

A COMPARISON OF IMIPRAMINE, CHLORPROMAZINE AND RELATED DRUGS IN VARIOUS TESTS INVOLVING AUTONOMIC FUNCTIONS AND ANTAGONISM OF RESERPINE

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Seven structurally-related compounds consisting of three antidepressant drugs (imipramine, desmethylimipramine and amitriptyline), three tranquillizing agents (promazine, chlorpromazine and chlorprothixene) and a hybrid, desmethylpromazine, have been examined in a series of tests involving autonomic functions and antagonism of reserpine. Activities of the compounds in antagonizing reserpine-induced ptosis in rabbits and prolongation of alcohol hypnosis in mice give good correlation with their clinical actions, whilst their activities in augmenting excitation of rats by amphetamine and yohimbine toxicity in mice, and in reversing reserpine-induced bradycardia in rats offer further evidence for drug-induced sensitization to adrenergic or tryptaminic mechanisms, which is not however specific for antidepressant agents. No evidence has been obtained to indicate that a central parasympatholytic action is an important component of the antidepressant activity of imipramine and related drugs.

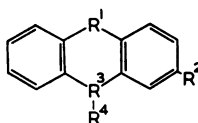
Imipramine, a drug originally synthesized as an antihistamine agent but now widely used in the treatment of endogenous depression, has been the subject of many investigations aimed at providing experimental evidence of an antidepressant action in animals, and at differentiating the drug in pharmacological and behavioural tests from the structurally-similar phenothiazine tranquillizing agents such as chlorpromazine and promazine (for references, see Discussion). The exact nature of the differences in the mode of action of imipramine and the phenothiazines is not clear. Both imipramine and chlorpromazine have been reported to interfere with the tissue uptake of noradrenaline at peripheral and central sites (Axelrod, Whitby & Hertting, 1961; Dengler & Titus, 1961). Chlorpromazine blocks adrenaline receptors at low doses (Martin, Riehl & Unna, 1960) whereas imipramine has been postulated to exert its antidepressant action by an activation of central adrenergic mechanisms (Sigg, 1959), but many workers have reported that imipramine exerts a weak chlorpromazine-like action.

We know of no published quantitative comparison between imipramine, promazine and chlorpromazine in a broad range of tests involving autonomic function, and the work reported here was initiated to provide such a comparison. In the course of it, two imipramine derivatives, desmethylimipramine (Brodie, Bickel & Sulser, 1961) and amitriptyline (Vernier, 1961), became recognized as clinically successful antidepressant drugs, and these compounds were studied also.

Chlorprothixene (Pellmont, Steiner, Besendorf, Bächtold & Läuppi, 1960), a tranquillizing agent which bears the same structural relationship to chlorpromazine as amitriptyline does to imipramine, and desmethylpromazine, which is similarly related to promazine as desmethylimipramine is to imipramine, were also included in the tests in order to gain further insight into structure-activity relationships in these classes of compound.

The chemical structures of the seven compounds examined are given in Table 1. Certain results obtained for imipramine have been reported previously (Monro, Quinton & Wrigley, 1963).

TABLE 1
CHEMICAL STRUCTURES OF IMIPRAMINE, CHLORPROMAZINE AND RELATED DRUGS



	R ¹	R ²	R ³	R ⁴
Imipramine	-CH ₂ .CH ₂ -	H	N	-[CH ₂] ₃ .N(CH ₃) ₂
Desmethylimipramine	-CH ₂ .CH ₂ -	H	N	-[CH ₂] ₃ .NHCH ₃
Amitriptyline	-CH ₂ .CH ₂ -	H	C	=CH.[CH ₂] ₂ .N(CH ₃) ₂
Promazine	-S-	H	N	-[CH ₂] ₃ .N(CH ₃) ₂
Desmethylpromazine	-S-	H	N	-[CH ₂] ₃ .NHCH ₃
Chlorpromazine	-S-	Cl	N	-[CH ₂] ₃ .N(CH ₃) ₂
Chlorprothixene	-S-	Cl	C	=CH.[CH ₂] ₂ .N(CH ₃) ₂

METHODS

Dog blood pressure preparation. The blood pressure was measured by a mercury manometer from a cannulated femoral artery of Beagle dogs anaesthetized with pentobarbitone sodium (30 mg/kg, intravenously). The effect of drugs, given in a range of doses from 0.016 to 5 mg/kg, was determined upon the pressor or depressor responses to noradrenaline (2.5 or 5 µg), adrenaline (5 or 10 µg), acetylcholine (20 or 40 µg), bilateral carotid arterial occlusion (for 20 to 25 sec) or stimulation of the peripheral cut end of the right cervical vagus nerve (32 shocks/sec, 1 msec duration, 1 to 4 V, 5 to 10 sec bursts). The minimal effective dose of drug to change the response by 25% was noted. Forty-one dogs were used. All drugs were given intravenously.

Excitation of rats by amphetamine. This test, previously described by Quinton & Halliwell (1963), measured the automatous, stereotyped behaviour induced by amphetamine rather than increased motility. At any one time the maximum excitation score for each group of four rats was 24; a total score for each group was derived by summing the scores measured half-hourly for 6 hr. This total, less that derived from a group of rats given amphetamine only, represented the net excitation score. The operator scoring the activity did not know what treatment the animals had received.

Yohimbine toxicity in mice. The method has been described elsewhere (Quinton, 1963b). Drugs were administered orally to groups of ten mice, 1 hr before a subcutaneous dose of yohimbine hydrochloride (20 mg/kg), a dose which is not lethal by itself (0/51 mice dead). The mice were kept in groups of five in compartments approximately 12×12 cm in floor area, at an ambient temperature of 21 to 25° C. ED₅₀ values (that is, doses of drug producing a 50% mortality in mice given yohimbine) were derived graphically from overnight mortality figures, at least twenty mice being used at each dose-level.

Reversal of reserpine-induced ptosis. The method of Maxwell & Palmer (1961) was used. Thirty-five rabbits were tested.

Reversal of bradycardia due to reserpine. Albino male rats (Tuck Wistar strain, 220 to 280 g) were anaesthetized with allobarbitone (32 mg/kg) and urethane (740 mg/kg) injected intraperitoneally, and placed on a table warmed to 30 to 32° C. Heart rates were measured from electrocardiograms recorded on a pen-recording oscillograph connected to two No. 16-gauge needle electrodes inserted through the skin on each side of the animals' chests. Thirty-six rats were tested at the same time, at least five animals being used for each dose-level of drug, and their heart rates averaged. Unless otherwise stated, drugs were injected subcutaneously 1.5 to 2 hr after induction of anaesthesia. In the case of animals which had been treated with reserpine (5 mg/kg injected intraperitoneally 18 hr previously), the response to a drug at any one time after injection was expressed as the difference in the mean increase in heart rate (beats per minute) over preinjection levels in animals injected with drug and those given 0.9% saline. This response is called "net increase in heart rate".

Reduction of reserpine-induced prolongation of alcohol sleeping-time. The method used was a modification of that of Sulser, Watts & Brodie (1960). Albino male mice (16 to 20 g) were deprived of solid food overnight but allowed to drink 10 to 20% glucose solution *ad libitum*. Reserpine (1 mg/kg) was injected intraperitoneally 1 hr after an oral dose of drug. Twenty mice were used for each dose. After 1 hr they received ethanol by mouth (25 ml/kg of a 20% solution in water). The duration of sleep (loss of righting reflex) was measured in those mice which lost their righting reflex within 30 min of administration of ethanol; 240 min was taken as the maximum duration of sleep. All values obtained from two or three experiments performed with the same dose of drug on different days, together with an equal number of values for control mice tested at the same times, were examined by the standard analysis of variance procedure, in order to determine the significance of the difference between the mean sleeping-times of the control and test mice. The mean response to the drug was expressed as the percentage difference in mean sleeping time between test and control mice. The ambient temperature was 21.5° C.

The duration of sleep (mean and standard error) in 495 control mice tested during the course of these experiments was 90.1 ± 4.7 min, with 172 mice (35%) not sleeping at all and 97 (20%) sleeping longer than 240 min.

Prolongation of sleeping-time due to alcohol. Albino male mice (16 to 22 g), deprived of solid food overnight, were given an oral dose of drug 30 min before receiving the dose of alcohol mentioned above. Since this dose of alcohol by itself induced sleep in only two out of fifty-five control mice (durations 6 and 9 min, mean 0.27 min), the response to a drug was expressed directly as the duration of sleep observed in mice given the drug as well as alcohol. Twenty mice were used for each dose of drug, and the experiment repeated at least once on another day. The statistical significance of any prolongation was assessed by Student's *t*-test. The ambient temperature was 21.5 ± 1.0 ° C.

