THE INFLUENCE OF MONOAMINE OXIDASE INHIBITORS AND SOME OTHER ANTIDEPRESSANTS ON THE ANTI-PARKINSONIAN ACTIVITY OF SUB-EFFECTIVE DOSES OF DIPHENYLHYDRAMINE IN RATS AND MICE

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Our earlier observations (1) with antidepressants (MAOIs and tricyclic) in protecting drug-induced Parkinson-like signs in animals showed that these agents were not so effective as the commonly employed antiparkinsonian drugs. It was, therefore, decided to combine these antidepressants i.e. sub-effective doses of these together with sub-effective doses of the commonly used antiparkinsonian drugs, in order to assess the therapeutic efficacy of such combinations, as undesirable sedative effects of antiparkinsonian drugs may be counteracted by antidepressants. In the present study such combinations with the well established antiparkinsonian drug, diphenylhydramine, were used and the degree of protection from parkinson-like signs induced by tremorine in mice and perphenazine in rats, were observed.

MATERIALS AND METHODS

Animal and drugs: Albino rats (125 to 175 g) and mice (25 to 40 g) of Haffkine strain were used. The drugs employed were diphenylhydramine hydrochloride (5 mg/kg); MAOIs such as, \( \alpha \)-methyltryptamine acetate (10 mg/kg), \( \alpha \)-ethyltryptamine acetate (10 mg/kg), phenelzine sulphate (5 mg/kg); tricyclic antidepressants (20 mg/kg) like, protriptyline hydrochloride, nortriptyline hydrochloride, proheptatriene hydrochloride, prothiadene hydrochloride, and hydrothiadene maleate. All drugs were administered intraperitoneally. A group of 10 rats or 10 mice was employed for each drug or drug combination.

Solutions: Perphenazine was dissolved in to a drop of N/10 HCl, diluted with distilled water and the pH adjusted to 6 with dilute N/10 NaOH before the required volume was made. All other drugs were dissolved in to distilled water. The volume administered was kept constant i.e. 4 ml/kg for rats and 10 ml/kg for mice.

Pretreatment: The animals were pretreated for 15 min with diphenylhydramine; \( \alpha \)-methyltryptamine and \( \alpha \)-ethyltryptamine 30 min; phenelzine 18 hr and all tricyclic antidepressants 30 min before administration of perphenazine or tremorine.

Perphenazine technique: The technique employed was essentially the same as described by Morpurgo (2). Effects were evaluated at various times by the following procedure a modification of that described by Dandiya and Bhargava (1).
The drug was considered to be fully protective (f), if more than 75% of animals failed to show perphenazine induced signs beyond stage I, partially protective (p), if 50 to 74% of the animals failed to show signs beyond stage I or 75% beyond stage II. It was considered ineffective (n), if it protected less than 50%, of the animals from stage III. The effect of drugs was recorded at various time intervals after administration of perphenazine (Table 1).

In order to evaluate the effect of drugs, the symbols for full protection (f) partial protection (p) and no protection (n) were given scores (f: 20, p: 10, n: 0) and the “Overall Protection” was calculated by the addition of the scores obtained at various time intervals. The values so obtained were then converted into a percentage score. A percentage score of 75% or more was considered as Overall Full Protection (F); score between 50 to 74% as Overall Partial Protection (P) and less than 50% as Overall No Protection (N).

**Tremorine technique:** The technique employed was essentially the same as described by Everett et al. (3). Tremorine was administered in a dose of 20 mg/kg in mice pretreated with test drug or solvent (control) or a combination. The degree of severity of the signs was

| Drugs | Time in min | “Overall protection” |
|-------|-------------|----------------------|
|       | 15 | 30 | 60 | 90 | 150 | 210 |
| Diphenhydramine (DPH) | p | p | n | n | n | n | 17% (N) |
| α-Methylytryptamine | p | n | n | n | n | n | 8% (N) |
| α-Methylytryptamine – DPH | f | f | p | p | n | n | 50% (P) |
| α-Ethyltryptamine | p | n | n | n | n | n | 8% (N) |
| α-Ethyltryptamine – DPH | p | p | n | n | n | n | 17% (N) |
| Phenergan | p | n | n | n | n | n | 8% (N) |
| Phenergan – DPH | f | p | p | p | n | n | 33% (N) |
| Protriptyline | f | p | n | n | n | n | 25% (N) |
| Protriptyline – DPH | f | f | p | p | p | p | 67% (P) |
| Nortriptyline | f | p | n | n | n | n | 25% (N) |
| Nortriptyline – DPH | f | f | p | p | p | p | 50% (P) |
| Proheptatriene | f | p | n | n | n | n | 25% (N) |
| Proheptatriene – DPH | f | p | p | n | n | n | 33% (N) |
| Prothiadeine | f | p | p | n | n | n | 33% (N) |
| Prothiadeine – DPH | f | f | p | p | p | p | 50% (P) |
| Hydrothiadeine | f | p | n | n | n | n | 25% (N) |
| Hydrothiadeine – DPH | f | p | p | p | p | n | 42% (N) |

