great variation. In contrast, the low standard error for hexamethonium and TEA combination is striking.

**DISCUSSION**

When responses of the rabbit ileum to DMPP and nicotine were recorded with auxotonic lever, "fade" reported by Paton (16) and Ariens (17) for the guinea pig intestine (and confirmed in the present study) was not observed. In isolated tissues made to contract under isometric (or auxotonic) conditions, high tensions are built up to which the organ may give way by change in shape i.e. elongate resulting in "fade" (17). The isolated rabbit ileum possesses strong rhythmic pendular movements which are not observed in the isolated guinea pig ileum. It is therefore possible that these strong rhythmic pendular movements inhibit the development of high tension and, therefore of "fade".

Hexamethonium, TEA and lower concentrations of mecamylamine, pempidine, chlorisondamine and pentolinium caused parallel shifts of the dose-response curves of nicotine and DMPP. With a higher dose of the latter four blockers, shifts of the dose-response curves were not parallel and there was a progressive flattening of the curves with reduction of the maximal responses. The slope values of regression lines of \( \rho A_x \), plots for hexamethonium and TEA were close to the theoretical value of unity for competitive antagonism.

It is possible that mecamylamine, pempidine, chlorisondamine and pentolinium act competitively at lower doses and noncompetitively at higher doses. It may be improbable but not impossible to invoke different mechanisms of action at different doses to achieve the same effect. In fact, there is no convincing evidence for "specific", noncompetitive antagonism (18). Another possibility is that these antagonists act noncompetitively at all concentrations but spare receptors for the agonist are present. A non-competitive site of action for mecamylamine, pempidine, chlorisondamine and pentolinium has to be ruled out. Except for two instances, the \( K_b \) values of all blockers were not significantly different from each other at the two lower doses. This is in accord with the observation that under equilibrium conditions the \( K_b \) values are independent of the concentration of an antagonist (10). Secondly, TEA or hexamethonium which act competitively protected the intestinal ganglia from blockade by these agents. In protected preparations the shifts of the dose-response curves were parallel and maximum responses were no longer depressed. Receptor protection design has been criticised by Wand (19) for two reasons. Firstly, high concentrations of agonists conventionally employed for protection can occupy sites other than the receptor. We used antagonists rather than agonists for protection. Moreover, even in high doses which were used for protecting nicotinic receptors, muscarinic receptors were slightly protected against blockade by atropine. On the other hand, hexamethonium and TEA did protect against the nicotinic
ganglion blocking action of high doses of atropine. The second objection of Waud related to the presence of "spare receptors". The dose ratios with lower doses of mecamylamine, pempidine, chlorisondamine and pentolinium (which produced equilibrium blockade) ranged between 1.2 to 10.2. Admittedly, these dose ratios obtained with partial agonists would not give quantitative estimate of receptor reserve in intestinal ganglia. The doses of hexamethonium (6.3 x 10^{-4} M) and TEA (4.6 x 10^{-3} M) used for receptor protection were fairly large and must have blocked all receptors, including spare receptors.

According to Paton and Rang (13) when two antagonists compete to occupy the same receptors, then the dose ratio \((DR_{1+2}) = DR_1 + DR_2 - 1\) and when they occupy different receptors \((DR_{1+2}) = (DR_1 \times DR_2)\) where \(DR_1\) and \(DR_2\) are the dose ratios of each of the antagonists given separately. Results depicted in Table 5 indicate that dose ratios obtained for the combination of hexamethonium and TEA or hexamethonium and chlorisondamine or hexamethonium and pentolinium are consistent with the same locus of action. Results obtained for the combination of hexamethonium and mecamylamine or hexamethonium and pempidine rule out different loci of action. The low \(DR\) values for hexamethonium and mecamylamine or hexamethonium and pempidine combination could be ascribed to a good proportion of the amines becoming intracellularly concentrated, the receptors having been protected by the simultaneous administration of shorter acting hexamethonium.

The lower \(K_a\) values and the delayed offset of action of mecamylamine, pempidine, chlorisondamine and pentolinium compared with those of TEA and hexamethonium indicate that the former four agents have a much higher affinity for the nicotinic ganglion receptor. Barlow and Franks (20) have suggested that in the case of hexamethonium, the second onium group is not likely to contribute significantly to binding. In the present study, hexamethonium and TEA were almost equipotent and mecamylamine and pempidine which have only one nitrogen were more potent than hexamethonium. All ganglion blocking agents investigated in the present study, can form an ionic bond with the receptor, the onium compounds because of the cationic head and amines because of steric crowding around nitrogen, which restricts the availability of its unshared electron pair. The greater affinity of the amines and pentolinium as compared to that of hexamethonium or TEA could be due to a greater hydrophobicity. The hydrocarbon rings possessed by all of them and the methyl groups of mecamylamine and pempidine could all make these compounds more hydrophobic. In the case of chlorisondamine, the four chlorines of tetrachlorobenzene moiety by creating a dense electron cloud could be assumed to form a sort of \(\pi\) complex with the receptor.

DMPP and nicotine are partial agonists. Nonparallel shifts of the dose-response curves of DMPP and nicotine with the higher doses of the four potent antagonists could be explained in terms of Thron and Waud's (21) access-limited model. This model has been proposed by them for the muscarinic receptor of the guinea pig ileum since there is evidence (13) that atropine is taken up by the muscarinic receptor of the guinea pig ileum. According to this model, a potent antagonist, because of its high affinity would be displaced.
by an agonist only with difficulty. Since a partial agonist requires occupancy of a large proportion of a receptor pool for maximal response, it would encounter fewer free receptors when high doses of a potent antagonist are used. The uptake of potent ganglion blockers by the nicotinic ganglion receptors has yet to be shown although the uptake of C\(^{14}\)-curarine or C\(^{14}\)-toxiferin by the endplates of mouse diaphragm has been demonstrated by Waser (22).

In a number of instances the \(K'_a\) values were 2 log units higher than \(K_a\) values. It is already well documented that if the ratio of \(K'_a/K_a\) values is higher, the compound behaves as a competitive antagonist over a wide dose range (12). The higher \(K'_a\) values compared with the much lower \(K_a\) values of the potent antagonists would suggest site(s) of loss for many antagonists. According to the model of Thron and Waud (21) the receptors would form a large "virtual" space for potent antagonists, since such antagonists would require lesser receptor occupancy for a given degree of blockade as compared with weaker antagonists. In the case of mecamylamine and pempidine, intracellular localization would be an additional site of loss. In general, the \(K'_a\) values with the highest doses of mecamylamine and pempidine were significantly higher than those with the lower doses implying greater losses of amines at higher doses than those of chlorisondamine and pentolinium. Presumably, at higher doses, larger quantities of the amines are finding their way into the cells.

Atropine at all concentrations produced a nonparallel shift of DMPP dose-response curves. In this respect it differed from mecamylamine, pempidine, chlorisondamine and pentolinium which produced parallel shifts at lower concentrations. The nonparallel shift even at the lowest concentration of atropine is presumably due to the antimuscarinic action of atropine. Hexamethonium gave a slight protection against the antimuscarinic action of atropine.

The present results, therefore, suggest that all ganglion blockers including high doses of atropine have nicotinic receptors as their site of action. Mecamylamine, pempidine, chlorisondamine and pentolinium may, therefore, be designated as nonequilibrium competitive antagonists as opposed to hexamethonium and TEA which are equilibrium competitive antagonists. The controversial literature reports cited in "Introduction" can easily be explained in light of the present study.

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