Protection against the toxicity of amphetamine in grouped mice. Each drug was administered orally in a dose of 2, 10 or 50 mg/kg to albino female mice (16 to 22 g), 30 min before subcutaneous injection of (\pm)-amphetamine sulphate. Five dose-levels of amphetamine were given (12.5, 25, 50, 100 and 200 mg base/kg), each to ten mice. Mice were kept in groups of ten in boxes 24 × 16 cm in floor area and 10 cm high. The ambient temperature was 21.5° C. The mortality rate was counted 24 hr later, and the amphetamine LD50 value in mice given the drug was calculated by the method of Kärber (1931) and compared with that derived in exactly the same way for mice given amphetamine only and tested at the same time. The drug response was expressed as the percentage difference in the amphetamine LD50 between treated and control mice.

Mydriasis in mice. The mydriatic response was measured as the net increase in the diameter of the right pupil of albino male mice (25 to 45 g) measured with a binocular microscope immediately before and at the time of peak effect after oral injection of drug. The method has been described elsewhere (Quinton, 1963a). At least five mice were used for each dose of

drug and their net mydriatic responses were averaged. In order that the observed mydriasis should be a measure of the parasympatholytic activity of the drug, any pupil dilatation likely to be produced by augmentation of sympathetic mechanisms was minimized by treatment of the mice with reserpine (5 mg/kg, 5 or 18 hr previously).

Antagonism of effects of Tremorine. Groups of five albino male mice (25 to 35 g) were injected intraperitoneally with Tremorine (1,4-dipyrrolidino-2-butyne hydrochloride, 20 mg/kg), 30 min after receiving an oral dose of drug. The degree of tremors produced in each mouse was scored on an arbitrary scale from 0 (nil) to 3 (severe) at 30 and 45 min after injection of Tremorine. Both assessments were made by an operator who did not know what treatment the mice had received. The percentage inhibition of tremors by a drug was calculated by reference to the response of mice injected with Tremorine only and tested at the same time. The ED₅₀ values quoted are the means derived graphically from results of at least two experiments performed on different days.

Antagonism of arecoline-induced "analgesia." The degree of "analgesia" induced in male albino mice (14 to 17 g) by subcutaneous injection of arecoline (5 mg/kg) was determined by a modification of the hot-plate method of Woolfe & Macdonald (1944). A mouse was placed on a metal plate kept at the temperature of boiling acetone (56° C) and the time measured till it lifted both front paws together; 30 sec was taken as the maximum response time. Drugs were administered orally 0.5 to 1 hr before the arecoline, the time for each drug being chosen as that found optimum for mydriatic activity. Mice were tested exactly 3 min after injection of arecoline. The peripheral parasympathomimetic symptoms of arecoline were prevented by subcutaneous injection of atropine methyl nitrate (2 mg/kg) 1 to 5 hr beforehand. The response to a drug was calculated as the percentage reduction in the arecoline prolongation of the response time from the formula:

$$\% \text{ reduction} = 100 \times (A - AD) / (A - D)$$

where A is the mean response time after arecoline only, D is that after the drug only and AD that after drug with arecoline. All three responses were obtained in the same mice. In this way allowance was made for any analgesic or narcotic action of the drugs themselves. Each drug-response was derived from observations on twenty mice. The same mice were used for several drugs, being tested three times a week for 3 weeks.

Antagonism of inhibition of a conditioned reflex by arecoline. The method was based on that described by Pfeiffer & Jenney (1957). Male hooded rats (initially 100 g) were trained to jump on to a small platform inserted through the wall of a wooden box 25 × 30 × 35 cm, in order to escape from an electric shock (50 cycles/sec a.c., 20 to 40 V, 5 sec bursts) delivered to the grid floor 5 sec later. This conditioned response was then tested at 2 min intervals immediately before and for 26 min after the subcutaneous injection of arecoline (5 mg/kg). The number of positive responses after the arecoline was compared with those elicited in the same six rats treated with drug 0.75 to 1 hr before the arecoline. Peripheral parasympathomimetic symptoms were prevented by the intraperitoneal injection of atropine methyl nitrate (40 mg/kg) 45 min before arecoline.

It was noted that if the atropine methyl nitrate and arecoline were injected into the same subcutaneous site, the inhibition of the action of arecoline did not appear. A somewhat similar interference by atropine with the absorption of an anticholinesterase from subcutaneous sites has been reported recently by Ramachandran & Agren (1963).

Drugs. The following seven drugs (given as hydrochloride) were compared: amitriptyline (Roche, and Merck, Sharp & Dohme), chlorprothixene (Merck, Sharp & Dohme), chlorpromazine (May & Baker), promazine (Wyeth), imipramine (Geigy), desmethylimipramine and desmethylpromazine. The last two drugs were synthesized in our Chemical Research Laboratories. In experiments on mice, doses of 10 and 50 mg/kg were generally used unless activity was maximal at the former dose. Solutions of phenothiazines tended to colour readily on standing; this was prevented by the addition of ascorbic acid (10⁻³) to all drug solutions which were not used immediately after preparation. Reserpine was dissolved in a few drops

of glacial acetic acid, diluted to volume with 10% propylene glycol in distilled water, and brought to pH 5 to 6 immediately before injection. Unless otherwise stated, imipramine, chlorpromazine and related drugs were administered orally.

Note. In the tests for excitation by amphetamine and antagonism of reserpine-induced bradycardia in rats, and for potentiation of yohimbine in mice (Shillito, personal communication), considerable variation in sensitivity was noted between different strains of animals. In the case of rats, evidence pointed towards dietary causes, and sensitivity differences were associated to a certain extent with differences in the catechol amine content of the brain but not of the adrenals.

RESULTS

Potentiation of agents acting on adrenergic mechanisms

Pressor response to noradrenaline. Imipramine and amitriptyline increased the pressor response to noradrenaline at approximately the same minimum effective dose (0.1 to 0.2 mg/kg). Desmethylinipramine was about five times as potent as imipramine. The other four drugs failed to increase the response consistently, although low doses of chlorpromazine (0.016 to 0.032 mg/kg) and desmethylpromazine (0.080 mg/kg) caused marginal increases in some but not all dogs. Doses of these four drugs which blocked the pressor response to adrenaline (see later) sometimes slightly depressed the height of the response to noradrenaline but prolonged its duration.

Responses to adrenaline and carotid arterial occlusion. A variable but generally slight augmentation of the pressor response to adrenaline was observed after imipramine, desmethylinipramine and occasionally chlorpromazine in doses around the minimum effective level for augmentation of the response to noradrenaline. A significant increase in the pressor response to carotid arterial occlusion was seen occasionally after low doses of desmethylinipramine (0.016 mg/kg).

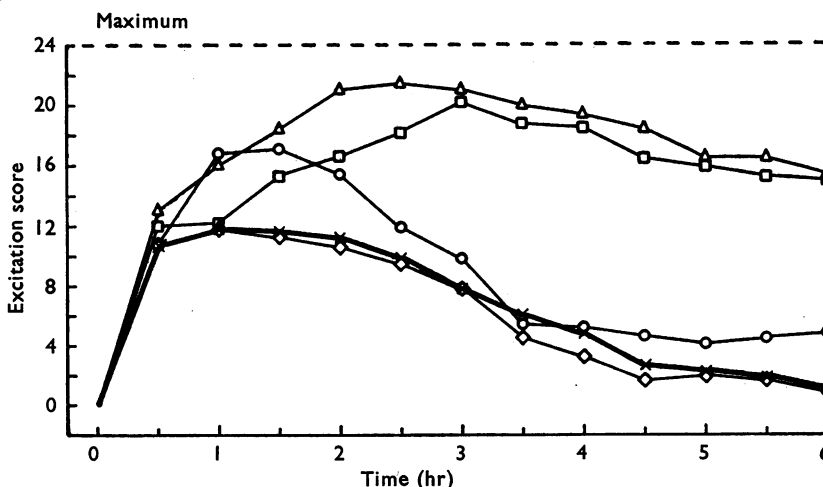


Fig. 1. Excitation induced in groups of 4 rats by (\pm)-amphetamine sulphate (5 mg/kg, intraperitoneally) with or without treatment with various oral doses of imipramine given 1.5 hr previously: \times , none; \diamond , 0.4 mg/kg; \circ , 2.0 mg/kg; \square , 10 mg/kg; and \triangle , 50 mg/kg.