DPH = Diphenhydramine hydrochloride
f = Full protection
p = Partial protection
n = No protection
F = Overall full protection
P = Overall partial protection
N = Overall no protection
A group of 10 rats was employed for each treatment or combination.
observed at various times.

Intensity of signs in every animal was noted at each time interval and a definite score i.e. absence of signs = 100, mild effect = 75, moderate = 50, marked effect = 25 and severe effect = 0, was given. The average score for a test drug was thus obtained at different times. A score of 75 or more, between 50 to 74 and less than 50 were assigned f, p and n symbols respectively. The scores of different times were pooled and the percentage protection was calculated. A percentage score of 75 or more was designated by f; of 50 to 74 by P, and of less than 50 by n.

The ‘Overall Protection’ offered by a drug or a combination was than calculated by giving a score of 20, 10 and 0 to symbol ‘f’, ‘p’ and ‘n’, respectively. A symbol ‘F’ (Overall Full Protection) was assigned to a drug when it scored 75% or more, a symbol ‘P’ (Overall Partial Protection) when it obtained 50 to 74% and ‘N’ (Overall No Protection) when it secured less than 50%.

RESULTS

Perphenazine induced catatonia (Table 1)

The antihistaminic drug diphenhydramine partially protected the animals for only the first 30 min.

All MAOIs partially protected the animals for the first 15 min. In combination with diphenhydramine, α-methyltryptamine gave full protection for the first 30 min and partial protection for the next 60 min while ε-ethyltryptamine failed to modify the response to diphenhydramine. Full protection for the first 15 min and partial protection for the next 54 min was observed with phenelzine when combined with diphenhydramine.

Analysis of observations led to α-methyltryptamine and diphenhydramine combination obtaining a final rating of “Overall Partial Protection” (P) while the other two MAOIs combined with diphenhydramine received the final rating of “Overall No Protection” (N).

The tricyclic antidepressants failed to protect the animals, while protriptyline, nortriptyline and prothiadene caused “Overall Partial Protection” (P) when given in combination with diphenhydramine.

Tremorine induced parkinsonian syndrome (Table 2)

Diphenhydramine fully prevented diarrhoea and partially lachrymation while it failed to antagonize other signs. Its overall activity was rated as ‘N’ (Overall No Protection).

ε-Methyltryptamine fully prevented tremor and diarrhoea but failed to protect against other signs. Overall activity was rated as ‘N’. This drug in combination with diphenhydramine fully prevented lachrymation and diarrhoea, partially tremor, while it failed to protect against rigidity and salivation. This combination received a rating of ‘P’. ε-Ethyltryptamine fully prevented lachrymation and diarrhoea, partially tremor and salivation but did not prevent rigidity. This drug alone was rated as ‘P’ and in combination with diphenhydramine, it fully prevented all signs except tremor which was partially antagonized and received a rating of ‘F’. Phenelzine fully prevented diarrhoea and partially prevented salivation and lachrymation but failed to offer any protection against tremor and rigidity.
**Table 2. The influence of antidepressants or DPH or their combination in protecting mice from tremorine induced Parkinson-like syndrome.**

