Brit. J. Pharmacol. (1964), 22, 154–161.

TRANQUILLIZING AND HYPOTENSIVE ACTIVITIES OF TWELVE PHENOTHIAZINES

BY

K. P. BHARGAVA AND OM CHANDRA

From the Department of Pharmacology and Therapeutics, K.G. Medical College, University of Lucknow, India

(*Received September* 24, 1963)

Twelve phenothiazine derivatives have been compared in relation to their central sedative, tranquillizing and hypotensive activities. The ED50's of the compounds to suppress the conditioned response, unconditioned response and forced locomotor activity were determined. Suppressions of the conditioned and unconditioned responses were taken as criteria for tranquillizing and central sedative activities respectively. The " tranquillizing ratio " (ED50 for unconditioned response/ED50 for conditioned response) was used as the criterion of tranquillizing activity in relation to sedative activity and for purposes of comparison this ratio was assigned a value of unity for chlorpromazine. Perphenazine was the most potent drug in suppressing the conditioned and unconditioned responses whereas fluopromazine had the maximal " tranquillizing index " activity. Six compounds had great, three moderate and three mild hypotensive activity when doses of 1 mg/kg were injected intravenously into dogs. The relationship of the chemical structure to the central sedative activity is discussed.

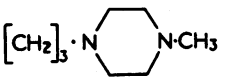
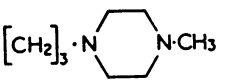
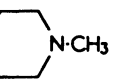
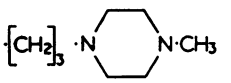
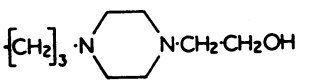
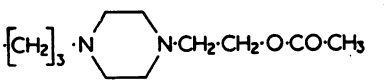
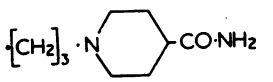
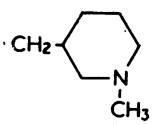
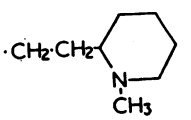
Central sedative properties of chlorpromazine were first reported by Courvoisier, Fournel, Ducrot, Kolsky & Koetschet (1953). During the last decade a large number of phenothiazine derivatives have been synthesized and tested for tranquillizing activity. The relative activity of some of these compounds has been determined in a series of clinical and experimental studies (Piala, High, Hassert, Burke & Craver, 1959 ; Sandberg, 1959 ; Taeschler & Cerletti, 1959 ; Haley, Flesher & Raymond, 1960 ; Dolivo & Maillard, 1961 ; Irwin, 1961). Although chlorpromazine has always been taken as the standard for comparison there are discrepancies in the reports. It was therefore considered of interest to compare the tranquillizing activities of a series of phenothiazine derivatives under identical experimental and environmental conditions. The phenothiazine compounds selected for the study are listed in Table 1 and are classified according to the substituent (R_2) at position 10 in the phenothiazine nucleus. The comparative antiemetic potencies of these agents in relation to their chemical structure have been reported (Bhargava & Chandra, 1963).

Hypotension is a major side effect of the phenothiazine compounds used clinically (Courvoisier *et al.*, 1953 ; Feldman & Kidron, 1957). Hence in this study, the compounds have also been compared for their hypotensive activities in dogs.

TABLE 1

PHENOTHIAZINE DERIVATIVES

Phenothiazine ($R_1 = R_2 = H$)

Name	R_2	R_1
3-Dimethylpropylamines		
Chlorpromazine	$\cdot[CH_2]_3 \cdot N(CH_3)_2$	Cl
Fluopromazine	$\cdot[CH_2]_3 \cdot N(CH_3)_2$	CF_3
Chlorpromazine sulphoxide*	$\cdot[CH_2]_3 \cdot N(CH_3)_2$	Cl
3-(4-Methylpiperazin-1-yl)propyl derivatives		
Prochlorperazine	$\cdot[CH_2]_3 \cdot$ 	Cl
Trifluoperazine	$\cdot[CH_2]_3 \cdot$ 	CF_3
Methyltrifluoperazine (SKF 5657-A_3)	$\cdot CH_2 \cdot CH(CH_3) \cdot CH_2 \cdot$ 	CF_3
Thioproperazine	$\cdot[CH_2]_3 \cdot$ 	$.SO_2.N(CH_3)_2$
3-[4-(2-Hydroxyethyl)piperazin-1-yl]propyl derivatives		
Perphenazine	$\cdot[CH_2]_3 \cdot$ 	Cl
Thiopropazate	$\cdot[CH_2]_3 \cdot$ 	Cl
Piperidine derivatives		
Pipamazine	$\cdot[CH_2]_3 \cdot$ 	Cl
Pecazine		H
Thioridazine		$.SCH_3$

*The bivalent sulphur of the phenothiazine nucleus is oxidized to $>SO_2$.