Excitation due to amphetamine. All drugs increased the excitation induced by amphetamine, in degree and/or in duration. The effect of various doses of imipramine is shown in Fig. 1. No augmentation of the response to amphetamine became apparent until about 1 hr after injection of amphetamine. From this time the excitation induced in imipramine-treated rats rose steadily, to reach a peak at about 3 hr, whereas that in control rats altered little from 0.5 to 2 hr after amphetamine and thereafter fell steadily to zero. Rats given imipramine (10 mg/kg or more) still showed gnawing or licking movements 8 hr after injection of amphetamine.

Dose/response curves for the seven drugs tested are given in Fig. 2. Imipramine, desmethyylimipramine and promazine all showed high potency, but the effects of the highest dose of imipramine and promazine tested (50 mg/kg) were less than those of a lower dose (10 mg/kg). This inversion of the dose/response curve was even more marked for chlorpromazine and chlorprothixene. In the case of these two drugs, low doses (2 to 10 mg/kg) caused a moderate increase in the response to amphetamine, by prolonging but not by augmenting it in degree, whereas a dose of 50 mg/kg abolished the response. Desmethylpromazine was somewhat less

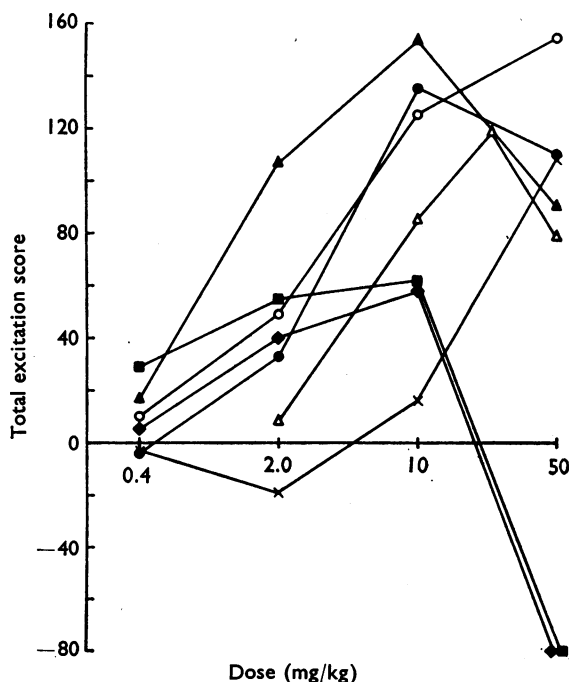


Fig. 2. Dose response curves for imipramine (●), desmethyylimipramine (○), amitypyline (×), promazine (▲), desmethylpromazine (△), chlorpromazine (■) and chlorprothixene (◆), in increasing the excitation caused in groups of four rats by (\pm)-amphetamine sulphate (5 mg/kg, intraperitoneally). The ordinate represents the total score obtained by summing readings every 30 min for 6 hr after injection of amphetamine, less the corresponding score for rats given amphetamine only.

potent than promazine and showed a similar biphasic dose/response relationship. Amitriptyline showed negligible activity except at 50 mg/kg.

A few experiments were performed with apomorphine hydrochloride (7.5 mg/kg, subcutaneously) which induced compulsive biting behaviour very similar to that observed after amphetamine, except in the shorter latency of onset and the relative rarity of licking movements (Janssen, Niemegeers & Jageneau, 1960). Imipramine and promazine (10 and 50 mg/kg) failed to influence this response to apomorphine, whereas chlorpromazine (10 and 50 mg/kg) reduced it.

Yohimbine toxicity in mice. All drugs increased the toxicity of yohimbine in doses well below those lethal by themselves. Dose/response curves for imipramine, amitriptyline, chlorpromazine and chlorprothixene have been published elsewhere (Quinton, 1963b). ED₅₀ values for all seven drugs are indicated in Fig. 3.

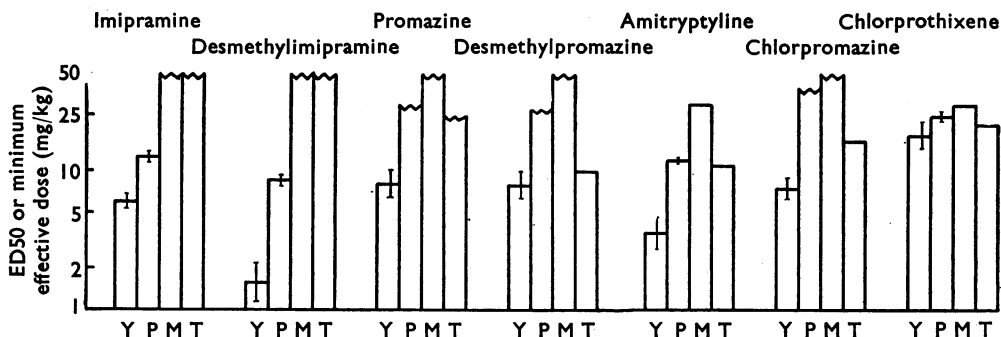


Fig. 3. ED₅₀ values in mg/kg for imipramine, chlorpromazine and related drugs given orally, in tests for increasing yohimbine toxicity (Y), dilating the pupil (M) and antagonizing Tremorine effects (T), in mice; and the mean minimum effective intravenous dose for reversing reserpine-induced ptosis (P) in rabbits. Vertical lines indicate 2× standard error, where appropriate.

Imipramine, promazine, desmethylpromazine and chlorpromazine were approximately equipotent (ED₅₀, 6 to 8 mg/kg); amitriptyline (3.6 mg/kg) and desmethylimipramine (1.6 mg/kg) were significantly more potent than imipramine; chlorprothixene displayed only weak activity (18 mg/kg).

Antagonism of reserpine actions

Antagonism of reserpine-induced bradycardia. The heart rate of rats anaesthetized with allobarbitone and urethane remained fairly steady for 1 to 6 hr after induction of anaesthesia (Fig. 4). Injection of reserpine (5 mg/kg) caused, after a delay of 1 hr, a persistent bradycardia. In rats given a previous injection of imipramine, which alone caused a moderate bradycardia lasting about 4 hr, a subsequent injection of reserpine induced a marked tachycardia. By 4 hr after reserpine, the mean heart rate of these rats approximated to that of control animals, being some 120 beats/min faster than that of rats which had received reserpine only.

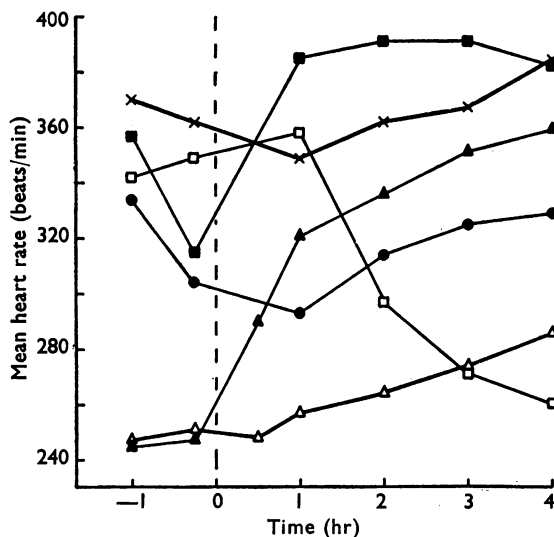


Fig. 4. Reversal by imipramine of reserpine-induced bradycardia in rats anaesthetized with allobarbitone-urethane. In four groups, injections were given at (i) -0.5 hr and (ii) 0 hr. *First group* (\square): (i) saline, 0.5 ml. subcutaneously, and (ii) reserpine, 5 mg/kg intraperitoneally; *second group* (\times): (i) saline and (ii) reserpine solvent, 1 ml.; *third group* (\bullet): (i) imipramine, 20 mg/kg, and (ii) reserpine solvent; and *fourth group* (\blacksquare): (i) imipramine and (ii) reserpine. The fifth and sixth groups of rats received reserpine 18 hr previously, and saline (Δ) or imipramine (\blacktriangle) at zero time. Points represent the mean values obtained from eleven to twenty rats.

A similar effect of imipramine could be demonstrated more strikingly in rats which had received reserpine 18 hr before anaesthesia. Such animals had a low and steady heart rate of 240 to 290 beats/min throughout the experiment. Injection of imipramine caused a marked and rapid increase in heart rate, which persisted over 5 hr (Fig. 4). Atropine sulphate (20 mg/kg) in reserpinized rats neither caused a tachycardia itself nor affected that induced by imipramine. Phenoxybenzamine (25 mg/kg), chlorisondamine chloride (10 mg/kg), guanethidine (25 mg/kg), bretylium (25 mg/kg) or α -methyldopa (400 mg/kg) did not significantly reduce the tachycardia induced by imipramine in reserpinized rats. It was, however, greatly reduced by pronethalol (15 mg/kg), and was not observed in rats adrenalectomized 3 hr previously.

