ANTAGONISTIC ACTIVITY OF SOME PHENOTHIAZINE DERIVATIVES AGAINST NEUROHUMORAL TRANSMITTERS

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Abstract—The antagonistic activity of eighteen phenothiazine derivatives against the neurohumors histamine, ACh, NA and 5-HT was studied in vitro and compared with accepted specific antagonists, viz., diphenhydramine, atropine, tubocurarine, phenoxybenzamine and LSD-25. The phenothiazines exhibited a dual antagonism (competitive and non-competitive) against histamine and muscarinic action of ACh. Many of them appeared more potent and more specific on histamine receptors than on ACh receptors while several others did not discriminate between the two receptor types. The phenothiazines showed a non-competitive antagonism against ACh (nicotinic action), NA and 5-HT. The activity against ACh (nicotinic action) was low while that against 5-HT was moderate. The α-adrenergic blocking activity was not shared by all the derivatives and some even potentiated the NA response. Only atropine and tubocurarine exhibited a pure competitive antagonism over a wide concentration range on the muscarinic and nicotinic receptors, respectively. Phenoxybenzamine possessed the widest spectrum of activity, antagonizing all the neurohumors. 5-HT receptors showed the maximum susceptibility to blockade, reacting to all the phenothiazines and reference antagonists.

The multiplicity of actions of phenothiazine derivatives suggests that they act on a number of receptor sites. Chlorpromazine, the most extensively investigated member of the series, has been shown to possess antihistaminic, anti-cholinergic, α-adrenergic blocking and anti-serotonergic activity (1-4). Reports concerning the type of antagonism exerted by the phenothiazines are, however, few. The present study aims at a qualitative and quantitative evaluation of the blocking effect of eighteen phenothiazines on receptors activated by histamine, acetylcholine (ACh), noradrenaline (NA), and 5-hydroxytryptamine (5-HT).

The competitive and non-competitive antagonistic activities of the phenothiazines against the foregoing agonists were evaluated in terms of pA₂ and pD₂ values and compared with those of an accepted specific antagonist (reference antagonist) namely, diphenhydramine, atropine, d-tubocurarine, phenoxybenzamine and lysergic acid diethylamide (LSD-25), for the respective receptor sites.

MATERIALS AND METHODS

The technique described by Van Rossum (5) for obtaining the cumulative dose-response curves in isolated organs and evaluation of drug parameters from these curves,
was generally followed. The following isolated tissues were employed: guinea pig intestine for histamine, rabbit intestine and frog rectus abdominis muscle for muscarinic and nicotinic actions, respectively, of ACh, longitudinal half of uterus of oestrogenized adult female rat (0.5 mg stilboestrol injected i.m. daily on two preceding days) for 5-HT and vas deferens of adult male rat for NA. Guinea pigs, rabbits and rats were stunned by a blow on the head and frogs were pithed before dissection.

A 50 ml bath was used for suspending a 2 cm long piece of intestine and a 20 ml bath for suspending other tissues. Tyrode solution was used for suspending the intestine, Ringer's solution for frog rectus, Tyrode solution as modified by Patil et al. (6) for Vas deferens and de Jalon's solution for rat uterus. The bath fluid was maintained at 37 ± 0.5 °C for intestine and vas deferens and 25 ± 0.5 °C for uterus. The bath was maintained at room temp. (22 ± 25 °C) for the rectus muscle. Air was bubbled through the fluid from an opening in the tissue holder. The contractile response of the intestine was recorded with a frontal writing lever and those of the other tissues with a straw lever, on a slow moving sooted drum.

Drugs were prepared in molar solutions. The total amount of drug added in sequence to the bath was kept below 1.2 ml for the 50 ml bath and below 0.6 ml for the 20 ml bath. Cumulative dose responses were obtained by adding the agonists in doses increasing in 1/2 log10 steps until the maximum contraction was obtained. A subsequent dose was added only when the response to the preceding dose was at its highest. The agonist was washed out by overflow with a volume of solution approx. equal to five bath values. A control cumulative dose-response curve of an agonist was obtained. A phenothiazine or reference antagonist was added to the bath and allowed to act for one min. Another cumulative dose-response curve was then obtained using the same agonist. Fig. 1 illustrates the typical cumulative dose-response curves of histamine (a) and ACh (b), in the absence (control)

![Typical records of cumulative dose-response curves for the agonists, histamine on guinea pig intestine (a) and ACh on the rabbit intestine (b) in the presence of constant but log10 increasing concentrations of thioproperazine.](image)

A competitive inhibition as a progressive shift of the curves to a higher concentration of the agonists with increasing doses of thioproperazine is better defined in (a) than in (b).