METHODS

Central sedative activity

Conditioned and unconditioned responses. The procedure used was essentially that described by Cook & Weidley (1957). Rats were trained to jump on a pole placed in the centre of a plastic box, in response to a buzzer (the conditioned stimulus) by means of electrical shocks to the paws (the unconditioned stimulus) applied after a 30 sec period of the buzzer alone. Rats which jumped in response to the buzzer alone after a few trials were selected for the study and were randomly divided into groups of ten.

In each test, four groups, each of ten rats, were treated with different dose levels of drug, injected intraperitoneally, and a fifth group served as a control. Treated and control groups were tested at 15 min intervals after administration of a drug until the peak effect was observed for both conditioned and unconditioned responses and the ED50 and 95% fiducial limits were calculated. After each test the animals were not used again for 5 days to avoid cumulative effects. Usually one such test provided sufficient information but occasionally it was necessary to repeat the test using the same animals after the 5 day rest period.

Forced locomotor activity. The test was based on the method described by Kinnard & Carr (1957). Mice were trained to remain on a rotating rod, divided into five compartments by cardboard discs, for 2 min in at least four consecutive trials before the start of the experiment. The drugs, dissolved in equal volumes of 0.9% saline, were injected intraperitoneally into the trained mice using ten animals at each dose level. The mice were tested, in sub-groups of five, at intervals of 15 min until the peak effect was noted. The "performance time," that is, the time for which each animal remained on the rod, was recorded up to a maximum of 2 min. When the summated "performance time" for a group reached a minimal value this was considered the peak drug effect. The percentage reduction in performance time was determined for at least three dose levels of each drug and the combined results subjected to probit analysis by the method of Finney (1952) and the ED50 and 95% fiducial limits were calculated.

Blood pressure studies

The hypotensive study was done in dogs weighing between 7 and 16 kg. The dogs were anaesthetized with pentobarbitone sodium (30 mg/kg, intravenously) and a femoral vein was cannulated with an indwelling polyethylene tube for injection of drug solutions. All animals were vagotomized and the trachea was intubated for positive pressure artificial ventilation to avoid any secondary effect of breathing on the blood pressure. The blood pressure was recorded from the right common carotid artery by a mercury manometer. The left common carotid artery was exposed to obtain the reflex pressor response to occlusion for 30 sec. Responses to noradrenaline were obtained by intravenous injection of 5 μg of the drug, irrespective of the weight of the animal. All the phenothiazines were injected intravenously in a dose of 1 mg/kg, and their effects on the pressor responses to carotid occlusion and noradrenaline were observed. For each drug the percentage recovery was noted after intervals of 1 and 3 hr.

The following drugs were used: chlorpromazine hydrochloride, fluopromazine hydrochloride, chlorpromazine sulphoxide, prochlorperazine acid maleate, trifluoperazine dihydrochloride, methyltrifluoperazine dihydrochloride, thioproperazine methanesulphonate, perphenazine sodium citrate, thiopropazate hydrochloride, pipamazine hydrochloride, pecazine hydrochloride and thioridazine hydrochloride. The structures of these compounds are shown in Table 1.