The effect of various doses of imipramine, chlorpromazine and related drugs were investigated on the heart rates of rats treated with reserpine (5 mg/kg). The time-courses of the "net increase in heart rate" (see Methods) are plotted in Fig. 5. The two secondary amino derivatives, desmethylimipramine and desmethylpromazine, were the most potent of the drugs tested, being approximately twenty- and ten-times more potent than their respective tertiary analogues. Desmethylimipramine caused a pronounced tachycardia lasting 5 hr at a dose of only 0.25 mg/kg. Imipramine, promazine and chlorpromazine were roughly equiactive; amitriptyline was less potent and chlorprothixene nearly inactive.

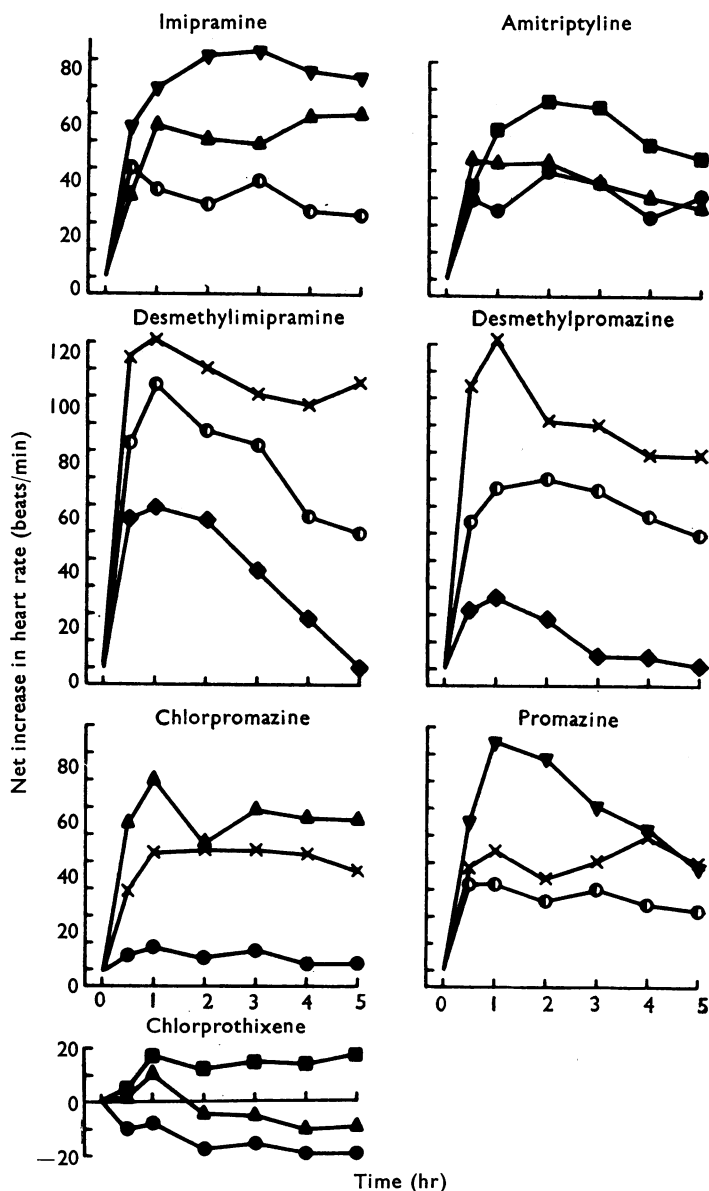


Fig. 5. Net increase in heart rate induced by subcutaneous injection of various doses of imipramine, chlorpromazine and related drugs in rats anaesthetized with allobarbitone-urethane and treated with reserpine, 5 mg/kg, 18 hr previously. Doses : 0.25 mg/kg (◆); 1.0 mg/kg (●); 2 mg/kg (○); 5 mg/kg (×); 10 mg/kg (▲); 20 mg/kg (▼); and 50 mg/kg (■).

Reversal of reserpine-induced ptosis. The values for the mean minimum effective dose of imipramine, chlorpromazine and related drugs are represented in Fig. 3. Desmethylimipramine, imipramine and amitriptyline were all approximately equipotent in this test, the first-mentioned being only slightly more potent than its

tertiary analogue. Chlorprothixene showed weak activity ($ED_{50}=25.6$ mg/kg), whereas promazine and chlorpromazine failed to reverse the ptosis at the highest doses given (30 to 40 mg/kg). Desmethylpromazine reversed ptosis in one rabbit at 27.2 mg/kg; it failed to do so in another after a dose of 24.7 mg/kg.

Reduction of reserpine-induced prolongation of sleeping-time due to alcohol. Values for the duration of sleep in individual mice showed a wide variation, and a percentage difference between the mean sleeping-time of twenty test and twenty control mice of less than $\pm 40\%$ was rarely significant. Dose/response curves for the seven drugs are represented in Fig. 6, with an indication of the significance

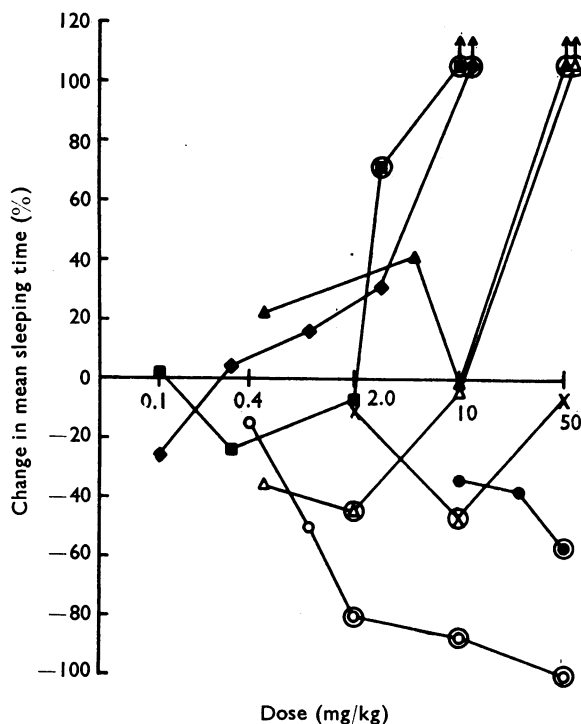


Fig. 6. The percentage change in the duration of sleep in mice injected with reserpine, 1 mg/kg intraperitoneally 1 hr before an oral dose of ethanol (25 ml./kg of a 20% solution), caused by various doses of imipramine (●), desmethylimipramine (○), amitriptyline (×), promazine (▲), desmethylpromazine (△), chlorpromazine (■) and chlorprothixene (◆). A ring around a point indicates significance ($P < 0.01$).

of each value. Highly significant reductions ($P < 0.01$) in sleeping-time were caused by imipramine (50 mg/kg), desmethylimipramine (2 to 50 mg/kg), amitriptyline (10 mg/kg) and desmethylpromazine (2 mg/kg). Higher doses of the last two drugs failed to give this reduction, and caused sedation by themselves. Promazine, chlorpromazine and chlorprothixene failed to shorten sleeping-time significantly at any dose tested, and in doses of 50, 3 and 10 mg/kg respectively significantly prolonged it.

In view of the observations made by Sulser, Watts & Brodie (1962) that imipramine, but not desmethylimipramine, needs to be injected several hours before alcohol in order to antagonize the reserpine-induced prolongation, a few experiments were performed in which the drugs were given 4 hr instead of 2 hr before alcohol. Under these conditions, the antagonism of the reserpine effect exerted by imipramine (25 to 50 mg/kg) was somewhat increased, and promazine caused a significant reduction in sleeping-time of 48 and 53% in two experiments ($P < 0.01$). Chlorpromazine (2 mg/kg) failed to shorten sleeping-time under these conditions. In a similar way to that proposed for imipramine by Sulser *et al.* (1962), this effect of promazine at the longer time-interval between injection and alcohol administration can presumably be related to the gradual accumulation in the brain of the demethylated product, desmethylpromazine, which in low doses antagonizes the reserpine-induced prolongation of alcohol sleep.

