

EXPERIMENTAL EVALUATION OF THE POSSIBLE NEUROLEPTIC ACTIVITY OF CLOMIPRAMINE*

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Summary: Clomipramine, a new antidepressant, differs from imipramine by having chlorine in position 3 of the aromatic ring and in this respect resembles chlorpromazine. Clomipramine was therefore tested for neuroleptic activity. Clomipramine and imipramine were ineffective in inhibiting the traction response and pinna reflex in mice and in inducing catalepsy in rat. Compared to chlorpromazine they were less potent in blocking conditioned avoidance response and in decreasing spontaneous motor activity and exploratory behaviour. In contrast to chlorpromazine, clomipramine like imipramine was found to enhance methamphetamine-induced stereotyped behaviour. Thus clomipramine like imipramine possesses negligible neuroleptic activity.

Key words: clomipramine imipramine chlorpromazine neuroleptic activity

INTRODUCTION

Clomipramine hydrochloride (ANAFRANIL) a newly introduced tricyclic antidepressant drug (2) differs from imipramine only by a chlorine substitution in position 3 of the aromatic ring (11). In the experimental tests commonly used to investigate antidepressant activity clomipramine was found to be less effective than imipramine in antagonising reserpine or tetrabenazine induced ptosis and catalepsy and in potentiating amphetamine induced hyperthermia (11).

In promazine molecule, substitution of a chlorine in position 2 increases its neuroleptic potency i. e. potency for depressing motor activity and conditioned avoidance responses in animals and for altering psychotic behaviour in man (1). As the chemical structure of imipramine bears some resemblance to that of promazine, a relevant question would be whether substitution of a chlorine in position 3 of the aromatic ring of imipramine imparts to it neuroleptic activity (Fig. 1). Hence on this basis the present investigation was undertaken to evaluate the neuroleptic activity of clomipramine and characterise it with reference to chlorpromazine a standard neuroleptic drug.

MATERIALS AND METHODS

Male albino mice and rats, weighing between 20 to 30 g and 120 to 180 g respectively, were used for the study.

Clomipramine hydrochloride (CIMI), imipramine hydrochloride (IMI), chlorpromazine hydrochloride (CPZ) and methamphetamine hydrochloride (MAMPH) were dissolved in distilled

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water. Doses quoted refer to the salts. The strength of the solutions was so adjusted that the requisite dose of a drug was injected intraperitoneally in a constant volume of 0.1 ml/10 kg body weight in mice and in a volume of 0.2 ml/100 g body weight in rats. Control animals received intraperitoneal injection of distilled water. Groups of 10 animals per dose level of the drug were used.

