MODIFICATION OF DRUG-INDUCED CATATONIA AND TREMORS BY QUIPAZINE IN RATS AND MICE

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Abstract—Administration of perphenazine, tremorine, nicotine and harmine induced Parkinson-like symptoms in rats and mice. The efficacy of quipazine, a serotonin agonist, in antagonizing these drug-induced Parkinsonian symptoms was assessed. Combinations of this drug with other antiparkinsonian agents such as scopolamine, diphenhydramine and amantadine were also studied in the manifestation of Parkinson-like symptoms in the animal models. The results indicate that quipazine, a central serotonergic agent, counteracted some of the drug-induced symptoms of pseudo-parkinsonism in laboratory animals. Cholinergic, dopaminergic and histaminergic receptors play an important role in the manifestations of these symptoms.

Quipazine, 2-1 (1-piperazinyl)-quinoline, stimulates both peripheral and central serotonergic receptors and closely simulates serotonin induced responses in isolated tissue preparations as well as in the intact organisms (1-3). These effects are antagonized by serotonin blockers such as methysergide, morphine and BOL 148 (1). Likewise, the behavioural excitation induced by quipazine in cats is blocked by prior administration of cyproheptadine, a relatively more specific serotonin antagonist (1). Quipazine has been reported to possess a weak and reversible monoamine oxidase inhibitory property (4), but its ability to reduce turnover rate of brain serotonin has been attributed primarily to its stimulatory effect on the central serotonergic receptors (5).

Although the involvement of dopaminergic and cholinergic systems in the manifestations of Parkinson’s disease has been fairly well established (6-8), the role played by serotonin in this disease has not been clearly defined, perhaps partially because a specific serotonin agonist was not available until recently. Since the introduction of quipazine as a prototype of serotoninergic agent (1), it has been possible to examine the role of central serotonergic receptors. In the present work, therefore, an attempt has been made to study the ability of quipazine to counteract the drug induced pseudo-parkinson-like symptoms in rats and mice. Its interaction with anticholinergic (scopolamine), antihistaminergic (diphenhydramine) and dopaminergic (amantadine) agents was also studied.

MATERIALS AND METHODS
Male albino Sprague-Dawley rats (150-200 g) and Swiss-Webster mice (25-30 g), main-
tained on a 12 hr light and dark cycle and *ad libitum* food and water, were used throughout. All drugs were dissolved in distilled water or saline in concentrations with which the i.p. administration of 1 ml/100 g of mice and 0.5 ml/100 g of rats could be kept constant. Each treated group included 8–30 animals. The following drugs and experimental models were used.

**Drugs used:** Quipazine maleate (Miles Laboratories) was tested at 5, 10, and 20 mg/kg dose levels. Scopolamine HBr (1 mg/kg), diphenhydramine HCl (20 mg/kg) and amantadine HCl (20 mg/kg) were used for drug combination studies with quipazine.

Serotonin creatinine-sulphate and quipazine were also administered intracerebroventricularly up to 300 μg dissolved in 10 μl of saline. The technique of Noble *et al.* (9) was used to cannulate the right lateral ventricle of rat for intraventricular drug administration. Briefly, rats were anesthetized with pentobarbital (35 mg/kg, i.p.) and a mid-sagittal incision was made and the bregma exposed. A small 4 mm optical metal screw was placed 2 mm deep at a site 1.5 to 2 mm medial to the crossing of sagittal and coronal sutures and a polyethylene cannula (PE 10) closed at one end was inserted 4 mm deep at a site 1.5 to 2 mm lateral to the crossing. The cannula was secured in position by applying dental acrylic. Four to six days after cannulation the animals were used for testing. Drugs were administered using a Hamilton microsyringe.

**Perphenazine catatonia:** Using the method of Morpurgo (10), various stages of catatonia were induced in rats with perphenazine (5 mg/kg). The drugs to be tested were administered 30 min prior to perphenazine injection and the scoring was made at 20, 40, 60, 90, 180 and 240 min after the injection. The development and severity of the four stages of induced catatonia were observed and scored as follows: Stage 1, rat moves when placed on the table, score=0; Stage 2, rat moves only when touched or pushed, score=0.5; Stage 3, rat placed on the table with front paws set alternately on a 3 cm high block fails to correct the posture in 10 sec, score=0.5 for each paw with a total of 1 for this stage; State 4, rat fails to move when the front paws are placed alternately on a 9 cm high block, score=1 for each paw with a total score 2 for this stage. Thus, for a single rat, the maximum possible score would be 3.5 reflecting total catatonia. Less scores would mean an apparently lesser degree of catatonia.