Adrenolytic or sympatholytic effects. Each of the seven drugs reduced the pressor response to adrenaline and carotid arterial occlusion in the dog over some upper part of the dose-range (0.016 to 5 mg/kg), even though in some instances lower doses caused slight augmentation of these responses (see above). Desmethylimipramine reduced the response to adrenaline only at the maximum dose, which depressed also responses to acetylcholine, histamine and dimethylphenylpiper-

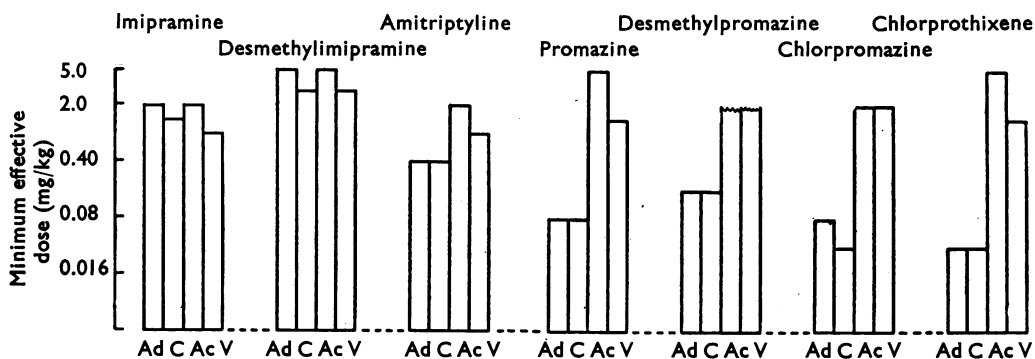


Fig. 7. Histograms showing minimum effective doses of imipramine, chlorpromazine and related drugs injected intravenously into dogs anaesthetized with sodium pentobarbitone, to reduce the pressor response to adrenaline (Ad) or the carotid occlusion reflex (C), or the depressor response to acetylcholine (Ac) or efferent vagal stimulation (V).

zinium bromide. The carotid occlusion response was generally slightly more sensitive to the depressant action of drugs than was the response to adrenaline (Fig. 7). The drugs showed the following descending order of potency in their depression of responses to adrenaline and carotid occlusion: chlorprothixene, chlorpromazine, promazine, desmethylpromazine, amitriptyline, imipramine and desmethylimipramine.

In doses up to 5 mg/kg, no drug markedly or consistently reduced the pressor response to noradrenaline (Martin *et al.*, 1960).

Sedative or "tranquillizing" effects

Prolongation of sleeping-time due to alcohol. Each of the drugs except desmethyl-imipramine prolonged the sleeping-time when given in adequate dosage, but their potencies varied markedly (Fig. 8). Chlorpromazine and chlorprothixene were very active, inducing sleep of 60 min duration in doses of about 1 mg/kg. Promazine and its desmethyl analogue were slightly less potent (4 to 6 mg/kg), with the

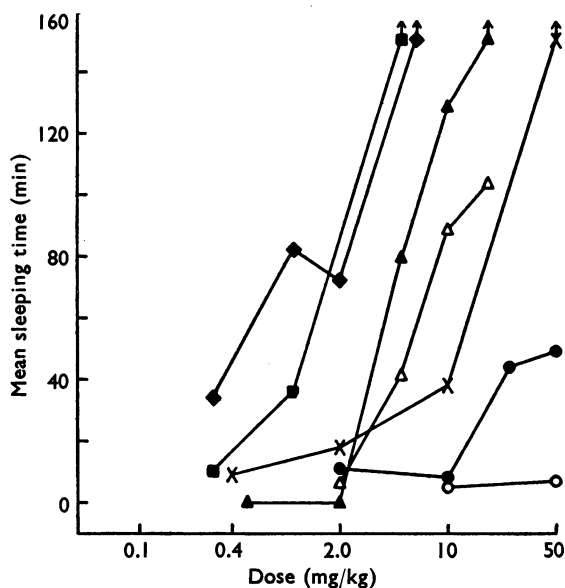


Fig. 8. The prolongation of sleep in mice given ethanol, caused by various oral doses of imipramine, chlorpromazine and related drugs. Ethanol dose and drug symbols are as in Fig. 6.

activity of the latter increasing less at higher doses than that of the former. Amitriptyline (12 mg/kg) showed weak activity. Imipramine and in particular desmethylimipramine were the least potent drugs in this test, doses of 50 mg/kg inducing sleep with durations of only 49.4 ± 9.5 and 7.1 ± 3.7 min (means and standard errors) respectively. (In Fig. 8, all responses indicated represent significant prolongations of sleeping-time ($P < 0.05$), except those for promazine (0.5 and 2.0 mg/kg), desmethylpromazine (2 mg/kg) and imipramine (10 mg/kg).

Protection against the toxicity of amphetamine in grouped mice. In our hands, this test for tranquillizing agents (Burn & Hobbs, 1958) yielded unsatisfactory results with drugs closely related to imipramine, although chlorpromazine gave consistently high protection against amphetamine toxicity. Higher doses (3 to 20 mg/kg) were required however than those which caused a significant prolongation of sleep induced by alcohol. Imipramine and amitriptyline even at 50 mg/kg gave variable protection. The values for the percentage increases in amphetamine LD₅₀ observed in three experiments with imipramine (50 mg/kg) were 81, 6 and 25% ; corresponding figures for amitriptyline (50 mg/kg) were increases of 45, 107 and 23%. After low doses (2 to 5 mg/kg) no consistent change in amphetamine toxicity was seen.

Parasympatholytic or antiacetylcholine effects

Prevention of depressor responses to acetylcholine or efferent vagal stimulation (Fig. 3). With the exception of desmethylimipramine and desmethylpromazine, differences in activity between the drugs were of doubtful significance in view of the unsuitability of the method for the statistical analysis of results. Imipramine and amitriptyline were marginally more potent than the phenothiazines in blocking vagal responses. Desmethylimipramine significantly reduced acetylcholine and vagal responses only at the highest dose tested (5 mg/kg). Insufficient material was available to fix with certainty the activity of desmethylpromazine; in the only dog into which doses of 2 and 5 mg/kg were injected, the response to acetylcholine was slightly reduced by the higher dose whereas the vagal response was unaffected by either dose.

The tertiary amines blocked vagal responses at slightly lower doses than those which reduced acetylcholine responses, in contrast to atropine which reduced the acetylcholine slightly more readily than the vagal response. Vagal blockade wore off only gradually, whereas reduction of the acetylcholine response was usually transient. Since, in the doses used here, acetylcholine did not reduce the heart rate but lowered the blood pressure presumably by a vasodilatation, whereas vagal stimulation slowed or stopped the heart by a direct action, this differentiation in the duration of block may be further evidence of a high specificity of imipramine for cardiac tissue, which has been noted in many cases of imipramine poisoning (for references, see Giles, 1963).

Mydriasis in mice. In normal mice all compounds induced some pupil dilatation at the highest dose tested (50 mg/kg) but only that produced by amitriptyline or chlorprothixene exceeded 50% of maximum (Fig. 3). When compared with atropine at their ED₂₅ levels, these compounds had relative potencies of 0.11 and 0.16 respectively (Table 2). Except in the case of chlorprothixene, after which pupil dilatation was at its peak 60 min after oral administration, responses to other drugs were at their highest 30 min after dosage.

The mydriasis caused by all compounds listed in Table 2 except atropine was significantly weaker in mice treated with reserpine, which was considered to reduce any pupil dilatation arising from augmentation by the compounds of sympathetic

TABLE 2
DOSES OF ATROPINE, IMPRAMINE, CHLORPROMAZINE AND RELATED DRUGS CAUSING PUPIL DILATATION 25% OF MAXIMUM POSSIBLE (ED₂₅) IN NORMAL AND RESERPINE-TREATED MICE

Drug	ED ₂₅ (mg/kg)	
	Normal	Reserpine-treated
Imipramine	27	> 50
Desmethylimipramine	> 50	> 50
Promazine	40	> 50
Desmethylpromazine	> 50	> 50
Amitriptyline	7	13
Chlorpromazine	50	> 50
Chlorprothixene	5	13
Atropine	0.72 (0.69-0.74)	0.30 (0.27-0.32)

Values in brackets indicate 95% limits of error

mechanisms. Under these conditions, only amitriptyline and chlorprothixene induced mydriasis exceeding 25% of maximum, the ED25 values of both drugs being 13 mg/kg. Imipramine (50 mg/kg) caused a mean mydriatic response of only 5 units (maximum possible, about 35); the corresponding figure for desmethyl-imipramine was 0.4 units.

Atropine was over twice as potent in mice given reserpine as in normal mice, the ED25 values being 0.30 and 0.72 mg/kg respectively. A similar augmentation of the mydriatic action of atropine by reserpine was reported by Tripod, Bein & Meier (1954).