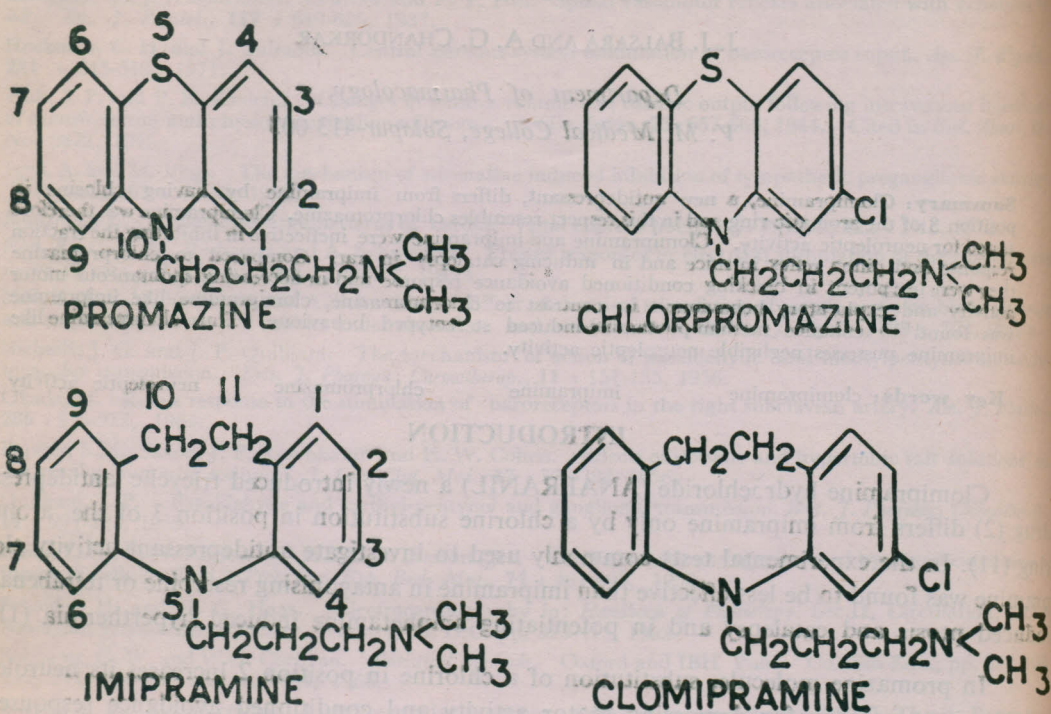


Fig. 1: Illustrates the structural resemblance of imipramine to promazine and of clomipramine to chlorpromazine.

Traction response in mice:

The method followed was that of Courvoisier *et al.* (5). Mice were suspended from a horizontal wire by their fore paws, and the animals which were able to draw themselves up to touch the wire with one hind paw within 5 sec of being placed on the wire were used for further study. Both control and drug treated groups were tested for the traction response at 30 and 60 min time interval after the injection. The response was said to be inhibited when the animal was unable to draw itself up to touch the wire within 5 sec of placement.

Pinna reflex in mice:

The pinna reflex was elicited by stimulating the external auditory meatus of each ear with a fine hair and involves the twitch tremor or laying back of the ear in response to stimulation of the external auditory meatus (13). Both control and drug-treated groups were tested for the pinna reflex at 30 and 60 min time interval after the injection. The reflex was said to be negative when no response could be elicited from either ear.

Conditioned avoidance response (CAR) in rats:

Effect on CAR was studied in trained rats by the technique of Cook and Weidley (3) using Cook's pole climbing response apparatus. On the day of the experiment, the animal had to make 3 consecutive correct avoidance responses prior to the intraperitoneal injection of distilled water (control group) or drug. The animals were tested again 30 min after the injection. The drug effect on CAR was expressed as the percentage of animals which failed to climb the pole on hearing the buzzer but did climb the pole in response to the electric shock.

The ED₅₀ of the drug for inhibiting the traction response, pinna reflex and the CAR was computed by the method of Miller and Tainter (8).

Methamphetamine-induced stereotyped behaviour (SB) in rats:

MAMPH was used in the dose of 4 mg/kg which in a preliminary trial had induced stereotyped behaviour, characterised by continuous sniffing and small head movements, in 100% of the animals. Control group received distilled water followed 30 min later by MAMPH injection. Drug-treated groups received CIMI, IMI or CPZ followed 30 min later by MAMPH.

The rats were kept in individual cages made of netting (floor area 21 cm x 27 cm, ht 20 cm) and were observed visually. The latency of onset and the duration of stereotypy were determined by the method of Lal and Sourkes (7). The onset of stereotypy was determined by the occurrence of continuous sniffing, small head movements with the rat sitting in a crouched posture.

The intensity of SB was assessed by observing the animals for 5 min at 30 min intervals. The SB was assessed according to the scoring system suggested by Costall *et al.* (4) and is as follows:

- Score:**
- 0 : The rats are hyperactive as seen by increase in exploratory activity, increase in forward locomotion and increase in rearing and responsiveness towards the experimenter.
 - 1 : Continuous sniffing and small head movements, periodic exploratory activity, and slight responsiveness towards the experimenter.
 - 2 : Continuous sniffing and small head movements, discontinuous gnawing, biting and licking, very brief periods of locomotor activity. No responsiveness towards the experimenter.
 - 3 : Continuous gnawing, biting and licking, no exploratory activity, occasional backward locomotion. No responsiveness towards the experimenter.

The termination of MAMPH induced SB was characterised by the animal entering a phase of hyperactivity and by the gradual emergence of normal activity like forward locomotion and grooming. The end point was evaluated by lifting the rat out of the cage and observing the failure of the rat to resume SB lasting 30 sec within 20 sec of replacement. The results were statistically analysed by the Student's unpaired t-test.