| Drugs                      | Signs | "Overall protection" |
|----------------------------|-------|----------------------|
|                            | Tremor | Rigidity | Salivation | Lachrymation | Diarrhea |               |
| Diphenhydramine (DPH)      | n<sub>1</sub> | n<sub>1</sub> | n<sub>1</sub> | p<sub>1</sub> | f<sub>1</sub> | 30% (N)       |
| α-Methyltryptamine         | f<sub>1</sub> | n<sub>1</sub> | n<sub>1</sub> | n<sub>1</sub> | f<sub>1</sub> | 40% (N)       |
| α-Methyltryptamine + DPH   | p<sub>1</sub> | n<sub>1</sub> | n<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | 50% (P)       |
| α-Ethyltryptamine          | p<sub>1</sub> | n<sub>1</sub> | p<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | 60% (P)       |
| α-Ethyltryptamine + DPH    | p<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | 90% (F)       |
| Phenelzine                 | n<sub>1</sub> | n<sub>1</sub> | p<sub>1</sub> | p<sub>1</sub> | f<sub>1</sub> | 40% (N)       |
| Phenelzine + DPH           | p<sub>1</sub> | p<sub>1</sub> | p<sub>1</sub> | p<sub>1</sub> | f<sub>1</sub> | 60% (P)       |
| Protriptyline              | f<sub>1</sub> | p<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | 90% (F)       |
| Protriptyline + DPH        | f<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | 100% (F)      |
| Nortriptyline              | f<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | 100% (F)      |
| Nortriptyline + DPH        | f<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | 100% (F)      |
| Proheptatriene             | f<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | 100% (F)      |
| Proheptatriene + DPH       | f<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | 100% (F)      |
| Prothiadeine               | f<sub>1</sub> | f<sub>1</sub> | p<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | 90% (F)       |
| Prothiadiene + DPH         | f<sub>1</sub> | f<sub>1</sub> | p<sub>1</sub> | p<sub>1</sub> | f<sub>1</sub> | 90% (F)       |
| Hydrothiadiene             | f<sub>1</sub> | f<sub>1</sub> | n<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | 80% (F)       |
| Hydrothiadiene + DPH       | p<sub>1</sub> | p<sub>1</sub> | n<sub>1</sub> | f<sub>1</sub> | f<sub>1</sub> | 60% (P)       |

DPH = Diphenhydramine  

- f<sub>1</sub> = Full protection = % score 75 or >75  
- p<sub>1</sub> = Partial protection = % score between 50 to 74  
- n<sub>1</sub> = No protection = % score <50  
- F = Overall full protection 75 or >75%  
- P = Overall partial protection between 50 to 74%  
- N = Overall no protection <50%

A group of 10 mice was employed for each treatment or combination.

This drug alone was rated ‘N’ and in combination with diphenhydramine although it gave full protection against diarrhea it brought about only partial protection against all other signs. This combination received a rating of ‘P’.

Nortriptyline and proheptatriene caused full protection against all the signs induced by tremorine. Protriptyline fully prevented all signs except rigidity which was partially antagonized. Prothiadeine and hydrothiadiene fully prevented tremor, rigidity, lachrymation and diarrhea whereas the former drug gave partial protection, the latter failing to protect against salivation. The overall activity of all these tricyclic antidepressants was given a rating of ‘F’.

In combination with diphenhydramine, protriptyline, nortriptyline and proheptatriene gave full protection against all signs. Prothiadeine combination fully prevented tremor, rigidity, lachrymation and diarrhea and partially protected against salivation while hydrothiadiene combination fully prevented lachrymation and diarrhea, partially protected against tremor and rigidity but failed to prevent salivation. All these combinations received the rating of ‘F’ except hydrothiadiene combination which received a rating of ‘P’.
ANTIDEPRESSANTS AS ANTIPARKINSONIAN AGENTS

DISCUSSION

Dandiya and Bhargava (1) reported earlier that \( \alpha \)-methyltryptamine and \( \alpha \)-ethyltryptamine exhibited antiparkinsonian activity in doses of 20 mg/kg, but potency was less than that shown by well established, currently employed antiparkinsonian agents such as scopolamine (10 mg/kg in rats, 5 mg/kg in mice), diphenylhydramine (20 mg/kg), benztropine (10 mg/kg) and trihexyphenidyl (5 mg/kg).

In the present study various MAOIs, in doses lower than those employed by Dandiya and Bhargava (1) and some tricyclic antidepressants have been employed singly or in combination with one of the most widely used antiparkinsonian agents (diphenylhydramine, 5 mg/kg), in order to compare the combined effect of these drugs with individual effects. Diphenylhydramine alone failed to offer any protection against the signs induced by both techniques, since this drug was employed in a sub-effective dose.