In (b) the shift of the curves to higher concentrations of the agonist is soon followed by depression of the maximum response as the thioproperazine dose is gradually increased, showing the onset of non-competitive antagonism.
and presence of increasing concentrations of thioproperazine. Phenoxybenzamine, fluphenazine and perphenazine were allowed to act in the bath for 5 min as their effect was inconsistent if applied for a shorter period.

The effect obtained for every cumulative dose was measured in millimeters and calculated in percentage of the maximum height of contraction obtained with the agonist. The percentages of the response, thus obtained, were plotted on the ordinate against the drug concentrations in the bath on a logarithmic scale on the abscissa on a millimeter graph paper to obtain the control log concentration—percent response curve of the agonist. Similar concentration—response curve of the agonist was drawn for the cumulative dose-response curve obtained after the tissue had been treated with a dose of the antagonist, the effect of every cumulative dose being expressed in percentage of the maximum height obtained in the control curve. Fig. 2 shows such curves for histamine (a) and ACh (b), plotted from the respective cumulative dose-response curves of Fig. 1.

The extent of shifting of the dose-response curve of the agonist at 50% response level in the presence of a constant concentration of the antagonist was used for calculating the pA₂ value of the antagonist. The extent of the depression of the maximum height of the dose-response curve of the agonist in the presence of a constant concentration of the antagonist was used for calculating the pD₂' value of the antagonist. Values were calculated from the cumulative dose-response curves obtained from at least two preparations of a tissue and two concentrations of the antagonist. Tables IV and VI from the paper of Van Rossum (5) were freely used for calculating these values.

**RESULTS**

Addition of an antagonist to the bath resulted in a shifting of the dose-response curve, or depression of the maximum response of the agonist, or shifting at low, and depression
at high doses of the antagonist. In a few experiments such treatment had either no effect on the dose-response curve or increased the response of the tissue to the agonist. Results are summarized in Tables 1 and 2.

Table 1 shows that phenothiazines exerted a strong competitive antagonistic effect against histamine (column 2) and a moderate effect against muscarinic action of ACh (column 4). Acetophenazine, fluphenazine, methoxypromazine, perphenazine, pipamazine, promazine, promethazine, triflupromazine and trimeprazine were over 10 times more potent in their activity against histamine than against ACh. Increase in concentration of the phenothiazine resulted in non-competitive antagonism (columns 3 and 5). This increase was of a higher magnitude for histamine (columns 2 and 3) than for ACh (columns 4 and 5) showing a greater specificity of the phenothiazines for histamine receptors.

A similar change from competitive to a non-competitive antagonism was exhibited by diphenhydramine and atropine against histamine and by diphenhydramine and tubocurarine against muscarinic action of ACh. The pA₂ values of diphenhydramine, atropine, tubocurarine, phenoxybenzamine and LSD-25 indicated a high degree of competitive an-