Antagonism of the effects of Tremorine (Fig. 3). Neither imipramine nor its desmethyl analogue reduced the tremors caused by Tremorine by 50%, even at doses of 50 mg/kg. The peripheral parasympathomimetic effects of the agent were similarly little reduced. All other drugs produced signs of severe sedation at the dose of 50 mg/kg, which tended to make assessment of tremor-inhibition difficult. Amitriptyline (ED50=11 mg/kg) and desmethylpromazine (10 mg/kg), however, produced 50% reduction in tremor at doses just below those causing these gross effects. Chlorpromazine and chlorprothixene showed approximately similar activity, but their ED50s caused appreciable sedation; promazine was less active.

TABLE 3

SUMMARY OF ACTIVITIES OF IMIPRAMINE, CHLORPROMAZINE AND RELATED COMPOUNDS IN VARIOUS TESTS

The degree of activity is represented by O, (+), + or ++; D = activity opposite in nature to that tested; S = sedation interfered with endpoint of test.

Test	Drug						
	Imi- pramine	Desmethyl- imi- pramine	Ami- tripy- line	Pro- mazine	Desmethyl- pro- mazine	Chlor- pro- mazine	Chlor- pro- thixene
Potiation of actions of							
Noradrenaline	+	++	+	0	(+)	0	0
Amphetamine	++	++	(+)	++	+	(+)/D	(+)/D
Yohimbine	+	++	++	+	+	+	(+)
Antagonism of reserpine-induced							
Prolongation of alcohol sleep	+	++	+/0	D	+/D	D	D
Bradycardia	+	++	+	+	++	+	0
Ptosis	++	++	++	0	(+)	0	(+)
Reduction of carotid occlusion reflex	+	(+)	+	++	++	++	++
Prolongation of sleep due to alcohol	(+)	0	(+)	+	+	++	++
Mydriasis after reserpine	0	0	+	0	0	0	+
Antagonism of acetylcholine	(+)	0	(+)	0	(+)	(+)	0
Inhibition of vagal stimulation	+	(+)	+	+		+	+
Antagonism of Tremorine	0	0	++	+	++	S	S

Hyoscine hydrobromide gave an ED₅₀ (mean and standard error) of 2.7 ± 0.8 mg/kg in five experiments. With all compounds the reduction of tremors ran approximately parallel to that of the peripheral effects of Tremorine.

Antagonism of arecoline-induced "analgesia." The gross sedative properties of the phenothiazines, chlorprothixene and, in high doses, amitriptyline interfered in the arecoline test to an even greater degree than in the Tremorine test. Thus whilst chlorpromazine (5 mg/kg), chlorprothixene (5 mg/kg) and amitriptyline (10 mg/kg) reduced "analgesia" due to arecoline by 20 to 40%, higher doses by themselves prolonged the response time as much as did arecoline. Imipramine could be tested at high doses and gave an ED₅₀ of 30 mg/kg (hyoscine hydrobromide=0.4 mg/kg). The two desmethyl compounds were not tested.

Antagonism of arecoline-induced block of a conditioned reflex. Arecoline itself caused an inhibition of the conditioned response of $58.5 \pm 4.2\%$ (mean and standard error). This was completely antagonized by atropine (10 mg/kg), but imipramine, desmethyylimipramine and amitriptyline (all at 50 mg/kg) did not significantly reduce the effect of arecoline.

In tests where no arecoline was administered, imipramine and amitriptyline (25 mg/kg) made the rats apparently more eager to respond to the conditioning stimulus (that is, to jump up onto the platform). A similar effect was reported for imipramine by Maxwell & Palmer (1961). Chlorpromazine and chlorprothixene (25 mg/kg) reduced the conditioned response by 25 and 42% respectively. Neither in these experiments, nor in those in which arecoline was used, did the rats fail to respond to the unconditioned stimulus (shock).

DISCUSSION

Imipramine and two structurally-related drugs clinically successful in the treatment of depression, three tranquillizing agents and a hybrid, desmethylpromazine, have been subjected to a series of tests to assess their effects upon peripheral and central autonomic nervous functions and upon the actions of reserpine. The responses of the seven drugs in the main tests are summarized in Table 3.

The three antidepressant drugs, imipramine, desmethyylimipramine and amitriptyline, potentiated noradrenaline and antagonized the ptosis and prolongation of alcohol hypnosis induced by reserpine. The tranquillizing agents, chlorpromazine, chlorprothixene and promazine, were either inactive or produced opposite effects in these tests. They markedly reduced the pressor response to carotid arterial occlusion, prolonged sleep due to alcohol at low doses, and chlorpromazine and chlorprothixene in high doses depressed excitation produced by amphetamine; imipramine-like antidepressants were less active on the carotid occlusion reflex, prolonged alcohol hypnosis only moderately and at high doses, and never depressed excitation due to amphetamine.

Some tests failed to differentiate between the two classes, however. All compounds except chlorprothixene antagonized reserpine-induced bradycardia and in low doses increased the toxicity of yohimbine. Furthermore the activities of the compounds in the various tests for central or peripheral parasympathetic blockade

were unrelated to their activities in other tests. The relative weakness of imipramine and especially of desmethylimipramine in the tests for parasympatholytic activity cannot easily be reconciled with the importance placed by Fink (1959) and by Benešová & Trinerová (1963) on a central anticholinergic component in the anti-depressant action of imipramine (see also Biel, Nuhfer, Hoya & Leiser, 1962).

Desmethylpromazine exhibited an interesting mixture of actions. In its antagonism (at low doses) of reserpine-induced prolongation of alcohol hypnosis it resembled desmethylimipramine; similarly in its antagonism of reserpine-induced bradycardia it approached the potency of the latter compound, being some ten-times more potent than the tertiary amine, promazine. On the other hand, like promazine, it reduced the carotid occlusion reflex at low doses, rarely increased the pressor response to noradrenaline, prolonged alcohol hypnosis and showed weak potency in reversing reserpine-induced ptosis.

These results agree with the conclusions of Brodie, Dick, Kielholz, Poeldinger & Theobald (1961), Vernier, Alleva, Hanson & Stone (1962) and Bickel, Sulser & Brodie (1963) that demethylation of tertiary amines related to imipramine increases antireserpine and decreases depressant activity. Bickel *et al.* (1963) reported that desmethylpromazine exhibited weak antagonism of reserpine-like symptoms induced in rats by Ro-4-1284 (whereas promazine was inactive) but gave no further details.

Many workers have attempted to differentiate the pharmacological actions of imipramine and chlorpromazine, with varying degrees of success. Domenjoz & Theobald (1959), Frommel & Fleury (1959), Herr, Stewart & Charest (1961), Costa, Garattini & Valzelli (1960) and Metyšová, Metyš & Votava (1963) concluded that differences in pharmacological activity between the two substances were mainly quantitative, arising particularly from the relative weakness of the central depressant actions of imipramine. Certain other workers, however, have reported methods for differentiating imipramine from chlorpromazine in pharmacological tests on laboratory animals. In particular emphasis has been laid on various aspects of the antagonism of reserpine effects by imipramine but not by chlorpromazine (Maxwell & Palmer, 1961; Askew, 1963; Bickel *et al.*, 1963). Stein & Seifter (1961), working with rats capable of "self-stimulation" by indwelling hypothalamic electrodes, have reported probably the most clear-cut and positive differentiation of imipramine and chlorpromazine effects, exerted furthermore at low doses, but their experimental technique is hardly suitable for general laboratory use.

Somewhat similar but not so clear-cut results have been obtained in the present work by simple observation and scoring of the compulsive licking and gnawing behaviour induced in rats by amphetamine. Imipramine increased this excitatory behaviour markedly, both in degree and in duration, whereas low to moderate doses (2 to 10 mg/kg) of chlorpromazine only prolonged it without accentuating it, and high doses (50 mg/kg) depressed it. Chlorprothixene acted similarly, but promazine showed a marked potentiation like that of imipramine. It would be interesting to know whether promazine acts like imipramine or like chlorpromazine in Stein & Seifter's (1961) test.

It is reasonable to ask how far the results obtained for imipramine, desmethyl-imipramine and amitriptyline in the laboratory tests described here may be con-

sidered indicative of a central antidepressant action comparable to that exerted by these compounds in human therapy.

The main tests may be considered separately:

(1) All those utilizing the dog blood pressure preparation are likely to detect peripheral actions only. In the absence of further evidence, the reduction of the reflex response to carotid arterial occlusion by the drugs tested in this work may similarly signify a peripheral site of action, since it was always accompanied by reduction of the pressor response to adrenaline.