Spontaneous motor activity (SMA) in rats:

The technique described by Vad *et al.* (12) was employed for recording SMA. Only one animal was placed in the activity cage at a time. After waiting for 10 min to allow the initial excitement to pass away, the up and down i.e. vertical movements of the animal were recorded by the lever of the Marey's tambour on a moving kymograph for the next 30 min. The animal was then administered drug or distilled water and was kept aside for 20 min. It was then placed in the activity cage and after 10 min interval, the record was again taken for 30 min.

Exploratory behaviour of mice:

Effect on exploratory behaviour of mice was studied by the method of Shillite (10). One control group was always used simultaneously with groups to which various doses of drugs had been administered. Mice were placed one at a time on the left hand corner of a wooden board measuring 61 X 61 cm onto which 12 tunnels 7.5 cm long and 4 cm in diameter were fixed arranged in a symmetrical pattern. The tunnels were numbered. Drugs were given 30 min before the observations, while the control group received distilled water. Each mouse was watched for 5 min after it was placed on the board. The number of different tunnels entered in the first minute, the total number of tunnels entered as well as the total number of different tunnels entered during the 5 min observation period, by the control and drug treated groups was noted. The experiments were conducted at the same time each afternoon.

The significance of differences between means was assessed by the use of Student's unpaired t-test.

Induction of catalepsy in rats:

The animal was tested for catalepsy 30 min after the drug injection. The animals were positioned with their front paws resting on a wooden block 12 cm high and scored as cataleptic if they maintained this unnatural position for atleast one min (9).

RESULTS

1. Influence on traction response in mice:

CIMI and IMI, upto 60 mg/kg body weight, were inactive in inhibiting the traction response while the $ED_{50} \pm S.E.M.$ of CPZ was 5.62 mg \pm 0.31.

2. Influence on pinna reflex in mice:

CIMI and IMI, upto 60 mg/kg body weight, were inactive in inhibiting the pinna reflex while the $ED_{50} \pm S.E.M.$ of CPZ was 3.54 mg \pm 0.15.

3. Effect on conditioned avoidance response (CAR) in rats:

$ED_{50} \pm S.E.M.$ of CIMI and IMI for inhibiting the CAR were 25.12 mg \pm 1.15 and 28.18 mg \pm 1.40 respectively while that of CPZ was 4.07 mg \pm 0.14. CIMI and IMI though

equipotent ($P > 0.05$) in blocking the CAR were, on a weight basis, about six times less potent than CPZ in this respect.

4. Effect on methamphetamine-induced SB in rats:

Pretreatment with either CIMI or IMI not only significantly ($P < 0.001$) decreased the time required for the onset of MAMPH induced SB but also significantly ($P < 0.001$) prolonged the duration and the intensity of SB. Stereotyped behaviour did not occur in CPZ pretreated rats (Table I).

TABLE I: Effect of clomipramine, imipramine and chlorpromazine on methamphetamine induced stereotyped behavior in rats.

Treatment dose mg/kg	Onset in min Mean \pm S.E.M.	Duration in min Mean \pm S.E.M.	Intensity cumulative score Mean \pm S.E.M.
1. MAMPH 4	19.3 \pm 104	2.11 \pm 6.70	1.7 \pm 0.08
2. CIMI 10 + MAMPH 4	12.8 \pm 0.87*	260 \pm 4.71*	2.6 \pm 0.12*
3. IMI 10 + MAMPH 4	12.1 \pm 0.64*	266 \pm 4.89*	2.8 \pm 0.08*
4. CPZ 10 + MAMPH 4	—	0	0

*Drug effect for a particular parameter when compared with the control was statistically significant ($P < 0.001$). Numerals following the drugs indicate their doses (mg/kg).

5. Effect on spontaneous motor activity (SMA) in rats:

CIMI and IMI in a dose of 10 mg/kg did not affect the SMA. However, an increase in dose to 20 mg/kg of CIMI and IMI slightly reduced the SMA and a further increase in dose to 40 mg/kg reduced the SMA markedly. CPZ in a dose of 2 and 4 mg/kg reduced the SMA markedly.