### Table 1. pA₂ and pD₂' values of the antagonists against histamine and ACh.

<table>
<thead>
<tr>
<th>Antagonists</th>
<th>Histamine</th>
<th>ACh (Muscarinic action)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pA₂</td>
<td>pD₂'</td>
</tr>
<tr>
<td>Phenothiazines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetophenazine</td>
<td>7.43±0.17</td>
<td>5.90</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>6.22±0.15</td>
<td>5.32</td>
</tr>
<tr>
<td>Ethopropazine</td>
<td>6.78±0.20</td>
<td>6.14</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>7.06±0.15</td>
<td>6.24</td>
</tr>
<tr>
<td>Methodilazine</td>
<td>8.33±0.18</td>
<td>7.02</td>
</tr>
<tr>
<td>Methoxypromazine</td>
<td>7.45±0.16</td>
<td>6.06</td>
</tr>
<tr>
<td>Perphenazine</td>
<td>8.04±0.17</td>
<td>6.14</td>
</tr>
<tr>
<td>Pipamazine</td>
<td>7.04±0.20</td>
<td>5.68</td>
</tr>
<tr>
<td>Prochlorperazine</td>
<td>6.61±0.23</td>
<td>5.61</td>
</tr>
<tr>
<td>Promazine</td>
<td>7.29±0.19</td>
<td>6.01</td>
</tr>
<tr>
<td>Promethazine</td>
<td>7.89±0.18</td>
<td>6.13</td>
</tr>
<tr>
<td>Thiopropazate</td>
<td>6.00±0.21</td>
<td>5.37</td>
</tr>
<tr>
<td>Thioproperazine</td>
<td>6.79±0.22</td>
<td>5.00</td>
</tr>
<tr>
<td>Thioridazine</td>
<td>6.00±0.20</td>
<td>5.28</td>
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<tr>
<td>Trifluperazine</td>
<td>6.88±0.22</td>
<td>4.73</td>
</tr>
<tr>
<td>Triflupromazine</td>
<td>7.44±0.24</td>
<td>6.28</td>
</tr>
<tr>
<td>Trimeprazine</td>
<td>7.93±0.21</td>
<td>6.27</td>
</tr>
<tr>
<td>TT 418</td>
<td>6.81±0.10</td>
<td>5.21</td>
</tr>
</tbody>
</table>

Reference antagonists

| Diphenhydramine  | 8.15±0.22 | 5.80 | 6.66±0.18 | 5.42 |
| Atropine         | 5.88±0.21 | 5.09 | 8.52±0.20 | None |
| Tubocurarine     | Potentiation Potentiation | Potentiation Potentiation | 5.05±0.20 | 4.07 |
| Phenoxybenzamine | None      | 6.58 | None      | 5.06 |
| LSD-25           | None      | 4.66 | No effect | No effect |

Figures after ± are standard errors of the mean.
As is apparent from Table 2, the antagonistic activity of the phenothiazines against ACh (nicotinic action), NA and 5-HT could not be resolved into competitive and non-competitive components as the depression of the maximum response occurred almost simultaneously with the shift in the position of the curve. Dose intervals of phenothiazines less than \(1/2\log_{10}\) were not attempted in this study. A comparison of the pD\(_2^*\) values of the phenothiazines in Tables 1 and 2 shows that they blocked the effect of histamine, ACh (muscarinic action), 5-HT, NA and ACh (nicotinic action) in that order of decreasing potency. Thioproperazine did not modify while acetophenazine, perphenazine, prochlorperazine and promethazine enhanced NA response.

Non-competitive antagonism was exhibited by all the antagonists against 5-HT, that is, diphenhydramine, phenoxybenzamine and LSD-25 against nicotinic action of ACh and by tubocurarine and phenoxybenzamine against NA. Atropine had no effect while diphenhydramine and LSD-25 enhanced the NA effect. Tubocurarine enhanced the effect of histamine.
The present study shows that phenothiazines exert an antagonist effect against histamine, ACh, NA and 5-HT in vitro.

Antagonism against histamine

The phenothiazines show a dualism in their antagonistic action against histamine in the isolated guinea pig ileum. Thus they possess affinity towards both specific and non-specific histamine receptor systems. The finding is in agreement with that of Takayanagi (4) who has demonstrated a similar dualism in antihistaminic activity of these compounds. This dualism is exhibited by the reference antagonists, diphenhydramine and atropine as well.

A study of the differences between the pA₂ and pD₂' values (column 2 and 3 of Table 1) shows, however, that the specificity of diphenhydramine is considerable (pA₂−pD₂' = 2.23). Promethazine shows the highest specificity amongst the phenothiazines (pA₂−pD₂' = 1.76). Although methdilazine is as effective as diphenhydramine in its anti-histaminic activity it is not as specific (pA₂−pD₂' = 1.31). The specificity of promethazine, trimoprazine, TT418, acetophenazine, methoxypromazine, pipamazine, methdilazine, promazine, thioproperazine and prochlorperazine can be described as moderate while that of fluphenazine, chlorpromazine, ethopropazine, triflupromazine and thiopropazate, as low, in comparison with that of diphenhydramine.