(2) The potentiation of amphetamine appears likely to be predominantly of central origin since the test relies on a behavioural response. However, the degree of excitation elicited by amphetamine in imipramine-treated animals is slightly reduced by the ganglion-blocking drug chlorisondamine, and by adrenalectomy. The response may therefore be augmented by a peripheral release of catechol amines, probably from the adrenal medulla as a result of hypothalamic stimulation. There seems no direct correlation between amphetamine potentiation and clinical antidepressant activity, since amitriptyline potentiates amphetamine only at high doses, promazine is as potent as imipramine and the two desmethyl compounds are not more active than their tertiary analogues.

(3) The toxic action of yohimbine in imipramine-treated mice is believed to be mediated by central stimulation of the hypophyseal-adrenal axis, producing a release of catechol amines both from the brain and from the adrenals (Quinton, 1963b). Ganglion-blocking agents or adrenalectomy reduce the toxicity. It is not known whether the toxicity arises from an augmentation by imipramine of the effects of the released amines at a central or a peripheral site, but cardiac actions at β -receptors may be implicated.

Imipramine-like drugs are not the only ones capable of increasing the toxicity of yohimbine, since the phenothiazines are also active though less potent than desmethylimipramine and amitriptyline. The test may however be specific for substances (other than those like reserpine which cause also the release of amines) that block catechol amine uptake by tissues.

(4) In the test for antagonism of reserpine-induced bradycardia, the response to imipramine is abolished by adrenalectomy or pronethalol, but is little reduced by chlorisondamine. Imipramine therefore appears to be acting peripherally, possibly by increasing the cardio-accelerator effects of circulating catechol amines derived from the adrenals, where under the influence of reserpine they are still synthesized but not stored. This action of imipramine may be ascribed to its interference with the uptake of catechol amines at storage sites in the heart (Axelrod, Hertting & Potter, 1962), as a result of which more amine is available to reach effector receptors. If this is so, imipramine and reserpine, which also blocks catechol amine uptake by the heart (Axelrod *et al.*, 1962), must prevent uptake at different sites, and those blocked by reserpine must account for at least 95% of the amine storage capacity of the tissue, since the catechol amine content may fall by this amount after treatment with reserpine (Bertler, Carlsson & Rosengren, 1956; Lee & Shideman, 1959). We have observed that, in dogs anaesthetized with pento-

barbitone, imipramine potentiates noradrenaline in its pressor action even after reserpine (2 mg/kg).

Support for a cardiac action of circulating catechol amines derived from the adrenal medulla in rats has been given recently by the work of Bhagat & Shideman (1964), who showed that removal of the adrenal medullae seriously reduced the rate of restoration of cardiac noradrenaline stores after depletion by reserpine. Administration of chlorpromazine also interfered with the restoration, presumably by inhibiting amine uptake into storage sites in the heart; Bhagat & Shideman did not test imipramine.

(5) Since imipramine and amitriptyline themselves prolong alcohol-induced hypnosis in mice, they would not antagonize the prolongation of the hypnosis by reserpine by some acceleration of alcohol metabolism. It is possible that they may antagonize a depression of alcohol metabolism induced directly by reserpine, but we know of no evidence for this. Although Garattini, Giachetti, Jori, Pieri & Valzelli (1962) and Askew (1963) have shown that imipramine, amitriptyline and desmethylimipramine can reverse reserpine-induced hypothermia in mice, hypothermia alone is unlikely to be the direct cause of a reduced rate of alcohol metabolism (Jaulmes, Delga & Bobo, 1956) in this case, since Lessin & Parkes (1957) demonstrated that reserpine, in the same dose used in the present work, causes little fall in mouse body temperature within 2 to 3 hr of injection. Possibly in this test imipramine is directly counteracting the sedation which, according to Costa, Gessa, Hirsch, Kuntzman & Brodie (1962), arises from low levels of brain 5-hydroxytryptamine resulting from release of stores of this amine by reserpine, by making more 5-hydroxytryptamine available to effector receptors in the brain. Imipramine may achieve this by a mechanism involving blockade of 5-hydroxytryptamine uptake at certain binding sites (Marshall, Stirling, Tait & Todrick, 1960), similar to that postulated above for the reversal by imipramine of reserpine-induced bradycardia by a potentiation of circulating noradrenaline. The failure of chlorpromazine to act similarly may be explained by its relative weakness in blocking tissue binding of 5-hydroxytryptamine *in vitro* (Stacey, 1961) or *in vivo* (Axelrod & Inscoc, 1963), possibly coupled with its greater potency in blocking the central stimulatory effects of tryptaminic agents (Garattini & Valzelli, 1960).

The results in the test for antagonism or augmentation of the reserpine-induced prolongation of alcohol hypnosis correlate well with clinical antidepressant or tranquillizing activity respectively. However, high doses of amitriptyline and desmethylpromazine, which cause sedation and markedly prolong alcohol hypnosis by themselves, fail to reverse the reserpine-induced prolongation.

(6) The site of action of imipramine in its reversal of reserpine-induced ptosis is uncertain, but indirect evidence points to a central effect. Reversal of ptosis by imipramine is accompanied by antagonism of the sedation due to reserpine, and the rabbits stay alert with eyes open for some time after cessation of imipramine injection (Maxwell, personal communication). Bogdanski, Sulser & Brodie (1961) have pointed out that reserpine induces not a passive ptosis but an active closure of the eyelids, which they suggest is due, at least in rabbits, to sensitization of the light reflex. If this is so, it would be unlikely to be affected by drugs acting only

on peripheral autonomic mechanisms, although adrenergic or tryptaminic pathways in the central nervous system may be involved (Garattini, Giachetti, Pieri & Re, 1960; Costa & Pscheidt, 1961; Vane, Collier, Corne, Marley & Bradley, 1961). Further support for a central site of action is given by our observation that *p*-hydroxyamphetamine, which is known to lack to a large extent the central stimulatory properties of amphetamine (Jacobsen, Christiansen, Eriksen & Hold, 1938), is less than a fifteenth as potent as amphetamine in antagonizing both the ptosis and the sedation induced by reserpine in mice.

The high potencies of imipramine, desmethylimipramine and amitriptyline and of amphetamine (Maxwell & Palmer, 1961), and the weakness or lack of action of the tranquillizing agents in this test correlate well with clinical antidepressant activity.

NOTE ADDED IN PROOF

Recent experiments on the antagonism of Tremorine-induced tremors in mice, performed under slightly different conditions, have indicated that imipramine may display a somewhat higher potency (ED₅₀ approximately 25 mg/kg) than that indicated in the text, especially when peripheral parasympathomimetic effects are blocked by atropine methyl nitrate. The ED₅₀ of desmethylimipramine in this test however is still greater than 50 mg/kg.

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REFERENCES

- ASKEW, B. (1963). A simple screening procedure for imipramine-like anti-depressant agents. *Life Sciences*, 725-730.
- AXELROD, J., HERTTING, G. & POTTER, L. (1962). Effect of drugs on the uptake and release of ³H-norepinephrine in the rat heart. *Nature (Lond.)*, 194, 297.
- AXELROD, J. & INSCOE, J. (1963). The uptake and binding of circulating serotonin and the effect of drugs. *J. Pharmacol. exp. Ther.*, 141, 161-165.
- AXELROD, J., WHITBY, L. G. & HERTTING, G. (1961). Effect of psychotropic drugs on uptake of ³H-norepinephrine by tissues. *Science*, 133, 383-384.
- BENEŠOVÁ, O. & TRINEROVÁ, I. (1963). The effects of psychotropic drugs on the cholinergic and adrenergic system. *Biochem. Pharmacol.*, Suppl. 12, 57.
- BERTLER, A., CARLSSON, A. & ROSENGREN, L. (1956). Release by reserpine of catecholamines from rabbit hearts. *Naturwissenschaften*, 43, 521.
- BHAGAT, B. & SHIDEMAN, F. E. (1964). Repletion of cardiac catecholamines in the rat; importance of the adrenal medulla and synthesis from precursors. *J. Pharmacol. exp. Ther.*, 143, 77-81.
- BICKEL, M. H., SULSER, F. & BRODIE, B. B. (1963). Conversion of tranquillisers to antidepressants by removal of one N-methyl group. *Life Sciences*, 247-253.
- BIEL, J. H., NUHFER, P. A., HOYA, W. K. & LEISER, H. A. (1962). Cholinergic blockade as an approach to the development of new psychotropic agents. *Ann. N.Y. Acad. Sci.*, 96, 251-262.
- BOGDANSKI, D. F., SULSER, F. & BRODIE, B. B. (1961). Comparative action of reserpine, tetrabenazine and chlorpromazine on central parasympathetic activity; effects on pupillary size and lachrimation in rabbit and on salivation in the dog. *J. Pharmacol. exp. Ther.*, 132, 176-182.
- BRODIE, B. B., BICKEL, M. H. & SULSER, F. (1961). Desmethylimipramine, a new type of anti-depressant drug. *Med. exp. (Basel)*, 5, 454-458.
- BRODIE, B. B., DICK, P., KIELHOLZ, P., POELDINGER, W. & THEOBALD, W. (1961). Preliminary pharmacological and clinical results with desmethylimipramine (DMI), G35020, a metabolite of imipramine. *Psychopharmacologia (Berl.)*, 2, 467-474.