6. Effect on exploratory behaviour of mice:

CIMI and IMI did not significantly affect the exploratory behaviour when used in the dose of 10 mg/kg body weight. However, CIMI (20 mg/kg), IMI (20 mg/kg) and CPZ (2 mg/kg) significantly decreased the exploratory behaviour of mice. The number of different tunnels entered during the first min during the 5 min of observation period and the total number of tunnels entered during the 5 min period was significantly lower than that of their respective control group (Table II).

7. Induction of catalepsy in rats:

CIMI and IMI when used in the dose range of 10 to 40 mg/kg, did not induce catalepsy

in rats. Doses beyond 40 mg/kg induced ataxia and motor incoordination and were therefore not tested. CPZ (10 mg/kg) induced catalepsy in 100% of the animals tested.

TABLE II: Effect of clomipramine, imipramine and chlorpromazine on the exploratory behaviour of mice.

Study	Treatment dose mg/kg	Total number of different tunnels entered during the first min (mean \pm S.E.M.)	Total number of different tunnels entered during the 5 min period (mean \pm S.E.M.)	Total number of tunnels entered during the 5 min period (mean \pm S.E.M.)
I	1. Control	2.0 \pm 0.33	7.0 \pm 0.39	15.6 \pm 1.07
	2. CIMI 10	1.9 \pm 0.31	7.4 \pm 0.47	16.1 \pm 0.86
	1. Control	2.1 \pm 0.44	6.7 \pm 0.49	15.1 \pm 0.87
	2. IMI 10	1.8 \pm 0.41	6.9 \pm 0.43	15.8 \pm 0.92
II	1. Control	2.1 \pm 0.23	8.0 \pm 0.39	18.0 \pm 0.83
	2. CIMI 20	1.1 \pm 0.17*	5.0 \pm 0.14*	14.2 \pm 0.75*
	1. Control	2.0 \pm 0.25	7.6 \pm 0.33	17.8 \pm 0.81
	2. IMI 20	1.2 \pm 0.19*	4.9 \pm 0.31*	14.7 \pm 0.81*
III	1. Control	2.0 \pm 0.21	7.4 \pm 0.47	17.6 \pm 0.77
	2. CPZ 2	0.7 \pm 0.15*	3.9 \pm 0.27*	12.4 \pm 0.73*

*Significant in relation to corresponding controls ($P < 0.05$ to 0.01).

Numerals following the drugs indicate their doses (mg/kg).

DISCUSSION

The neuroleptic drugs like chlorpromazine are comparatively more potent and selective in inhibiting the traction response and the pinna reflex, in blocking CAR, in antagonising amphetamine effects, in decreasing SMA and exploratory behaviour and in inducing catalepsy and hence these test procedures are commonly used to evaluate neuroleptic activity of a drug (6).

Clomipramine and imipramine (upto 60 mg/kg ip) were found to be ineffective in inhibiting the traction response and pinna reflex. Further upto 40 mg/kg dose they did not induce catalepsy in rats. These results are similar to those of Theobald *et al.* (11) who have reported that clomipramine (upto 100 mg/kg, sc) did not inhibit the traction response and (upto 50 mg/kg, sc) produced only slight cataleptic effect in mice.

Clomipramine and imipramine, though equi-effective in blocking CAR were, on weight basis, six times less potent than chlorpromazine. Even Theobald *et al.* (11) have reported that clomipramine (upto 20 mg/kg, ip) did not significantly alter the rate of key pressing in the Sidman's conditioned avoidance test.

Clomipramine and imipramine were found, on weight basis, to be about 10 times less potent than chlorpromazine in decreasing SMA and exploratory behaviour. The orientation motility in mice is also affected only by high doses (ED_{50} 40 mg/kg, ip) of clomipramine (11).

Clomipramine, like imipramine, but unlike chlorpromazine was found to enhance the methamphetamine-induced stereotyped behaviour. In the study of Theobald *et al.* (11) clomipramine was found to potentiate amphetamine-induced hyperthermia in rat and only at very high doses (ED_{50} 75 mg/kg, sc) it protected grouped mice against amphetamine toxicity. Neuroleptics like chlorpromazine however, are capable of protecting grouped mice against amphetamine toxicity, when used in a low dose range of 2.5 to 5 mg/kg (6).

On the basis of our findings, we therefore, conclude that clomipramine like imipramine possesses negligible neuroleptic activity and the 3 chlorine substitution has not imparted neuroleptic activity to imipramine.

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