RESULTS

Effects on conditioned and unconditioned responses and on forced locomotor activity

The results are presented in Table 2; in all the above tests perphenazine was the most active drug. With the other drugs the order of efficacy varied but the similarity was greatest between the conditioned response and the forced locomotor activity

TABLE 2

ED50'S WITH 95% FIDUCIAL LIMITS OF THE PHENOTHIAZINE COMPOUNDS ON THE SUPPRESSION OF THE CONDITIONED AND UNCONDITIONED RESPONSES AND FORCED LOCOMOTOR ACTIVITY

	Conditioned response		Unconditioned response		Forced locomotor activity	
Compound	ED50 (mg/kg)	95% fiducial limits	ED50 (mg/kg)	95% fiducial limits	ED50 (mg/kg)	95% fiducial limits
Chlorpromazine	2·7	2·38–3·02	13·70	12·27–15·13	5·01	4·15–5·87
Fluopromazine	0·72	0·42–0·96	6·60	5·74–7·46	2·82	1·92–3·72
Prochlorperazine	0·72	0·61–0·83	3·77	3·13–4·41	3·31	2·85–3·77
Trifluoperazine	0·58	0·50–0·66	2·82	2·34–3·30	2·09	1·59–2·59
Thioproperazine	2·83	2·39–3·27	6·58	5·82–7·34	11·22	9·62–12·82
Methyltrifluoperazine	2·82	2·44–3·20	15·80	13·11–18·49	13·18	11·77–14·39
Perphenazine	0·30	0·28–0·32	1·80	1·63–1·97	1·32	0·72–1·92
Thiopropazate	0·41	0·35–0·47	3·37	3·12–3·62	2·51	2·11–2·91
Pipamazine	2·81	2·35–3·27	15·55	13·88–17·22	14·12	12·94–15·30
Pecazine	2·81	2·35–3·27	9·52	9·03–10·01	3·98	3·08–4·88
Thioridazine	3·43	3·14–3·72	16·07	13·74–18·40	15·85	14·41–16·29
Chlorpromazine sulphoxide	17·60	16·72–18·48	Ineffective		Ineffective	

test. Chlorpromazine sulphoxide had a very low activity in the conditioned response test and in the other tests was inactive even in doses ten-times greater than the ED50 for chlorpromazine itself.

Effect on blood pressure

The results are summarized in Table 3. Perphenazine, fluopromazine, pipamazine, trifluoperazine, thiopropazate and thioridazine had great hypotensive activity, producing a 31 to 45% reduction in the blood pressure at the time of their peak

TABLE 3

EFFECTS OF PHENOTHIAZINE COMPOUNDS ON BLOOD PRESSURE IN ANAESTHETIZED DOGS

The compounds were given in doses of 1 mg/kg, intravenously. Carotid arterial occlusion was applied for 30 sec. Noradrenaline was given in a dose of 5 μg

	Control blood pressure (mm Hg)				Peak blood pressure effect				Recovery in blood pressure (% of control) after	
	Before		After			% of control				
Compound	Range	Mean	Carotid occlusion	Noradrenaline	Time (min)	Before	Carotid occlusion	Noradrenaline	1 hr	3 hr
Chlorpromazine	96–112	104	50	26	5	76	48	92	79	81
Fluopromazine	122–136	129	60	20	10	68	20	95	84	100
Prochlorperazine	88–94	91	22	24	15	72	100	100	79	94
Trifluoperazine	108–120	114	68	48	10	66	44	80	79	98
Thioproperazine	104–118	111	86	18	10	72	25	96	91	100
Methyl-trifluoperazine	68–80	74	52	40	10	97	100	104	100	100
Perphenazine	120–134	127	50	26	15	59	10	90	83	87
Thiopropazate	102–108	105	52	24	5	56	31	110	62	80
Pipamazine	86–104	95	28	50	20	62	25	80	62	73
Pecazine	140–160	150	26	18	5	96	96	96	100	100
Thioridazine	82–86	89	60	42	10	60	40	100	60	77
Chlorpromazine sulphoxide	80–100	90	50	44	10	89	90	96	98	100

effect. Chlorpromazine, prochlorperazine and thioproperazine produced a moderate fall in blood pressure (16 to 30% reduction); methyltrifluoperazine, pecazine and chlorpromazine sulphoxide had mild hypotensive activity (0 to 15% reduction).