The pA₂ values of atropine and diphenhydramine against histamine obtained in this study are in close agreement with those reported by Wilson and Schild (7). Potentiation of histamine response by tubocurarine may be due to release of histamine by this agent from the tissue. The antihistaminic activity of phenoxybenzamine has been reported by earlier workers (8). This study shows the activity to be of a non-competitive character. The antihistaminic activity of LSD-25 demonstrated in this study has not been previously reported.

Antagonism against ACh

Muscarinic action: The phenothiazines exhibit a dual antagonism on the muscarinic receptors (columns 4 and 5, Table 1) similar to that exhibited on the histamine receptors, an observation earlier reported by Takayanagi (4). In addition, the phenothiazines generally exhibit a greater difference between pA₂ and pD₂' values against histamine than between similar values against ACh, both in this study and in that of Takayanagi. This supports the existing concept that the phenothiazines are more specific against histamine than against ACh.

Dualism in antagonism is also exhibited by diphenhydramine and tubocurarine. Atropine shows a purely competitive antagonism of a high order at these receptors. The pA₂ values of atropine and diphenhydramine obtained in this study are in close agreement with those reported by Wilson and Schild (7) using guinea pig ileum. The non-competitive antagonism exhibited by phenoxybenzamine demonstrated in this study confirms the finding of Befney and Grillo (9) who have shown that phenoxybenzamine antagonized ACh in the guinea pig ileum and the antagonism was not overcome by increasing the ACh
concentration. ACh response on the rabbit ileum is not affected by LSD-25.

Ethopropazine shows the highest specificity (pA₂ – pD₂’ = 2.68). Prochlorperazine, pipamazine and methdilazine stand next in order of specificity and equal diphenhydramine in this respect. Phenothiazines, which require at least 10 times the concentration for giving pD₂’ values than for giving the pA₂ values, include promethazine, chlorpromazine, promazine, thiopropazate, trifluperazine and TT418. They equal tubocurarine in specificity. Acetophenazine, trimetropazine, perphenazine, triflupromazine, thiohperazine, methoxypromazine, fluphenazine and thioridazine, are even less specific.

It is observed from the pA₂ and pD₂’ values of the phenothiazines against muscarinic actions of ACh (as also against histamine) that a high specificity is not necessarily associated with a high potency. For example, perphenazine shows a high potency on the muscarinic receptors (pA₂ = 7.27, pD₂’ = 6.69), its specificity for these receptors is lower (0.58), than the less potent ethopropazine (2.68). Further, several of the phenothiazines—fluphenazine, triflupromazine, TT418, ethopropazine, chlorpromazine, prochlorperazine, thiohperazine, thioridazine and thiopropazate—do not appear to discriminate between histamine and ACh receptors as their pA₂ as values against both these agonists are almost equal.

Nicotinic action: The phenothiazines block the nicotinic effect of ACh on the frog rectus abdominis in relatively high concentrations (column 2, Table 2). The antagonism is of a non-competitive nature. This is in agreement with the observation of Ryall (10) who, using the phrenic nerve-diaphragm preparation of the rabbit in vitro, demonstrated an inhibition of neuromuscular transmission by chlorpromazine and considered the blockade to be non-competitive as it could not be reversed by neostigmine.

The potencies of the different phenothiazines vary in a narrow range. Trimeprazine, thiopropazate and acetophenazine are the most active while TT418, ethopropazine and chlorpromazine the least active members in the series. The lack of appreciable variation in potency among the phenothiazines as well as among the reference antagonists of widely different chemical structures and the high concentrations of the antagonists required to produce effect, suggest a rather direct effect of these compounds on the skeletal muscle. In this context, the observation of Kopera and Armitage (11) appears relevant. They reported that chlorpromazine intra-arterially produced a blockade of both direct and indirect stimulation of the cat sciatic-gastrocnemius neuromuscular preparation, thus suggesting a direct action of chlorpromazine on skeletal muscle.