**Tremorine-induced tremors:** Various doses of quipazine were administered 30 min prior to tremorine and the scoring was made at 5, 15, 30, 45, 60, 90 and 120 min after tremorine administration. Various parasympathetic activities such as tremors, salivation and lachrymation were induced in mice with 25 mg/kg of tremorine (11) and the severity of these symptoms was scored as intensive, severe, moderate or simple presence designated by 4, 3 2 and 1 pluses, respectively. The cumulative scores of all the activities for each treatment were plotted against time.

**Harmine and nicotine tremors:** Tremors were induced in mice with harmine (15 mg/kg) and nicotine (5 mg/kg) (12, 13). Various doses of quipazine were administered 30 min prior to harmine or nicotine. In some of the harmine experiments, cyproheptadine (0.5 mg/kg) was included to verify, as a control, that harmine does indeed act through serotonergic
receptors. The severity of response was measured essentially in the same way as described under tremorine section, except that the tremors were scored at 5, 7, 10, 15, 20, 30, 45 and 60 min as the only symptoms seen.

Statistical analysis: For comparison of various treatment groups in each set of experiments, a nonparametric rank sum approach of Kruskal-Wallis (14) was used, employing a 95% confidence level. Fisher's exact probability approach was used to determine if the combination of drugs exhibited a potentiating effect, i.e., if the effect was greater than the sum of the individual effects of the two drugs.

RESULTS

Effect of quipazine on perphenazine catatonia: Preadministration of quipazine showed a dose-dependent antagonism of perphenazine induced catatonia. With 20 mg/kg dose, the appearance of marked catatonia was blocked and the effect lasted up to 2 hr. Optimum effectiveness of quipazine occurred during the first 20 min of perphenazine administration (Fig. 1). Scopolamine, though in itself effective, in combination with quipazine showed a potentiation as evidenced by a near complete blockade of the perphenazine effect (Fig. 1; p<0.05). On the other hand, the combination of quipazine with diphenhydramine and amantadine, a known antiparkinsonian agent, showed a synergistic effect rather than a potentiation (Fig. 1). The effect of amantadine was comparable to that of diphenhydramine and disappeared gradually, delaying somewhat the appearance of full catatonia. Intracerebroventricular administrations of both quipazine and serotonin up to 300 μg did not modify the perphenazine-induced catatonia.

Fig. 1. Effect of various drugs on perphenazine (5 mg/kg)-induced catatonia in rats. A, in all groups represents animals given perphenazine only. Quipazine: Dose-response curve of quipazine; B, 20 mg/kg; C, 10 mg/kg; D, 5 mg/kg. Scopolamine B, quipazine (20 mg/kg); C, scopolamine (1 mg/kg); D, quipazine+scopolamine. Diphenhydramine: B, quipazine (20 mg/kg); C, diphenhydramine (20 mg/kg); D, quipazine+diphenhydramine. Amantadine: B, quipazine (20 mg/kg); C, amantadine (20 mg/kg); D, quipazine+amantadine. Each point represents the mean activity scores of 10–15 animals.
Effect of quipazine on tremors: Tremorine-induced parasympathetic hyperactivity in mice were only partially blocked by quipazine, at the dose levels investigated (Fig. 2). The onset and severity of nicotine-induced hyperkinesia were not modified by quipazine, but the onset of the tremors in the quipazine group was delayed significantly (p<0.05). Also quipazine partially protected mice against nicotine-induced mortality, but not against the severity of convulsions.

In contrast to the effect on tremorine- and nicotine-induced tremors, the harmine-induced tremors were potentiated by quipazine (p<0.05) so far as the intensity and duration were concerned. The animals showed severe head and body movements, repetitive vocalisation, and stereotypic biting of the cages. Unlike the mice treated with harmine alone, the maximum intensity of tremors occurred within a few minutes of harmine administration in quipazine pretreated mice while this intensity was 3 to 4 times longer in the mice given harmine alone. Further, quipazine in higher doses prolonged the duration of action of harmine (Fig. 3).