The peak hypotensive effect was earliest (within 5 min) with chlorpromazine, thiopropazate and pecazine. Fluopromazine, trifluoperazine, thioproperazine, methyltrifluoperazine, thioridazine and chlorpromazine sulphoxide produced their peak effects in approximately 10 min. The maximal time (15 to 20 min) to produce the hypotensive response was taken by prochlorperazine, perphenazine and pipamazine. With the exception of prochlorperazine, methyltrifluoperazine, pecazine and chlorpromazine sulphoxide, all the compounds depressed the carotid occlusion response significantly at the time of their maximal activity. The response to noradrenaline was not significantly diminished by any of the phenothiazine compounds in the dose used (1 mg/kg). There was complete recovery in the blood pressure level in less than 1 hr with pecazine, methyltrifluoperazine and chlorpromazine sulphoxide. Fluopromazine, trifluoperazine and thioproperazine showed only partial recovery after 1 hr but there was complete recovery at the end of 3 hr. With the rest of the phenothiazines (chlorpromazine, perphenazine, thiopropazate, pipamazine and thioridazine) there was only partial recovery at the end of 3 hr.

DISCUSSION

Central sedative activity

The conditioned and unconditioned avoidance responses have commonly been used to test the tranquillizing activity of central nervous system depressants (Cook & Weidley, 1957; Tedeschi, Tedeschi, Cook, Mattis & Fellows, 1959). The conditioned avoidance suppression was taken as a criterion for the tranquillizing activity while the block of the unconditioned response was taken as a criterion for the central sedative activity. Irwin, Slabock, Debiase & Govier (1959) suggested that the efficacy of the potent tranquillizers in the treatment of psychoses can be inferred from the ability of the drugs to suppress avoidance behaviour. This would imply that an effective tranquillizer should block the conditioned response at a much lower dose than that at which it blocks the unconditioned response and produces central sedation. The greater the difference between the dose required to block the conditioned response and that required to block the unconditioned response for a given drug the better tranquillizing agent it should be. Such a drug could be used as a tranquillizer with minimal sedation. The " tranquillizing ratio " (ED50 for unconditioned response/ED50 for conditioned response) of the phenothiazines was therefore calculated. The " tranquillizing index " was obtained for each drug by comparing it with chlorpromazine, the " tranquillizing ratio " of which was taken as unity. Thus the drugs with a " tranquillizing index " of more than 1 are likely to be better tranquillizers than is chlorpromazine. The relative " tranquillizing indices " of the phenothiazines are shown in Fig. 1. Fluopromazine had the maximum " tranquillizing index " of 1.8. Although fluopromazine may not be the most active drug it is a more effective tranquillizer than perphenazine.

Forced locomotor activity has been used to test the tranquillizing activity of a drug (Riley & Spinks, 1958). Kinnard & Carr (1957), Irwin *et al.* (1959) and Tedeschi