- BURN, J. H. & HOBBS, R. (1958). A test for tranquillizing drugs. *Arch. int. Pharmacodyn.*, **113**, 290-295.
- COSTA, E., GARATTINI, S. & VALZELLI, L. (1960). Interactions between reserpine, chlorpromazine and imipramine. *Experientia (Basel)*, **16**, 461-463.
- COSTA, E., GESSA, G. L., HIRSCH, C., KUNTZMAN, R. & BRODIE, B. B. (1962). On the current status of serotonin as a brain neurohormone and in action of reserpine-like drugs. *Ann. N.Y. Acad. Sci.*, **96**, 118-133.
- COSTA, E. & PSCHIEDT, G. R. (1961). Correlations between active eyelid closure and depletion of brain biogenic amines by reserpine. *Proc. Soc. exp. Biol. (N.Y.)*, **106**, 693-696.
- DENGLER, H. J. & TITUS, E. O. (1961). The effect of drugs on the uptake of isotopic noradrenaline in various tissues. *Biochem. Pharmacol.*, **8**, 64.
- DOMENJOZ, R. & THEOBALD, W. (1959). Zur Pharmakologie des Tofranil. *Arch. int. Pharmacodyn.*, **120**, 450-489.
- FINK, M. (1959). Electroencephalographic and behavioural effects of Tofranil. *Canad. psychiat. Ass. J.*, **4**, S166-S171.
- FROMMEL, E. & FLEURY, C. (1959). De la pharmacodynamie différentielle du Tofranil d'avec la Chlorpromazine. *Med. exp. (Basel)*, **1**, 223-228, 264-268.
- GARATTINI, S., GIACHETTI, A., JORI, A., PIERI, L. & VALZELLI, L. (1962). Effect of imipramine, amitriptyline and their monomethyl derivatives on reserpine activity. *J. Pharm. Pharmacol.*, **14**, 509-514.
- GARATTINI, S., GIACHETTI, A., PIERI, L. & RE, R. (1960). Antagonists of reserpine-induced eyelid ptosis. *Med. exp. (Basel)*, **3**, 315-320.
- GARATTINI, S. & VALZELLI, L. (1960). Sulla valutazione farmacologica delle sostanze antidepressive. *Symposium, Estratto da le Sindromi Depressive, Rapallo*, pp. 7-30.
- GILES, H. M. (1963). Imipramine poisoning in childhood. *Brit. med. J.*, **ii**, 844-846.
- HERR, F., STEWART, J. & CHAREST, M. P. (1961). Tranquillizers and antidepressants; a pharmacological comparison. *Arch. int. Pharmacodyn.*, **134**, 328-342.
- JACOBSEN, E., CHRISTIANSEN, J. T., ERIKSEN, F. & HOLD, J. (1938). Analeptic action of sympathomimetic amines. *Skand. Arch. Physiol.*, **79**, 258-281.
- JANSSEN, P., NIEMEGEERS, C. & JAGENEAU, A. (1960). Apomorphine antagonism in rats. *Arzneimittel-Forsch.*, **10**, 1003-1005.
- JAULMES, C., DELGA, J. & BOBO, C. G. (1956). Metabolisme de l'alcool chez les animaux soumis à l'hibernation artificielle. *C.R. Soc. Biol. (Paris)*, **150**, 1094-1097.
- KÄRBER, G. (1931). Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmacol.*, **162**, 480-483.
- LEE, W. C. & SHIDEMAN, F. E. (1959). Role of myocardial catecholamines in cardiac contractility. *Science*, **129**, 967-968.
- LESSIN, A. W. & PARKES, M. W. (1957). The relation between sedation and body temperature in the mouse. *Brit. J. Pharmacol.*, **12**, 245-250.
- MARSHALL, E. F., STIRLING, G. S., TAIT, A. C. & TODRICK, A. (1960). Effect of iproniazid and imipramine on blood platelet 5-hydroxytryptamine level in man. *Brit. J. Pharmacol.*, **15**, 35-41.
- MARTIN, W. R., RIEHL, J. L. & UNNA, K. R. (1960). Chlorpromazine III. The effects of chlorpromazine and chlorpromazine sulfoxide on vascular responses to 1-epinephrine and levarterenol. *J. Pharmacol. exp. Ther.*, **130**, 37-45.
- MAXWELL, D. R. & PALMER, H. T. (1961). Demonstration of anti-depressant or stimulant properties of imipramine in experimental animals. *Nature (Lond.)*, **191**, 84-85.
- METÝŠOVÁ, J., METÝŠ, J. & VOTAVA, Z. (1963). Farmakologické Eigenschaften einiger neuen Tranquillizers und antidepressiven Substanzen. *Arch. int. Pharmacodyn.*, **144**, 481-513.
- MONRO, A. M., QUINTON, R. M. & WRIGLEY, T. I. (1963). Some analogs of imipramine. *J. med. pharm. Chem.*, **6**, 255-261.
- PELLMONT, B., STEINER, F. A., BESENDORF, H., BÄCHTOLD, H. P. & LÄUPPI, E. (1960). Zur Pharmakologie des "Taractan", eine Neurolepticums mit besondere Wirkungscharakter. *Helv. physiol. pharmacol., Acta*, **18**, 241-258.
- PFEIFFER, C. C. & JENNEY, E. H. (1957). The inhibition of the conditioned response and the counteraction of schizophrenia by muscarinic stimulation of the brain. *Ann. N.Y. Acad. Sci.*, **66**, 753-764.
- QUINTON, R. M. (1963a). The mydriatic response of mice to atropine. *J. Pharm. Pharmacol.*, **15**, 239-250.
- QUINTON, R. M. (1963b). The increase in the toxicity of yohimbine induced by imipramine and other drugs in mice. *Brit. J. Pharmacol.*, **21**, 51-66.
- QUINTON, R. M. & HALLIWELL, G. (1963). Effects of α -methyl dopa and dopa on the amphetamine excitatory response in reserpinized rats. *Nature (Lond.)*, **200**, 178-179.

- RAMACHANDRAN, B. V. & AGREN, G. (1963). The retarding effect of atropine on the absorption of subcutaneously injected DF³²P in the rat. *Life Sciences*, 820-824.
- SIGG, E. B. (1959). Pharmacological studies with Tofranil. *Canad. psychiat. Ass. J.*, 4, S75-S83.
- STACEY, R. S. (1961). Uptake of 5-hydroxytryptamine by platelets. *Brit. J. Pharmacol.*, 16, 284-295.
- STEIN, L. & SEIFTER, J. (1961). Possible mode of antidepressive action of imipramine. *Science*, 134, 286-287.
- SULSER, F., WATTS, J. & BRODIE, B. B. (1960). Antagonistic actions of imipramine (Tofranil) and reserpine on the central nervous system. *Fed. Proc.*, 19, 268.
- SULSER, F., WATTS, J. & BRODIE, B. B. (1962). On the mechanism of the antidepressant action of imipramine-like drugs. *Ann. N.Y. Acad. Sci.*, 96, 279-288.
- TRIPOD, J., BEIN, H. & MEIER, R. (1954). Characterization of central effects of reserpine and of their antagonistic reactions. *Arch. Int. Pharmacodyn.*, 96, 406-425.
- VANE, J. R., COLLIER, H. O. J., CORNE, S. J., MARLEY, E. & BRADLEY, P. B. (1961). Tryptamine receptors in the central nervous system. *Nature (Lond.)*, 191, 1068-1069.
- VERNIER, V. G. (1961). The pharmacology of antidepressant agents. In *Symposium on Depression*, pp. 7-13. *Dis. nerv. Syst.*, Suppl. 22.
- VERNIER, V. G., ALLEVA, F. R., HANSON, H. M. & STONE, C. A. (1962). Pharmacologic actions of amitriptyline, noramitriptyline and imipramine. *Fed. Proc.*, 21, 419.
- WOOLFE, G. & MACDONALD, A. D. (1944). The evaluation of the analgesic action of pethidine hydrochloride (Demerol). *J. Pharmacol. exp. Ther.*, 80, 300-307.