DISCUSSION

There is no single laboratory model which adequately simulates Parkinsonism and in which a proper evaluation of antiparkinsonian activity can be carried out. However, there is a positive correlation between catatonia in the laboratory animals and the extrapyramidal symptoms produced by neuroleptics in humans (10). Although phenothiazines do not produce all of the pseudo-parkinsonian symptoms in the rat, they do nonetheless affect the
extrapyramidal system, the dysfunction of which is considered to be a causative factor in Parkinson's disease. Therefore, the perphenazine-induced catatonia, along with the other tests adopted in the present studies may be considered a reasonable, though not total, approximation of the disease symptoms, providing at least one approach to evaluating the antiparkinsonian activity of quipazine.

Dopamine-acetylcholine imbalance hypothesis in Parkinson's disease has been substantiated by experimental evidence (8, 15, 16), including the recent report of Spehlmann and Stahl (17) who speculated that the degeneration of dopaminergic nigrostriatal fibres might induce sprouting of cholinergic terminals. However, little is known about the participation of serotonin and histamine in this balance or imbalance leading to the clinical picture of Parkinson's disease.

In the present study, quipazine, a serotonin agonist, blocked the effect of perphenazine-induced catatonia in a dose-dependent manner. A nonparametric multiple comparison of sets of data by Kruskal-Wallis one-way analysis of variance indicated that the 20 mg/kg dose of quipazine exhibited effects which were significantly (p<0.05) different from those seen with lower doses, as observed over a 90 min period. This anticatatonic action of quipazine may be due to the serotonergic property of this drug (1, 4, 5, 18).

The combination of quipazine with scopolamine exhibited a potentiation, as compared to the synergistic effect observed with the combinations of quipazine with amantadine or diphenhydramine. The potentiation in the combination treated animals was distinctly obvious at the longer observation period, i.e., at 180–240 min when the effects had all disappeared in the quipazine treated group and were gradually disappearing in the scopolamine treated group, while a potent blockade of the perphenazine-induced catatonia remained in the combination group. This potentiation suggests that the serotonergic out-flow affects the cholinergic regulatory mechanism in the nigrostriatal region and *vice versa*. Supporting this is a recent paper by Euvrard et al. (19) that striatal serotonergic neurones exhibit an inhibitory control over cholinergic interneurones in the region. On the contrary, the different mechanisms of pharmacologic action of amantadine, diphenhydramine and quipazine may be accounted for by their synergistic effect.

The intracerebroventricular administration of quipazine or serotonin had no significant effect on perphenazine-induced catatonia. The failure of these agents to diffuse from the cerebrospinal fluid (20) or the fact that quipazine may be acting through one of its metabolites generated in the systemic circulation, most likely by the liver, may serve as an explanation for these effects.

The effects of tremorine are considered to be due to its muscarinic activity and can be blocked by atropine and related agents (21). Quipazine is only one-tenth thousandth as active as atropine on the isolated guinea pig ileum (1). Whether the antitremorine activity of quipazine involves central serotonergic receptors (22) or is merely the result of a possible anticholinergic property of quipazine is debatable. Similarly, if nicotine produces convulsions and death as a result of stimulation of pre-synaptic cholinergic fibres, then the protection by quipazine against nicotine-induced tremors and mortality could also be
regarded as a result of stimulation of the serotonergic neurones which in turn exert an inhibitory control over the cholinergic interneurones (19).

Structural similarities between harmine and serotonin suggest that a modification of serotonin function in the central nervous system may be involved in eliciting the pharmacologic effects of harmine (23, 24). The exaggerated effects of harmine in quipazine-pretreated mice may well be explained on the basis of their common site of pharmacologic action, namely, the serotonergic receptors.

In conclusion, our data indicate that quipazine, presumably a 5-HT agonist, possesses an anticyatonic and anti-tremor activity in rats and mice. Furthermore, it appears that other receptors, especially those which are cholinergic and dopaminergic in nature, play an important role in the manifestation of the drug-induced pseudoparkinsonian symptoms.

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