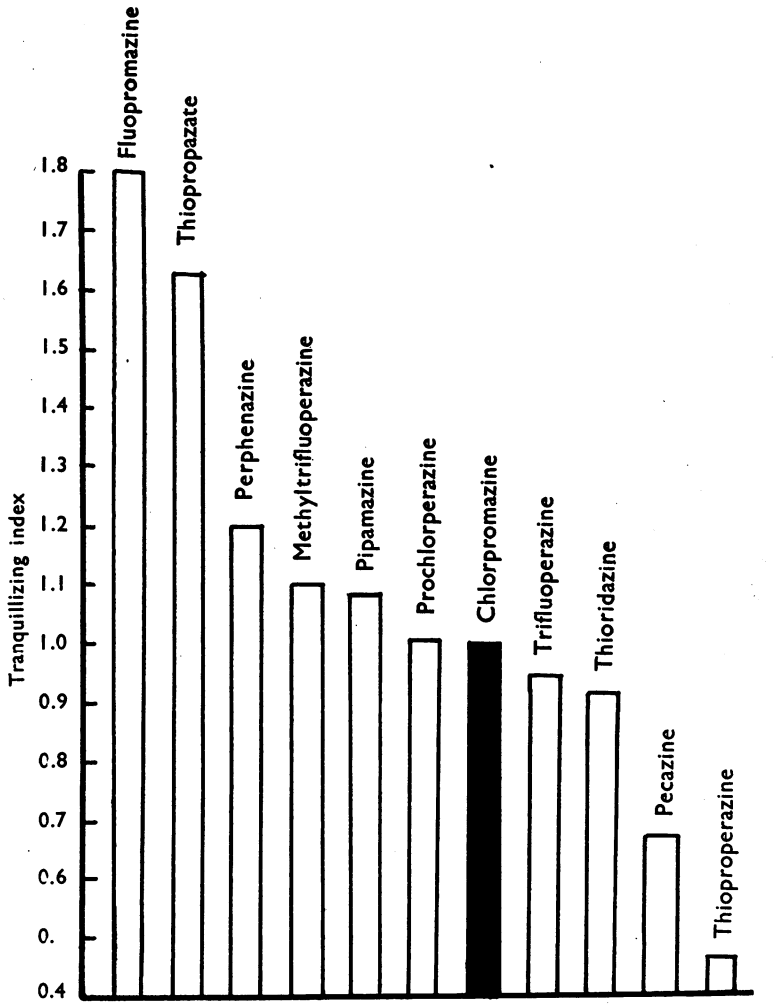


Fig. 1. Tranquillizing indices of phenothiazines. The ratio (ED50 for unconditioned response/ ED50 for conditioned response) for chlorpromazine has been taken as unity.

et al. (1959) have observed a high degree of correlation between drug-induced locomotor suppression and conditioned avoidance suppression. However, Irwin (1961) reported dissociation of the two properties with promazine. In our series of phenothiazines the doses required to suppress forced locomotor activity were close to the doses required to suppress the conditioned response.

Hypotensive activity

In Table 3 are shown the results of the hypotensive study. The hypotensive activities of the phenothiazines were compared by using the same dose (1 mg/kg) of each drug in dogs. As far as possible the depth of anaesthesia was the same in each experiment. With chlorpromazine, hypotension occurs without actual

adrenergic blockade and this is an action at the hypothalamic level (Tangri & Bhargava, 1960). Of the phenothiazines tested, fluopromazine, trifluoperazine, perphenazine, thiopropazate, pipamazine and thioridazine were the most active hypotensive drugs. Chlorpromazine, prochlorperazine and thioproperazine were moderately hypotensive, and methyltrifluoperazine, pecazine and chlorpromazine sulphoxide were less active. The more active hypotensive phenothiazines uniformly caused a considerable reduction in the reflex response to occlusion of the carotid artery (Table 2), although no significant depression of the response to noradrenaline was observed ; this indicates a central action. Although in general the hypotensive activity of the phenothiazines ran parallel with their tranquillizing property pipamazine and thioridazine were potent hypotensive compounds with little central sedative activity. These compounds may prove to be useful clinical hypotensive agents.

Central sedative activity and chemical structure

The phenothiazine ring and the chemical substituents of the compounds tested are shown in Table 1. The following relationships between the chemical structure of the compounds and their central sedative activity seem applicable.

(1) Central sedative activity is absent in chlorpromazine sulphoxide, the major metabolic product of chlorpromazine. It seems that the activity is lost when the sulphur atom is oxidized.

(2) Replacement of the hydrogen atom at position 2 (R_1) in the phenothiazine nucleus by electronegative radicals enhances the activity. Thioproperazine [$R_1 = SO_2.N(CH_3)_2$], prochlorperazine ($R_1 = Cl$) and trifluoperazine ($R_1 = CF_3$) are the piperazinyl derivatives listed in increasing order of potency. Similarly fluopromazine ($R_1 = CF_3$) is more active than chlorpromazine ($R_1 = Cl$) in the dimethylaminopropyl series.

(3) Of the compounds with a three carbon atom chain, which is optimal for tranquillizing activity (Parkes, 1961), the 3-piperazin-1-ylpropyl derivatives (trifluoperazine and perphenazine) are more active than the corresponding 3-dimethylaminopropyl compounds (chlorpromazine and fluopromazine). The activity of the piperidine derivatives (pipamazine, pecazine and thioridazine) is of the same order as that of chlorpromazine (Table 2). Of the 3-piperazin-1-ylpropyl phenothiazines the 4-(2-hydroxyethyl)piperazin-1-yl group of perphenazine confers greater activity than the corresponding 4-methyl group of prochlorperazine. Acetylation of the free hydroxyl group of perphenazine, as in thiopropazate, reduces activity. Branching of the three carbon chain interposed between the terminal and phenothiazine nitrogen atoms, as in methyltrifluoperazine, reduces activity compared with that with the unbranched chain in trifluoperazine.