Tubocurarine and atropine exhibit only competitive antagonism. The finding that atropine is more potent in antagonizing the ACh effect on the ileum (pA₂ = 8.52) than on the rectus abdominis (pA₂ = 5.95) is in agreement with that of Whittaker (12).

Antagonism against NA

The α-adrenergic blocking action of the phenothiazines is non-competitive and is not shared by all members (column 3, Table 2). The relatively small range of their pD₂’ values (4.50–5.56) indicates that even the active members do not differ appreciably in their antiadrenergic potency. This finding is in contrast to that of Takayanagi (4) who was
able to resolve the two components of the antiarterenergic activity of the phenothiazines. This may be due to difference in the experimental conditions.

Thioproperazine does not affect the NA dose-response curve in concentrations up to $10^{-3}$ M. The mechanism of potentiation of NA effect by acetophenazine, perphenazine, prochlorperazine and promethazine cannot be explained from this study. Chlorpromazine has been reported to prolong and enhance the pressor response to NA under appropriate conditions (13). Webster (2) has reported a low antiadrenergic activity (9-30% that of chlorpromazine) in the last three of the above compounds, using the pressor response to adrenaline in spinal rabbit. Triflupromazine, thiopropazate, chlorpromazine, methoxypromazine, pipamazine, TT418 and trimeprazine are the more potent among the members having pD$_2$' value of 5.00 or more.

Phenoxybenzamine demonstrates a characteristic antagonistic action of a dual nature, a competitive (pA$_2$ = 6.99) and a non-competitive (pD$_2$' = 4.40), manifesting a marked specificity for the $\alpha$-adrenergic receptors (pA$_2$ - pD$_2$' = -2.95). Tubocurarine blocks the NA effect non-competitively and diphenhydramine and LSD-25 potentiate NA effect. The absence of any effect of atropine on NA dose-response curve is in contrast to the finding of Burn and Dutta (14) that atropine blocks the excitatory effect of catecholamines. This may be due to different tissue, as the blood vessels of the rabbit ear were utilized by these investigators.

**Antagonism against 5-HT**

All the phenothiazines tested antagonize in vitro contractions of the rat uterus produced by 5-HT. The antagonism is non-competitive in nature. As non-competitive antagonists, their activity against 5-HT (column 4, Table 2) does not differ significantly in magnitude from that against histamine and muscarinic action of ACh (columns 3 and 5, Table 1). This activity is, however, much less compared to the overall antagonistic action against histamine and ACh (pA$_2$ values of columns 2 and 4, Table 1). Methdilazine, acetophenazine, thioproperazine and trifluperazine appear to be the least potent members in this respect.

LSD-25 exhibits a dual antagonistic action against 5-HT. It possesses a high specificity as is shown by the great difference in the pA$_2$ and pD$_2$' values (2.29). The phenothiazines are much less active than LSD-25 this finding being in agreement with the reported potency (15).

The other reference antagonists show a non-competitive blocking action of a moderate potency. The findings of Hawker et al. (16) that atropine diminishes and phenoxybenzamine abolishes the in vitro 5-HT induced contractions of the rat uterus and of Doepfner and Cerletti (17) that diphenhydramine has a similar effect, support the present findings on these agents.

The present study demonstrates that phenothiazine derivatives are capable of reacting with a wide variety of receptors. Boyd et al. (18) have suggested the existence of a considerable chemical and positional overlap between adrenaline and acetylcholine receptor sites which is sufficient to make pharmacological differentiation difficult. A similar
overlap can be conceived to exist between adrenaline and 5-HT receptor sites (15) and between histamine and acetylcholine receptor sites (7). It is, therefore, not surprising that phenothiazines exhibit a multiple affinity to varied receptor sites and any attempt to look at one receptor site for their action will be an over-simplification. In this study, such lack of selectivity is also shown by phenoxybenzamine in its antagonistic action involving all the neurohumors.

The variations in potency amongst the phenothiazines against an agonist may partly result from differential penetration barriers. The longer contact (5 min) with the tissues required by phenoxybenzamine, fluphenazine and perphenazine to produce consistent effect, adds support to this view.

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