The present work has indicated (Tables 1 and 2) that out of the three MAOIs, \( \alpha \)-methyltryptamine and phenelzine failed to prevent the parkinson-like signs induced by perphenazine or tremorine, although \( \alpha \)-ethyltryptamine brought about at least a partial protection against tremorine induced signs. It is also well established that MAOIs increase the level of norepinephrine, dopamine and 5-HT and if the hypothesis of McGeer et al. (4), is accepted, it could be predicted that all drugs belonging to this group will be useful in preventing parkinson-like signs. This was partially confirmed by Dandiya and Bhargava (1). Failure of these agents when given singly, is therefore, perhaps due to the sub-effective does used. There are also individual variations in response to chemically different MAOIs. \( \alpha \)-Methyltryptamine takes a much longer time to produce effects while, with \( \alpha \)-ethyltryptamine the onset of action is quicker with only negligible changes observed in blood pressure and heart rate (5). This is in agreement with our observations that \( \alpha \)-methyltryptamine offered no protection when tested using both techniques, as effects are produced rather slowly, whereas \( \alpha \)-ethyltryptamine partially prevented tremorine induced parkinson-like signs. Phenelzine, in a sub-effective i.p. or s.c. dose of 5 and 10 mg/kg respectively, was not expected to produce any marked inhibition of brain MAO, since the drug is reported to have equal effects on brain, liver and heart when given i.p. in contrast to a greater affinity when given by other routes (5). The partial protection offered by the combination of \( \alpha \)-methyltryptamine and diphenylhydramine in both techniques could possibly be due to the well established observation that \( \alpha \)-methyltryptamine increases catecholamines and 5-HT content and at the same time diphenylhydramine counteracts the histamine response. The combination thus makes a two point attack as against that of a single attack when the drugs are individually administered. The full protection given by \( \alpha \)-ethyltryptamine and partial protection given by phenelzine in combination with the antihistamine in tremorine technique could also be explained on the same basis.

The fact that the majority of combinations of the MAOIs with diphenylhydramine have shown a better protective effect in tremorine technique as against perphenazine technique, is indicative of the absence of parallelism in the mechanism by which these two agents i.e. perphenazine and tremorine, induced the parkinson-like signs.
All tricyclic antidepressants employed in this study, are dimethylamino derivatives except for nortriptyline which is a desmethyl derivative. Metys and Metysova (6) have demonstrated on oxotremorine antagonistic property in dimethylamino derivatives. The results obtained in this present study employing tremorine are consistent with those obtained with oxotremorine. It can be presumed that the full protection brought about by these antidepressants alone or in combination with diphenylhydramine is due to their central anticholinergic property (7).

The exact mechanism by which perphenazine induces rigidity, akinesia and depression is not fully understood at present but because of antiadrenergic properties, it can be expected that catecholamines play a role (8). The failure of tricyclic antidepressants in preventing perphenazine induced parkinson-like signs could possibly be due to inability to change the monoamine concentration in the body (9). In this connection one may also quote Meyers et al. (10) who have reported that nortriptyline and amitriptyline pretreatment did not raise the lethal dose of chlorpromazine. In our findings the tricyclic antidepressants did not block the activity of perphenazine which makes the findings consistent (10).

At least three tricyclic antidepressants i.e. protriptyline, nortriptyline and prothiadene showed a partial protection when combined with diphenylhydramine in perphenazine induced parkinsonian syndrome and this could be due to combination of the anticholinergic effect of these drugs and the antihistaminic and anticholinergic effects of diphenylhydramine. The failure of hydrothiadene in protecting rats from perphenazine induced signs could be due to the paralysis of extremities and mild central depression caused by this drug (11), the latter action being responsible for the partial antagonism shown by a combination with diphenylhydramine, which produced in additive effect.

This study has clearly demonstrated that MAOIs and to some extent a number of tricyclic antidepressants when employed in combination with diphenylhydramine increase the ability of this latter drug in protecting drug induced parkinson-like signs in animals while at the same time counteracting its sedative effects and has thus warranted a clinical study on humans in order to exploit the use of similar combinations in preference to the use of the antihistaminic drug alone in the treatment of Parkinsonism. This could enable lower doses of the latter drug to be used. Such a combination would prevent hazards of excessive sedation produced by diphenylhydramine.

SUMMARY

An estimation of protective ability of a number of antidepressants, belonging to the monoamine oxidase inhibitors and the tricyclic structure, was made singly and in combination with diphenylhydramine in preventing drug induced parkinson-like signs in rats and mice. When some of these drugs were given in combination with sub-effective doses of diphenylhydramine, the protective effect of the combination was much more superior than the effect of the individual drug. This potentiating ability was particularly prominent in α-methyltryptamine, α-ethyltryptamine and phenelzine against tremorine induced signs. All tricyclic antidepressants, except hydrothiadene, gave full protection singly and in combination with
diphenylhydramine in tremorine induced parkinson-like signs. Among these drugs only protriptyline, nortriptyline and prothiadene offered partial protection against perphenazine induced catatonia when combined with subeffective doses of diphenylhydramine. The possibility of a clinical evaluation of combinations of diphenylhydramine and antidepressants in the treatment of parkinsonism is discussed.

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