We gratefully acknowledge gifts of chlorpromazine (Largactil), prochlorperazine (Stemetil), thioproperazine (Majeptil) and chlorpromazine sulphoxide from May & Baker ; trifluoperazine (Eskazine) and methyltrifluoperazine (SKF 5657 A-2) from Smith, Kline & French ; fluopromazine (Siquil) from Sarabhai Chemicals ; perphenazine (Trilafon) from Schering ; pecazine (Pacatal) from Warner Chilcott ; thioridazine (Melleril) from Sandoz ; and pipamazine (Mornidine) and thiopropazate (Dartal) from Searle Laboratories.

REFERENCES

BHARGAVA, K. P. & CHANDRA, O. (1963). Anti-emetic activity of phenothiazines in relation to chemical structure. *Brit. J. Pharmacol.*, **21**, 436–440.

COOK, L. & WEIDLEY, E. (1957). Behavioral effects of some psychopharmacological agents. *Ann. N.Y. Acad. Sci.*, **66**, 740–752.

COURVOISIER, S., FOURNEL, J., DUCROT, R., KOLSKY, M. & KOETSCHET, P. (1953). Pharmacological properties of 3-chloro-10(dimethylaminopropyl)phenothiazine hydrochloride. Experimental study of a new substance used in potentiating anaesthesia and artificial hibernation. *Arch. int. Pharmacodyn.*, **92**, 305–345.

DOLIVO, P. M. & MAILLARD, J. M. (1961). Pharmacologie des medicaments psycho- et neurotropes. *Helv. Med. Acta*, **28**, 339–366.

FELDMAN, S. & KIDRON, D. P. (1957). Experimental studies with chlorpromazine. Effect on cerebrospinal fluid pressure, blood pressure, respiration and pial vessel diameter in the cat. *Arch. int. Pharmacodyn.*, **111**, 70–85.

FINNEY, D. J. (1952). *Probit Analysis*, 2nd ed. Cambridge University Press.

HALEY, T. J., FLESHER, A. M. & RAYMOND, K. (1960). Pharmacological comparison of chlorpromazine and mellaril, 3-methylmercapto-10[2-(*N*-methyl-2-piperdyl)-ethyl]-phenothiazine hydrochloride. *Arch. int. Pharmacodyn.*, **124**, 455–460.

IRWIN, S. (1961). Correlation in rats between the locomotor and avoidance suppresant potencies of eight phenothiazine tranquillizers. *Arch. int. Pharmacodyn.*, **132**, 279–286.

IRWIN, S., SLABOK, M., DEBIASE, P. L. & GOVIER, W. M. (1959). Perphenazine (Trilafon)—a new potent tranquillizer and anti-emetic. I. Behavior profile, acute toxicity and behavioral mode of action. *Arch. int. Pharmacodyn.*, **118**, 358–374.

KINNARD, W. J. & CARR, C. J. (1957). A preliminary procedure for the evaluation of central nervous system depressants. *J. Pharmacol. exp. Ther.*, **121**, 354–361.

PARKES, M. W. (1961). *Tranquillizers: Progress in Medicinal Chemistry*, vol. 1, pp. 72–131 London: Butterworth.

PIALA, J. J., HIGH, J. P., HASSERT, G. L., BURKE, J. C. & CRAVER, B. N. (1959). Pharmacological and acute toxicological comparisons of triflupromazine and chlorpromazine. *J. Pharmacol. exp. Ther.*, **127**, 55–65.

RILEY, H. & SPINKS, A. (1958). Biological assessment of tranquillizers. Part I. *J. Pharm. Pharmacol.*, **10**, 657–671.

SANDBERG, F. (1959). A comparative quantitative study of the central depressant effect of seven clinically used phenothiazine derivatives. *Arzneimittel-Forsch.*, **9**, 203–206.

TAESCHLER, M. & CERLETTI, A. (1959). Differential analysis of the effects of phenothiazine-tranquillizers on emotional and motor behaviour in experimental animals. *Nature (Lond.)*, **184**, 823–824.

TANGRI, K. K. & BHARGAVA, K. P. (1960). Localization of the central site of hypotensive action of chlorpromazine. *Arch. int. Pharmacodyn.*, **127**, 274–284.

TEDESCHI, D. H., TEDESCHI, R. E., COOK, L., MATTIS, P. A. & FELLOWS, E. J. (1959). The neuropharmacology of trifluoperazine: a potent psychotherapeutic agent. *Arch. int. Pharmacodyn.*, **122**, 129–143.